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Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.01/A1

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH 2 R56 NS063228-04

NIH R01 NS085993-01

Title: RNA export through nuclear envelope budding in premature aging disorders

Authors: *Y. LI, L. HASSINGER, J. ASHLEY, J. NUNNARI, M. MOORE, V. BUDNIK; UMMS, Worcester, MA

Abstract: We have recently uncovered a novel, nuclear pore complex-independent mechanism by which large ribonucleoprotein (megaRNP) granules exit the nucleus (Speese et al., (2012) Cell 149:832) This process, called nuclear envelope budding, is similar to the means by which Herpes-type viral nucleocapsids escape the host cell nucleus and is essential for postsynaptic development. An important process during nuclear envelope budding is the local reorganization of the nuclear lamina, a protein network localized beneath the inner nuclear membrane, and formed by the intermediate filament proteins, the lamins. Mutations in the *Drosophila* A-type lamin gene, *lamC*, prevent nuclear envelope budding, and result in abnormal synaptic development. In humans, mutations in the A-type lamin gene, *LMNA*, result in severe hereditary conditions, the laminopathies, affecting a number of tissues and leading to various dystrophies and premature aging disorders. In particular, specific mutations in *LMNA* cause dominant progeroid syndromes, including Hutchinson-Gilford progeria (HGPS) and Werner syndrome. To determine if progeroid phenotypes were related to abnormalities in nuclear envelope budding, we examined the nucleus of fibroblasts from HGPS patients, and generated the corresponding progeria mutations in fly *lamC.* Our preliminary ultrastructural studies reveal similar nuclear abnormalities both in HGPS fibroblasts and *Drosophila* cells expressing progeria-causing *lamC* mutant transgenes. In particular, the nuclear lamina is grossly abnormal, likely leading to abnormal nuclear envelope budding. We are currently using approaches to test the impact of these mutations in synaptic development and aging. These studies may shed new light on our current understanding of premature aging disorders, as well as their relationship to mRNA export through nuclear envelope budding and synaptic development.

Disclosures: Y. Li: None. **J. Ashley:** None. **J. Nunnari:** None. **M. Moore:** None. **V. Budnik:** None. **L. Hassinger:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.02/A2

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Austrian Science Fund

P24079

F4402

F4406

W01101

Title: Presynaptic α2δ subunits are essential for synapse formation and calcium channel trafficking

Authors: *C. L. SCHÖPF[1], M. CAMPIGLIO[1], S. GEISLER[1], R. STANIKA[1], A. LIEB[2], B. E. FLUCHER[1], B. NIMMERVOLL[1], G. J. OBERMAIR[1];
[1]Div. of Physiol., Med. Univ. Innsbruck, Innsbruck, Austria; [2]Dept. of Pharmacol. and Toxicology LFU Innsbruck, Innsbruck, Austria

Abstract: Auxiliary $\alpha_2\delta$ subunits modulate membrane trafficking and current properties of voltage-gated Ca^{2+} channels and have recently been implicated in neuronal functions like synaptic transmission and synapse formation. The CNS stably expresses $\alpha_2\delta$-1, -2, and -3 however, whether and how the individual isoforms contribute to specific functions in neurons simultaneously expressing all isoforms is elusive. In order to elucidate $\alpha_2\delta$ subunit-specific neuronal functions we studied the subcellular distribution of all three epitope-tagged $\alpha_2\delta$ isoforms in hippocampal neurons, and we established double knockout mice by crossbreeding single knockout ($\alpha_2\delta$-1 and -3) or mutant (*du*, $\alpha_2\delta$-2) mice. Moreover we established a cellular $\alpha_2\delta$ triple knock-out model by employing $\alpha_2\delta$-1 shRNA knock-down in cultured hippocampal neurons derived from *du*/$\alpha_2\delta$-3 double-knockout mice. Live-cell staining of cultured wildtype neurons expressing HA-tagged $\alpha_2\delta$-1, -2, and -3 revealed somato-dendritic, axonal, and presynaptic surface expression of all isoforms. Nevertheless expression levels of presynaptic $Ca_V2.1$ Ca^{2+} channels were mainly increased by $\alpha_2\delta$-2 and -3. This isoform specificity correlated with their propensity to increase $Ca_V2.1$ current density upon heterologous coexpression in tsA201cells (ΔI_{Ca}: $\alpha_2\delta$-3>$\alpha_2\delta$-2>>$\alpha_2\delta$-1). All double knockout mouse models showed strongly

reduced life spans, although to different degrees. This indicates that $\alpha_2\delta$ subunit functions are partly redundant, but essential for survival. Ca^{2+} current densities in double (*du*/$\alpha_2\delta$-3) and triple knockout neurons were significantly reduced to 65% and 50%, respectively, when compared to single $\alpha_2\delta$-3 knockout ($p<0.05$) or wildtype neurons ($p<0.01$). Axons from triple knockout neurons displayed axonal varicosities resembling presynaptic boutons, however, strongly reduced presynaptic synapsin and $Ca_V2.1$ content suggested a failure in synapse formation. Concomitant reduction in postsynaptic PSD-95 labeling indicated a role of $\alpha_2\delta$ subunits in the transsynaptic organization. In contrast, postsynaptic triple-knockout neurons still developed dendritic spines containing PSD-95 clusters opposite functional presynaptic boutons, which were formed by axons from $\alpha_2\delta$-1 containing neurons. Thus we conclude that the defect in proper induction and differentiation of synapses is caused by a presynaptic mechanism. Taken together our data demonstrate that $\alpha_2\delta$ isoforms partly differ with respect to trafficking and regulation of presynaptic Ca^{2+} channels. However, the essential role of $\alpha_2\delta$ subunits in presynaptic synaptogenesis is highly redundant.

Disclosures: C.L. Schöpf: None. **M. Campiglio:** None. **S. Geisler:** None. **R. Stanika:** None. **A. Lieb:** None. **B.E. Flucher:** None. **G.J. Obermair:** None. **B. Nimmervoll:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.03/A3

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Impaired synaptogenesis and learning in MARK2/Par1b knockout mice

Authors: *Q. WU[1], X. WANG[2], Z. PANG[2], G. WAGNER[3], H. ZHANG[1];
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Abstract: Partitioning defective1 (Par1), also called Microtubule-affinity regulating kinase (MARK), plays an essential role in many cellular contexts including embryogenesis, asymmetric cell division, directional migration, and epithelial morphogenesis. Previously, we found Par1 is required for normal spine morphogenesis in cultured hippocampal neurons. In addition, we found that Par-1 functions through phosphorylating the synaptic scaffolding protein PSD-95 at Ser561 in this process. However, it remains unclear what role Par1 plays during neuronal development

in vivo. Here we show that MARK2/Par1b knockout mice showed impaired dendritic spine moprhogensis, similar to what was observed in cultured hippocampal neurons expressing a Par1b shRNA. Additionally, Par1b knockout mice showed a decrease in mEPSC frequency but not mEPSC amplitude. Finally, we show that Par1b knockout mice exhibit poor performance in the Morris Water Maze as compared with wild type or heterozygotic controls. Together, our findings showed an important role of Par1 in learning and memory processes by regulating dendritic spine morphogenesis and synaptic transmission.

Disclosures: Q. Wu: None. **X. Wang:** None. **Z. Pang:** None. **G. Wagner:** None. **H. Zhang:** None.

Poster

777. Synapse Development

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Program#/Poster#: 777.04/A4

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Brain Canada

CIHR (84241)

Title: Role of LRRTM1/2 in synaptic plasticity, memory, and region-selective synapse development

Authors: *S. A. CONNOR[1], T. J. SIDDIQUI[2], F. MILLS[3], P. TARI[2], S. AU-YEUNG[2], H. KAWABE[5], S. BAMJI[3], N. BROSE[5,6], Y. WANG[4], A. CRAIG[2];
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Abstract: The proper wiring of neuronal networks required for normal brain development is crucially dependent upon regulated synapse formation. Synaptic organizing proteins mediate synapse formation by orchestrating pre- and postsynaptic differentiation. The leucine-rich repeat transmembrane proteins (LRRTMs) are synaptic organizing proteins that regulate synapse development and function in a cell type and brain region specific manner. LRRTMs have been linked to both schizophrenia (LRRTM1) and bipolar disorder (LRRTM2) suggestive of a critical role in neuropsychiatric disorders. Additionally, LRRTM1 and LRRTM2 are highly expressed in

the hippocampus, a brain structure essential for memory formation. To elucidate the function of LRRTMs in synapse regulation, we generated LRRTM1-/- LRRTM2-/- double knockout mice (LRRTM1/2 DKO). In LRRTM1/2 DKOs we observed a reduction in excitatory synapse number as indicated by a reduction in punctate immunofluorescence for VGlut1by confocal imaging, reduced dendritic spine density by Golgi staining, and decreased asymmetric synapse density by electron microscopy. mEPSC frequency was similarly decreased consistent with reduced excitatory synapse numbers. To determine if LRRTMs mediate synaptic plasticity, long-term potentiation was induced in CA1 of WT and DKO hippocampal slices. The duration of LTP was significantly reduced in DKO mice relative to wild-type (WT) littermate controls. To determine if reductions in LTP were recapitulated at a behavioral level, DKO and WT mice were trained in a contextual fear conditioning task which assays CA1-dependent memory performance. DKO mice exhibited impaired memory function at a long but not short time after training. Taken together, these data implicate LRRTMs as mediators of synapse formation, synaptic plasticity and memory and suggest mechanisms by which mutations in LRRTMs may contribute to neuropsychiatric disorders.

Disclosures: **S.A. Connor:** None. **T.J. Siddiqui:** None. **F. Mills:** None. **P. Tari:** None. **S. Au-Yeung:** None. **H. Kawabe:** None. **S. Bamji:** None. **N. Brose:** None. **Y. Wang:** None. **A. Craig:** None.

Poster

777. Synapse Development

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Program#/Poster#: 777.05/A5

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: a Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science (22390036)

Title: Localization of kirrel3 in the synapses during postnatal and adult stages in the mouse cerebellum

Authors: ***T. HISAOKA**[1], T. KITAMURA[2], Y. MORIKAWA[1];
[1]Dept. of Anat. & Neurobiology, Wakayama Med. Univ., Wakayama, Japan; [2]Div. of Cell. Therapy, Advanced Clin. Res. Center, The Inst. of Med. Science, The Univ. of Tokyo, Tokyo, Japan

Abstract: In the previous study, we have demonstrated that a member of the immunoglobulin superfamily, kirrel3, is expressed in the brain, including the cerebellum using *in situ* hybridization histochemistry. Recently, Kong *et al*. (Neurosci Lett 543: 22-26, 2013) have reported that a rat homolog of *kirrel3* is expressed in the granule cell layer (GCL) and Purkinje cell layer (PCL) during postnatal development. However, the detailed expression pattern and the function of kirrel3 in the mouse cerebellum remain unknown. In order to gain insights into the role of kirrel3 in the cerebellum, we characterized kirrel3-expressing cells in the postnatal and adult cerebellum using *kirrel3-lacZ* knockin mice, in which kirrel3 expression is detected as β-galactosidase (β-gal) activity. From postnatal day (P) 0 to P70 (adult mice), β-gal$^+$ cells were observed in both deep cerebellar nuclei and cerebellar cortex. In the cerebellar cortex, the expression of β-gal was found in the internal GCL and PCL from P0 to P70. On the other hand, β-gal$^+$ cells were observed in the external GCL at P0 and in the molecular layer (ML) from P7 to P70. The expression levels of β-gal reached a peak at P0 in the PCL, at P14 in the GCL, and at P21 in the ML, which are peak periods of the synaptogenesis in each region. In the PCL, small clusters of β-gal$^+$ cells were arranged as parasagittal bands in the posterior and nodular zones of the vermis. In the PCL of the nodular zone (vestibulo-cerebellum), Hsp25, a marker for a specific stripe of Purkinje cells (PCs) in the central and nodular zones, was expressed in β-gal$^+$ cells, suggesting that β-gal$^+$ cells may form a specific vestibulo-cerebellar circuit. Thus, kirrel3 is highly expressed in the projection neurons and interneurons of specific circuits in the cerebellum during the development of synapse. We further investigated the localization of kirrel3 protein in the cerebellum using immunohistochemistry. At P14, intense expression of kirrel3 protein was observed in the GCL and gradually decreased as development proceeded. Double-immunofluorescence staining for kirrel3 with PSD-95 revealed that kirrel3 was colocalized with PSD-95 in synapses and attachment plaques of the glomeruli of GCL at P14. Between P14 and P70, the expression of kirrel3 protein was also observed in the HCN1$^+$ basket cell terminals, which form pinceau around the axon initial segment of PCs. These findings suggest that kirrel3 may be involved in the synaptic formation/plasticity in granule cells and interneurons of the cerebellum during postnatal and adult stages.

Disclosures: **T. Hisaoka:** None. **Y. Morikawa:** None. **T. Kitamura:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.06/A6

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Circuit integration of VIP+ interneurons during development

Authors: *D. VAN VERSENDAAL, S. LEE, S. N. TUNCDEMIR, X. JAGLIN, B. WAMSLEY, G. MIYOSHI, G. FISHELL;
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Abstract: The diversity of interneurons is most readily evident in their unique gene expression and physiological properties. Nonetheless, it is their precise connectivity that allows them to achieve specialized functional roles in cortical processing, such as balancing network activity, gain control and gating of signal propagation. As such unraveling the mechanisms that drive cell-type specific connectivity are paramount to understanding how brain assembly is achieved. Interneurons that express vasointestinal peptide (VIP+) are positioned in the superficial layers of the cortex, predominantly receive long-range input from other brain areas and primarily act to mediate disinhibition. How they achieve their connectivity and function is unknown. Circuit assembly is tightly temporally and spatially coordinated and dependent on both genetic programs as well as neural activity. This study aims to resolve how these competing processes are coordinated to integrate VIP+ interneurons into a well-described circuit. We have begun by examining the events associated with the establishment of VIP+ connectivity. To this end, we used rabies tracing to identify the the sources of their afferent inputs and their developmental profile. Furthermore, we have utilized fluorescently-tagged synaptic proteins to study the structural dynamics of the afferent and efferent connections of VIP+ interneurons and explored how transcriptional regulation and activity contribute to their establishment during development.

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Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSC99-2321-B-010-002

NSC102-2321-B-010-018

NSC102-2911-I-010-506

Title: Foxp2 is required for morphological development of medium-sized spiny neurons in the mouse striatum

Authors: Y.-C. CHEN, *F.-C. LIU;
Inst. Neurosci, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: A severe speech and language disorder was found in the KE family. The genetic linkage study shows that the affected family members carry a missense R553H mutation of FOXP2 which causes the spoken language disorder. FOXP2 is therefore the first gene identified to be involved in speech and language. Clinical neuroimaging studies indicate that the caudate nucleus of striatum and the inferior frontal gyrus are structurally and functionally abnormal in the KE patient's brain. FOXP2R553H allele may function as a null allele which causes haploinsufficiency of FOXP2. In the present study, we studied the neurobiological function of the mouse homologue Foxp2 which differs three amino acids from human FOXP2. We focused on the morphological changes of striatal neurons by studying Foxp2 knockout (KO) mice. The Golgi's stain showed that the neurite lengths were dramatically decreased in the medium-spiny striatal neurons (MSN) of postnatal day (P12) Foxp2 KO brains. Moreover, the dendritic spine density in MSN was also significantly reduced in Foxp2 KO striatum. In parallel to the decreased dendritic spine density, the expression of PSD95, a post-synaptic marker, was also reduced in Foxp2 KO striatum. Taken together, our study suggests that Foxp2 is essential for morphological and dendritic spines/synapse development of MSN in the mouse striatum.

Disclosures: Y. Chen: None. **F. Liu:** None.

Poster

777. Synapse Development

Location: Halls A-C

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant MH086032

NIH Grant DK083452

Title: Leptin controls synaptogenesis by regulating the expression of Kruppel-like factor 4 (KLF4) and STAT3 signaling

Authors: *G. A. WAYMAN, M. DHAR, H. SHIINA, K. TYSON, M. ZHU, S. M. APPLEYARD;
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Abstract: Kruppel-like factor 4 (KLF4) is a transcription factor first identified in the gut and skin epithelium. It has been studied mostly in pre-mitotic cells, where it can act as either a transcription repressor or a transcription activator depending on the gene and the context. In the CNS, KLF4 levels rise significantly in the hippocampus of post-natal rats during a time of rapid synapse formation as well as in response to neuronal activity, which is required for synaptogenesis. Using high-throughput genome-wide screens we have identified KLF4 as an important target of neurotrophic factors such as leptin and BDNF, both of which stimulate synaptogenesis. Previously, KLF4 has been shown to inhibit axonal regeneration in nerve-injury models. Developing neurons polarize an axonal projection before they develop a dendritic arbor and form of synapses. We hypothesize that KLF4 acts a developmental switch from a period of axonal growth to a period of increased dendritic arborization and synapse formation in response to neurotrophic factors in hippocampal neurons. To study this we measured synaptogenesis in neonatal rat hippocampal cultures. We found that over-expressing KLF4 in dissociated hippocampal cultures increased spinogenesis. Whole-cell voltage-clamp experiments showed KLF4 over-expression also increased the frequency of miniature excitatory post-synaptic currents (mEPSCs), consistent with the formation of functional excitatory synapses. Over expression of KLF4 also increased spinogenesis in organotypic slice cultures. The hormone leptin, which increases synaptogenesis in hippocampal neurons, transiently increased KLF4 mRNA expression 1-2 hour post-treatment and KLF4 mRNA levels returned to baseline levels 4 hour post-leptin treatment. This increase in KLF4 transcription is required for leptin-induced synapse formation since reducing KLF4 expression using a targeted shRNA inhibits leptin-stimulated spine formation. KLF4 induces synaptogenesis in part by suppressing STAT3 dependent transcription. Transfection of KLF4 decreased pSTAT3 and nuclear localized STAT3, as well as STAT3 dependent transcription. Furthermore, expression of a constitutively active STAT3 attenuates leptin stimulated spinogenesis. In conclusion, we have identified KLF4 as a factor whose overexpression stimulates synaptogenesis and that is required for leptin-induced synapse formation, in part through suppression of STAT3.

Disclosures: G.A. Wayman: A. Employment/Salary (full or part-time):; Washington State 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.; NIMH MH086032. **M. Dhar:** None. **H. Shiina:** None. **K. Tyson:** None. **M. Zhu:** None. **S.M. Appleyard:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.09/A9

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Neuron specific gene 2 (Nsg2): a novel AMPA receptor interacting protein involved in secretory vesicle transport

Authors: P. CHANDER, *J. P. WEICK;
Neurosci., Univ. of New Mexico, Albuquerque, NM

Abstract: AMPA receptor (AMPAR) trafficking is a well-established mechanism underlying transient and enduring changes in synaptic efficacy during learning and memory. Altered glutamatergic neurotransmission has been implicated in a variety of neurological disorders such as Autism, Down syndrome, and Schizophrenia among others. Surprisingly, little is known about how newly-synthesized AMPARs are trafficked through the secretory pathway and delivered to nascent synaptic sites during development. We discovered that NSG2, along with the GluA2 AMPAR subunit, are two of the most highly-upregulated transcripts during functional maturation of human pluripotent stem cell-derived neurons (hPSNs). Based on sequence homology, NSG2 (also known as HMP19) has been classified as a member of a family of three, single transmembrane domain-containing proteins including Calcyon and NEEP21 (NSG1). Both Calcyon and NEEP21 are endosomal proteins shown to regulate AMPA receptor (AMPAR) trafficking during synaptic plasticity. Thus, we hypothesized that NSG2 was involved in trafficking newly-synthesized AMPARs to sites along neuronal arbors that are destined to form synaptic junctions during functional development. In addition to previously demonstrated golgi localization, overexpression of NSG2-mCherry resulted in small fluorescent punctae located throughout developing neuronal arbors. Time-lapse imaging revealed dynamic antero- and retrograde transport (~1μm/sec), consistent with active transport along microtubules. Endogenous and overexpressed NSG2 was found co-localized with intracellular compartments including endosomes and golgi apparatus. Co-immunoprecipitation experiments showed that NSG2 bound to both overexpressed and endogenous forms of the GluA2 subunit of AMPARs *in vitro* and from whole brain lysates from neonatal mouse pups. Lastly, modulation of NSG2 protein levels revealed predicted alterations in AMPAR-mediated glutamatergic neurotransmission. Together, these data support the notion that NSG2 is a critical mediator of AMPAR trafficking in developing neurons. In addition, these data support the use of hPSNs for the discovery of relatively late neurodevelopmental processes that occur *in vivo*.

Disclosures: **P. Chander:** None. **J.P. Weick:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.10/A10

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: University at Buffalo Rehabilitation Science Collaborative

Title: Neuromuscular NMDA receptors modulate developmental synapse elimination

Authors: ***S. B. UDIN**[1], B. SLUSHER[3], C. GARCIA[2], M. BANCONE[2], M. MORALES[1], K. PERSONIUS[2];
[1]Physiol. and Biophysics, [2]Rehabil. Sci., SUNY, BUFFALO, NY; [3]Neurol., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Motor neurons form excess synaptic connections with muscle fibers during normal development. After birth, the extraneous connections are lost, leading to mono-innervation. This synapse elimination is controlled by differential activity: the more active motor inputs gain synaptic territory, while the less active are deleted. The field has focused on acetylcholine as the mediator of activity, but our data indicate that NMDA receptors are the pivotal system activated by differential activity. NMDA and AMPA receptors have previously been described at the adult neuromuscular junction (NMJ) by Berger et al. (1995) and Mays et al. (2009). Using immunohistochemical methods in postnatal day 4 (P4) and P18 CD-1 mice, we find post-synaptic expression of GluA1, GluN1 and GluN2A at the NMJ. To test the role of ionotropic glutamate receptors in synapse elimination, we inhibited those receptors by implanting slices of the slow-release polymer Elvax infused with CNQX and AP-5 between the anterior and posterior compartments of the leg at P4. Contralateral muscles were implanted with saline-infused Elvax. At P11, immunostaining to reveal the extent of polyneuronal innervation demonstrated a highly significant slowing of synapse elimination in the CNQX-AP5 treated muscles. Another test of the hypothesis that NMDA receptors promote synapse elimination of weak inputs, and/or stabilization of the strongest input, utilized a morpholino to knock down expression of the NMDA receptor GluN1 subunit. Anti-GluN1 or scrambled control morpholinos were injected at the endplate region at P4. At P11, there again was highly significant slowing of synapse elimination in the experimental muscles vs control. The glutamate at the NMJ is likely to originate from N-acetyl-aspartylglutamate (NAAG) released from the motor neuron. NAAG can

be enzymatically cleaved by glutamate carboxypeptidase II (GCPII) to yield glutamate. We thus hypothesized that blocking GCPII would have an effect comparable to blocking glutamate receptors. We infused the GCPII inhibitor 2-(Phosphonomethyl)pentane-1,5-dioic acid via Elvax from P4-P11 and again obtained highly significant slowing of synapse elimination. To assess the response of the EDL muscle to glutamate from P3-adult, we have begun to use calcium imaging. Our results to date indicate much more vigorous response in muscles up to P14 than at older ages. Our data support the hypothesis that glutamate, produced from enzymatic breakdown of NAAG, activates NMDA receptors and thus contributes to reduction of polyneuronal innervation at the developing NMJ. This work was supported by University at Buffalo Rehabilitation Science Collaborative Grants to KEP.

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Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Global Center for Education and Research in Integrative Membrane Biology

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Title: Afadin plays a role in formation of puncta adherentia junction and differentiation of presynapses in hippocampal neurons

Authors: *D. TOYOSHIMA[1,2,3], K. MANDAI[2,3], T. MARUO[2,3], I. SUPRIYANTO[4,3], M. MORI[4,3], Y. TAKAI[2,3];
[1]Kobe Univ. Grad. Sch. of Med., Dept. of Pediatrics Kobe Univ. Grad., Kobe/Hyogo, Japan; [2]Dept. of Biochem. and Mol. Biol., Kobe Univ. Grad. Sch. of Med., Kobe, Japan; [3]CREST, Japan Sci. and Technol. Agency, Kobe, Japan; [4]Fac. of Hlth. Sciences, Kobe Univ. Grad. Sch. of Hlth. Sci., Kobe, Japan

Abstract: A synapse, a specialized form of a cell-cell adhesion site in neurons, consists of synaptic junctions and puncta adherentia junctions (PAJs). PAJs are highly developed in mossy fiber-CA3 pyramidal cell synapses in the hippocampus. The formation and remodeling of the mossy fiber-CA3 pyramidal cell synapses are implicated in the cellular basis of learning and memory. Although afadin and its binding cell adhesion molecules such as nectin-1, nectin-3, and N-cadherin, are concentrated at PAJs in these synapses, the function of these molecules has remained elusive. Here, we investigated the roles of afadin in PAJ formation and presynaptic differentiation in the mossy fiber-CA3 pyramidal cell synapses. In the mice of which afadin gene was conditionally inactivated before synaptogenesis, the immunofluorescence signals for the PAJ components such as nectin-1, nectin-3 and N-cadherin, disappeared almost completely, while those for the presynaptic components such as VGLUT1 and bassoon, were markedly decreased. In addition, these signals were significantly decreased in cultured afadin-deficient hippocampal neurons. Furthermore, the interevent interval of miniature excitatory postsynaptic currents (mEPSCs) was prolonged in the cultured afadin-deficient hippocampal neurons compared with control neurons, indicating that presynaptic release functions were suppressed or the number of synapses was reduced in the afadin-deficient neurons. Analyses of presynaptic vesicle recycling and paired recordings revealed that the cultured afadin-deficient neurons showed impaired presynaptic functions. These results indicate that presynaptic functions were suppressed or the number of synapses was reduced in the afadin-deficient neurons. Taken together, afadin regulates both PAJ formation and presynaptic differentiation in most mossy fiber-CA3 pyramidal cell synapses, while in a considerable population of the neurons, afadin regulates only PAJ formation but not presynaptic differentiation.

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Poster

777. Synapse Development

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Program#/Poster#: 777.12/A12

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Maintenance of the large-scale depolarization wave in the embryonic chick brain against deprivation of the rhythm generator

Authors: *Y. MOMOSE-SATO[1], K. SATO[2];
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Abstract: Widely correlated spontaneous activity in the developing nervous system is transiently expressed and is considered to play a fundamental role in neural circuit formation. The depolarization wave, which spreads over a long distance along the neuraxis, maximally extending to the lumbosacral cord and forebrain, is an example of this spontaneous activity. Although the depolarization wave is typically initiated in the spinal cord in intact preparations, spontaneous discharges have also been detected in the isolated brainstem. Although this suggests that the brainstem has the ability to generate spontaneous activity, but is paced by a caudal rhythm generator of higher excitability, a number of questions remains. Does brainstem activity simply appear as a passive consequence, or does any active change occur in the brainstem network to compensate for this activity? If the latter is the case, does this compensation occur equally at different developmental stages? Where is the new rhythm generator in the isolated brainstem? To answer these questions, we optically analyzed spatio-temporal patterns of activity detected from the chick brainstem before and after transection at the obex. The results revealed that the depolarization wave was homeostatically maintained, which was characterized by an increase in excitability and/or the number of neurons recruited to the wave. The wave was more easily maintained in younger embryos. Furthermore, we demonstrated that the ability of brainstem neurons to perform such an active compensation was not lost even at the stage when the depolarization wave was no longer observed in the intact brainstem.

Disclosures: Y. Momose-Sato: None. **K. Sato:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.13/A13

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Title: Optical survey of initial expression of synaptic function in the embryonic chick trigeminal sensory nucleus

Authors: *K. SATO[1], Y. MOMOSE-SATO[2];
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Abstract: We examined the initial expression of synaptic function in the embryonic chick trigeminal nucleus using voltage-sensitive dye recording. Brainstem preparations with three trigeminal nerve afferents, the ophthalmic nerve (N. V1), maxillary nerve (N. V2) and mandibular nerve (N. V3), were dissected from 5.5- to 6.5-day-old chick embryos. In our previous study [Sato et al., 1999], we detected slow signals corresponding to glutamatergic excitatory postsynaptic potentials and identified the principal sensory nucleus of the trigeminal nerve (Pr5), spinal sensory nucleus of the trigeminal nerve (Sp5) and trigeminal motor nucleus. In this study, we examined the effects of removing Mg2+ from the physiological solution, which enhanced N-methyl-D-aspartate receptor function in the sensory nuclei. In 6.5-day-old (St 29) embryos, the slow signal was observed in Pr5 and Sp5 only when N. V1 was stimulated, whereas it appeared in Mg2+-free solution with every nerve stimulation. In 6-day-old (St 28) embryos, the slow signal was observed in Sp5 with N. V1 stimulation, and the appearance of synaptic function in Mg2+-free solution varied, depending on the nerves and preparations used. In 5.5-day-old (St 27) embryos, synaptic function was not detected even when external Mg2+ was removed. These results indicate that the initial expression of synaptic function in the trigeminal system occurs earlier than previously considered, and that the developmental organization of synaptic function differs among the three trigeminal nerves and between the two sensory nuclei.

Disclosures: K. Sato: None. **Y. Momose-Sato:** None.

Poster

777. Synapse Development

Location: Halls A-C

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Program#/Poster#: 777.14/A14

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant Ey021222

Title: Matricryptins derived from collagen XIX induce inhibitory synapse formation

Authors: *J. SU, M. FOX;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Although inhibitory synapses comprise only ~20% of the total synapses in the mammalian cerebrum, they play essential roles in controlling neural activity. In fact, perturbing inhibitory synapse assembly or function has been associated with autism, epilepsy and schizophrenia. Although many types of inhibitory synapses exist, these developmental disorders have been strongly linked to defects in inhibitory synapses formed by parvalbumin (PV)-expressing interneurons. Despite their importance we lack a complete understanding of the mechanisms that underlie the formation of these inhibitory synapses. With that in mind our attention has been drawn to collagen XIX, an unconventional collagen expressed by interneurons during synaptogenesis. Mice lacking collagen XIX exhibit spontaneous generalized motor and absence seizures and are more susceptible to drug-induced seizure induction, both phenotypes associated with defects in inhibitory signaling. Here we show that these collagen XIX-deficient mice exhibit defects in PV+ synapse formation in subiculum, visual cortex and prefrontal cortex. Like other unconventional collagens, the C-terminal domain of collagen XIX is proteolytically shed and functions as a matricryptin (i.e. a fragment of an ECM molecule that exhibits a unique function from the full length molecule from which it was released from). Since other matricryptins have been shown to be synaptogenic, we speculated that collagen XIX-derived matricryptins (termed NC1[XIX]) are synaptogenic. Here, *in vitro* assays show that NC1[XIX] induces the formation of functionally active inhibitory nerve terminals and is sufficient to rescue synaptic defects in the absence of full-length collagen XIX. Moreover, the synaptogenic activity of NC1[XIX] can be blocked with RGD-containing peptides, indicating that integrins are required for NC1[XIX] function. Taken together, these results reveal a novel set of mechanisms governing the formation of inhibitory synapses in the mammalian cerebrum.

Disclosures: J. Su: None. **M. Fox:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.15/A15

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant EY021222

Title: Loss of synaptogenic collagen XIX results in spontaneous seizures, increased seizure susceptibility, and schizophrenia-related behaviors

Authors: *K. M. LIPPOLD, J. SU, J. CHEN, M. FOX;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Inhibitory synapses comprise only a small fraction of the total synapses in the mammalian cerebrum, but play essential roles in controlling neural activity. Perturbing inhibitory synapse assembly or function has been associated with autism, epilepsy and schizophrenia. We recently identified collagen XIX as a trans-synaptic synaptic organizing cue that is expressed by cerebral interneurons and is necessary for the proper assembly of inhibitory nerve terminals in hippocampus and cortex. In humans, deletion of the genomic region encoding this unconventional collagen has been linked to familial schizophrenia. Here, we discovered that genetically modified mice lacking collagen XIX exhibit spontaneous generalized motor and absent seizures. Video EEG/EMG recordings were employed to document rapid spiking waves and spike-wave complexes during seizures in collagen XIX-deficient mutants. In addition to spontaneous seizures, deletion of collagen XIX increased susceptibility to PTZ-induced and significantly impaired the ability to terminate drug-induced seizures. This result is particularly interesting to us since we hypothesize that collagen XIX is essential for the formation of inhibitory PV+ synapses and these synapses regulate seizure termination. Finally, based on these phenotypes and the mapping of familial schizophrenia to the genomic region encoding collagen XIX, we asked whether mice lacking collagen XIX exhibit schizophrenia-related behaviors. Data presented here demonstrate that mice lacking this synaptogenic collagen display impaired responses to pre-pulse inhibition assays and a striking lack of nest-building activity. Taken together with data showing a role of collagen XIX in being critical for inhibitory synapse formation, these data reveal that collagen XIX-deficient mutants exhibit anatomical, physiological and behavioral defects consistent with defects associated with schizophrenia. Moreover, these studies are beginning to provide cellular and molecular data that increase our understanding of why mutations of collagens lead to complex brain disorders.

Disclosures: K.M. Lippold: None. **J. Su:** None. **J. Chen:** None. **M. Fox:** None.

Poster

777. Synapse Development

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Program#/Poster#: 777.16/A16

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: CNPq

CAPES

FAPERJ

DECIT/MS

Title: Astrocyte transforming growth factor beta 1 promotes inhibitory synapse formation via CAM kinase II signaling

Authors: L. P. DINIZ[1], V. TORTELLI[1], M. N. GARCIA[1], A. BÉRGAMO[1], H. M. MELO[2], G. S. S. DA SILVA[2], F. G. DE FELICE[2], S. V. ALVES-LEON[3], J. M. DE SOUZA[3], L. F. ROMÃO[4], *F. C. GOMES[1];
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Abstract: Introduction: Astrocytes play an important role in the development and function of neuronal circuitry. Recent studies have indicated that, in addition to regulating excitatory synaptogenesis, astrocytes also regulate inhibitory synapse formation. However, the molecule(s) and the molecular mechanisms that control this event remain unknown. One putative candidate belongs to the Transforming Growth Factor Beta (TGF-β) superfamily, which multifunctional polypeptide members are critical for the modulation of GABAA synaptic transmission and dendritic homeostasis. Recently, we demonstrated that TGF-β1 induces formation of excitatory synapses between cortical neurons (Diniz et al., J. Biol. Chem. 2012). However, its effect on inhibitory synapses is unknown. Objective: To verify if TGF-β derived from murine and human astrocytes regulates the formation of inhibitory synapses between cortical neurons. Methods and Results: Conditioned media derived from human and murine astrocytes induced formation of inhibitory synapses between cerebral cortex neurons *in vitro*, an event inhibited by both pharmacologic and genetic manipulation of the TGF-β pathway. TGF-β1 induction of inhibitory synapses was dependent on glutamatergic activity and activation of CaM kinase II, which thus increased the levels of the synaptic adhesion protein, Neuroligin 2, and induced its localization to the inhibitory postsynaptic terminals. Additionally, intraventricular injection of TGF-β1 enhanced inhibitory synapse number in the cerebral cortex. Our results identify TGF-β1/CaMKII pathway as a novel molecular mechanism underlying astrocyte control of inhibitory synapse formation. We propose here that the balance between excitatory and inhibitory inputs might be provided by astrocytes signals, at least partly achieved via TGF-β1 downstream pathways. Our work contributes to the understanding of the GABAergic synapse formation and may be of relevance to further the current knowledge on the mechanisms underlying the development of various neurological disorders, which commonly involve impairment of inhibitory synapse transmission.

Disclosures: **L.P. Diniz:** None. **V. Tortelli:** None. **M.N. Garcia:** None. **A. Bérgamo:** None. **H.M. Melo:** None. **G.S.S. da Silva:** None. **F.G. de Felice:** None. **S.V. Alves-Leon:** None. **J.M. de Souza:** None. **L.F. Romão:** None. **F.C. Gomes:** None.

Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH/NINDS KO1 Award 1K01NS085071-01

Title: FGF22 and FGFBP1 regulate presynaptic differentiation

Authors: ***M. J. TENGA**[1], H. UMEMORI[2], G. VALDEZ[1,3];
[1]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [2]Boston Children's Hospital, Harvard Univ., Boston, MA; [3]Dept. of Biol. Sci., Virginia Polytechnic Inst. and State Univ., Blacksburg, VA

Abstract: Synaptic development is a multistep process that includes the selective aggregation of molecular machineries during the transformation of nerve terminals to mature presynaptic sites. Previous findings have shown that signaling mediated by the FGF receptor 2b (FGFR2b) is required for the proper maturation of presynaptic terminals in the central and peripheral nervous systems. In this study, we assessed the roles of FGF22, a ligand for FGFR2b, and the FGF binding protein 1 (FGFBP1), a molecule that enhances the activity of FGF22, in promoting presynaptic differentiation. Using animals that lack FGF22, FGFBP1 or both (dKO), we examined presynaptic differentiation at early postnatal stages at the neuromuscular junction and at parvalbumin-positive brain synapses. Compared to control animals, presynaptic differentiation was delayed at neuromuscular junctions in single and double knockout animals. These defects were characterized by delayed apposition of presynaptic vesicles to the postsynapse, with synaptic vesicles found aggregated along the axon and outside of the nerve terminal. Although these developmental defects are short lived in FGF22 knockout animals, they persist in FGFBP1 knockout and dKO mice. In addition to presynaptic defects, neuromuscular junctions in FGFBP1 and dKO mice exhibit additional structural alterations, including fragmentation. These findings suggest important roles for FGF22 and FGFBP1 during presynaptic differentiation. In addition, the long-lasting synaptic defects seen in FGFBP1 and dKO mice indicate that other FGFs, regulated by FGFBP1, are involved in synaptic maturation.

Disclosures: **M.J. Tenga:** None. **H. Umemori:** None. **G. Valdez:** None.

Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Dr. Ralph and Marian Falk Medical Research Trust

Title: Excitatory and inhibitory synaptogenesis during development of neuronal networks *in vitro*

Authors: ***J. SURESH**[1,2], J. WANG[1], V. P. BINDOKAS[3], J. D. MARKS[1], W. VAN DRONGELEN[1];
[1]Dept of Pediatrics, [2]Committee on Computat. Neurosci., [3]Dept Pharmacol Physiol Sci., The Univ. of Chicago, Chicago, IL

Abstract: Evolution of network behavior is determined both by excitatory and inhibitory synaptogenesis. Attempts have been made to count synapses at different developmental stages in cell cultures, however, little is known about the individual densities of excitatory and inhibitory synapses. To quantify synaptogenesis, we counted the number of excitatory and inhibitory synapses at four critical stages during development: 5, 8, 14 and 20 days *in vitro* (DIV). We used immunofluorescence to label: 1) dendrites (MAP-2), 2) pre- and 3) postsynaptic puncta of excitatory synapses (vGlut/PSD-95) and inhibitory synapses (VGAT/gephyrin), 4) nuclei in cell bodies (DAPI). High resolution (50nm x 50nm pixel) images were acquired on Leica confocal microscope and deconvolved using Huygens software to remove distortions arising from a microscope's point spread function. Subsequently, ImageJ software was employed to obtain the synaptic counts from colocalization of dendrites, pre- and postsynaptic puncta. We developed a novel method to detect these colocalizations and to obtain a noise estimate associated with the detections. First, we created binary masks of the a) dendrites by tracing the outline structure, b) pre- and c) postsynaptic puncta, by extracting single pixel local-maxima of fluorescence intensity and expanding them by two pixels in all directions. Overlap detected from binary AND of the three masks was counted as a colocalized synapse on dendrites. Next, to estimate colocalizations that occur by chance, we repeated the detection procedure after destroying the spatial correlation between pre- and postsynaptic puncta by a) randomizing images of local-maxima, b) shifting the original pre- and postsynaptic masks relative to each other (cross-correlation). Both these

methods produced similar results for colocalizations by chance, which was used as detector baseline noise. We thus estimated excitatory and inhibitory synaptic density as number of synaptic counts per unit dendritic area, normalized by total cell count. Our preliminary results show that the density of excitatory synapses increases rapidly from 5 DIV to 14 DIV and decreases during the last developmental stage, 20 DIV. In contrast, the density of inhibitory synapses grows steadily with age and approaches the density of excitatory synapses at the last stage. Given that the typical ratio of excitatory to inhibitory populations is 80:20 in hippocampal networks, these data suggest that excitatory synaptogenesis naturally predominates during the first three weeks *in vitro*, while inhibitory synaptogenesis increases steadily to eventually balance the excitation towards the fourth week of maturation.

Disclosures: J. Suresh: None. **J. Wang:** None. **V.P. Bindokas:** None. **J.D. Marks:** None. **W. van Drongelen:** None.

Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH RO1EY019498

NSF GFRP

Title: Development of asymmetric inhibition required for direction selectivity in the retina

Authors: *R. D. MORRIE, M. B. FELLER;
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Abstract: In the murine retina, direction selective ganglion cells (DSGCs) fire action potentials maximally to objects in the visual scene moving in their preferred direction, but minimally to objects moving in the opposite, or null, direction. These directional responses are produced by an asymmetry in overall inhibitory conductance onto DSGCs, such that object motion in the null direction elicits a greater amount of GABAergic inhibition from starburst amacrine cells (SACs.). Though it has been established that this asymmetry in inhibition emerges during the second postnatal week, the synaptic basis of the asymmetry remains to be determined. Here we assess the quantal properties of inhibitory transmission between SACs and DSGCs during

development. First, we performed paired voltage-clamp recordings between genetically labeled SACs and DSGCs in the mouse and measured the amplitude and time course of paired pulse depression to compare the probability of release. We found no significant differences between null and preferred side synapses at P7 or P14. In addition, we found no significant difference in the kinetics of the GABA-A receptor mediated responses, indicating there were not differences in quantal content. Last, we have conducted analysis on spontaneous events, which we previously determined increase in frequency but not amplitude between these two ages. Preliminary data indicates that across development there is a significant change in frequency of events. Together, these findings suggest that the over the course of development there is an asymmetric increase in the number, but not the strength, of SAC to DSGC synapses. This implicates synaptogenesis from null side SACs as the driving force for the development of the direction selective circuit.

Disclosures: R.D. Morrie: None. **M.B. Feller:** None.

Poster

777. Synapse Development

Location: Halls A-C

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

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National Basic Research Program of China (2013CB530900)

Title: The role of Axin in dendrite and spine development

Authors: *Z. LIANG[1,2,3], Y. CHEN[1,2,3], E. FEI[1,2,3], W. FANG[1,2,3], W.-Y. FU[1,2,3], A. FU[1,2,3], N. IP[1,2,3];
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Abstract: Precise coordination of extracellular and intrinsic factors is critical for various developmental processes during the formation of the mammalian cerebral cortex. Among the scaffold proteins which serve as platforms for the formation of multi-signaling components to

transduce extracellular signals to intracellular targets, Axin (axis inhibitor) is one that is expressed at high levels in mouse brain at embryonic and postnatal stages. Our laboratory recently reported that Axin and its phosphorylation at Thr485 by cyclin-dependent kinase 5 (Cdk5) is crucial for the cerebral cortex development, particularly in the regulation of embryonic neurogenesis and axon outgrowth. However, the roles of Axin in other neurodevelopmental processes remain unclear. Interestingly, we found that Axin and its Thr485 phosphorylated form were enriched in the post-synaptic compartments in mouse brain, and well co-localized with post-synaptic marker PSD-95. Whereas knockdown of Axin by targeted shRNA impaired dendritic growth and reduced the density of dendritic spines in cultured hippocampal neurons, these developmental defects could be rescued by re-expression of the RNAi-resistant Axin construct. In addition, stabilization of Axin increased the phosphorylation of GluA receptors at Ser831. Taken together, our study suggests that Axin plays a critical role in dendrite and spine development in the hippocampus.

Disclosures: Z. Liang: None. **Y. Chen:** None. **E. Fei:** None. **W. Fang:** None. **W. Fu:** None. **A. Fu:** None. **N. Ip:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.21/A21

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: Fellowships for Excellence

Title: Exploring cell-type specific alternative splicing activity in neurons using bichromatic reporters

Authors: *T.-M. NGUYEN, D. SCHREINER, P. SCHEIFFELE;
Biozentrum, Univ. of Basel, Basel, Switzerland

Abstract: Neurexins represent a highly polymorphic family of receptors that contributes to the differentiation and function of neuronal synapses. The large molecular diversity results from combinatorial usage of alternative exons at six alternative spliced segments. The respective alternative splicing modulates protein structures and regulates the interaction with different synaptic ligands. The polymorphic nature and selective biochemical interactions of Neurexins raise the question whether specific isoforms are linked to the genetic identity of neurons.

Furthermore it remains unknown if neurons of same population display similar preference of the exon usage at an alternatively spliced segment or if cell-to-cell heterogeneity exists in the alternative splicing activity. Due to the cellular complexity of the brain as well as the molecular diversity of Neurexins the analysis of isoform expression with the single-cell resolution level using mRNA methods or histological tools has been challenging. To address these questions we have developed a bichromatic reporter system to monitor and quantify alternative splicing of individual cells *in situ*. Through introduction of a single nucleotide insertion in the alternatively spliced exon, two different translational reading frames are generated. Therefore changes in the alternative splicing choice are converted into changes in the reading-frame that can be detected at the protein level. We will present our results of validating and applying these reporters in cell cultures and in mouse brain *in vivo*.

Disclosures: T. Nguyen: None. **D. Schreiner:** None. **P. Scheiffele:** None.

Poster

777. Synapse Development

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Program#/Poster#: 777.22/A22

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: KAKENHI26430063

Title: Spikar, a novel transcriptional coactivator, regulates the formation and stabilization of dendritic spines dependent on drebrin

Authors: *H. YAMAZAKI, T. SHIRAO;
Gunma Univ., Maebashi, Japan

Abstract: Dendritic spines are small, actin-rich protrusions on dendrites whose development is fundamental for the formation of neural circuits. The actin cytoskeleton is a central player in dendritic spine morphogenesis. Drebrin is an actin-binding protein, which is thought to initiate the spine formation through forming unique drebrin-actin complex at postsynaptic sites. In this study, we have isolated a novel drebrin-binding protein, spikar. Spikar has bromo domain, two zinc-finger domains, and LXXLL nuclear-receptor box. A reporter gene assay demonstrated that spikar acts as a transcriptional coactivator for thyroid hormone receptor and estrogen receptor. In cultured neurons, spikar was localized mainly in nuclei and dendritic spines. Using RNA interference (RNAi) approaches, we found that spikar knockdown in cultured neurons resulted in

decrease of dendritic spines and filopodia. In addition, electrophysiological study demonstrated that Spikar knockdown resulted in a decrease of mEPSC frequency. These data suggested that spikar is involved in the spine formation. Rescue and overexpression experiment using mutated-NLS spikar (mNLS-spikar) showed that spikar is involved in spine formation in cytoplasm by modulating de novo spine formation and retraction of existing spines. We then examined whether the spine-formation activity of mNLS-spikar would require the presence of drebrin. We cotransfected mNLS-spikar and drebrin-shRNA into cultured neurons and measured the numbers of spines and filopodia. The drebrin knockdown abolished the mNLS-spikar-induced increase in spine and filopodium density. The inhibition of mNLS-spikar function by drebrin knockdown was rescued by the co-expression of an RNAi-resistant drebrin mutant. We further employed fluorescent recovery after photobleaching (FRAP) to evaluate the stability of spikar in dendritic spines. In drebrin-knockdown neurons, the stable fraction of mNLS-spikar was smaller than that of control neurons. These data indicate that extranuclear spikar facilitates spine and filopodium formation depending on drebrin.

Disclosures: H. Yamazaki: None. **T. Shirao:** None.

Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

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FEDER

COMPETE

FCT - PTDC/SAU-NEU/104100/2008

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Marie Curie Actions - International Reintegration Grant, 7th Framework Programme, EU

Title: Intra-axonal translation is required for synapse formation

Authors: R. O. COSTA[1], J. R. PEDRO[1], M. PINTO[1,2], H. R. RYU[3], N. L. JEON[3,4], S. R. JAFFREY[5], *R. D. ALMEIDA[1];
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Abstract: The mechanism of local protein synthesis in dendrites and axons is currently under intense investigation. Recent studies identified a large number of mRNAs localized at distal axons and growth cones, suggesting that local axonal translation may play an important role in different steps of neuronal development. In line with these evidences, early studies in axons demonstrated the requirement of local translation during axon chemotrophic responses to guidance cues. Moreover, it was demonstrated that local axonal translation is required for other neurodevelopmental mechanisms, such as axonal outgrowth, neuronal survival and axon regeneration. Interestingly, recent studies in *Aplysia*, suggest that local translation might be important for synapse formation. However, the role of local protein synthesis in presynaptic differentiation is largely unknown. Using a novel platform, a microfluidic chamber system which allows the physical separation of axons from cell bodies and dendrites, we were able to specifically manipulate axons without the cell body contribution. Our results demonstrate that FGF22 induced the clustering of synaptic vesicles, a hallmark of presynaptic assembly, when added specifically to axons. Additionally, when protein synthesis is inhibited specifically in axons, SV2 clustering is reduced to basal levels. FGF22 leads to the phosphorylation of 4E-BP1, a translational repressor, in an asymmetric pattern and induces intra-axonal translation of a beta-actin reporter, a destabilized form of EGFP fused to the 3'UTR of beta-actin. Taken together our results show that FGF22 activates cap-dependent translation and that this intra-axonal mRNA translation is required for presynaptic differentiation.

Disclosures: R.O. Costa: None. **J.R. Pedro:** None. **M. Pinto:** None. **H.R. Ryu:** None. **N.L. jeon:** None. **S.R. Jaffrey:** None. **R.D. Almeida:** None.

Poster

777. Synapse Development

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 777.24/A24

Topic: A.02. Neurogenesis and Gliogenesis

Support: CNPq

FAPERJ

CAPES

PCM-UFRJ

Title: Thyroid hormones induce neural differentiation and synapse formation in the cerebral cortex

Authors: *R. S. DEZONNE, SR, J. STIPURSKY, F. GOMES;
Inst. de Ciências Biomédicas-ICB, Bloco B, Sala F-15, Federal Univ. of Rio De Janeiro, Rio De Janeiro, Brazil

Abstract: Thyroid hormones (TH) are crucial for brain morphogenesis, acting in several steps like proliferation of progenitor cells, neuronal differentiation, maturation and migration, and synapse formation. We have previously shown that TH effects in neonatal cerebral cortex are mediated by astrocytes, although the mechanisms underlying these events during early phases of neural development are still unknown.In this study, we investigated the role of TH in neural cells (neuronal and glial progenitors) differentiation and maturation. By using cultures of neurons and radial glia cells (RG) derived from the cerebral and *in vivo* model of hypothyroidism, we analyzed neuronal and RG differentiation, neurite outgrowth and synaptogenesis.We observed that T4 significant reduced neuronal death and increased neuronal progenitors' proliferation and terminal differentiation. This was followed by neuronal morphological maturation, revealed by increased number and size of neurites in response to hormone treatment. Further, T4 induced excitatory and inhibitory synaptogenesis *in vitro*. These *in vitro* data was corroborated by the observation that hypothyroidism reduced the number of excitatory synapses in the cerebral cortex. In addition,THs induce astrocyte differentiation form RG. RG treated cells switch their neurogenic state to a gliogenic one, and change their radial morphology to resembles mature astrocytes.Together, our data suggest an important role for TH during neuronal maturation, and contribute to the understanding of the cellular and molecular mechanisms associated with cognitive deficits that accompany neuroendocrine disorders.

Disclosures: R.S. Dezonne: None. **J. Stipursky:** None. **F. Gomes:** None.

Poster

777. Synapse Development

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NINDS 1R01NS078164

Title: Rewiring inhibitory microcircuits in mouse motor cortex by direct lineage reprogramming of pyramidal neurons *in vivo*

Authors: *Z. YE[1], M. A. MOSTAJO-RADJI[2], J. R. BROWN[2], C. ROUAUX[4], G. SRUBEK TOMASSY[2], T. K. HENSCH[3], P. ARLOTTA[2];
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Abstract: Proper brain function requires balanced inhibitory and excitatory microcircuitry within the neocortex. To establish this balance, precise connections matching cell identity and spatial distribution are required. Previous work has shown that loss of the transcription factor, Fezf2, converts the fate of corticofugal projection neurons (CFuPN) to callosal projection neurons (CPN) in the deep layers, which in turn leads to abnormal lamination of inhibitory neurons and altered GABAergic inhibition (Lodato et al, 2011). It remains unclear whether the identity of individual pyramidal neuron targets or extracellular signals contained within different cortical layers instruct the local connectivity of inhibition. Here, we took the approach of direct lineage programming of CPN to CFuPN by *in utero* expression of Fezf2 in post-mitotic layer 2/3 neurons to dissect these possibilities. Reprogramming of CPNs to CFuPNs did not disturb gross lamination of cortical structure. Using single-cell real-time PCR, we first confirmed that reprogrammed CPNs acquired molecular signatures of deep layer CFuPNs. By whole-cell electrophysiology in brain slices, Fezf2-expressing layer 2/3 neurons were classified into two groups by supervised learning using a generalized linear model. The intrinsic membrane properties of reprogrammed CPNs were found to be distinct from neighboring CPNs, but similar to CFuPNs. Further, Fezf2-expressing layer 2/3 neurons showed higher frequency of miniature inhibitory post-synaptic events, indicating that changing the identity of individual pyramidal neurons from CPN to CFuPN recruited more inhibitory synapses. In particular, the functional inputs from Parvalbumin expressing interneurons onto Fezf2-expressing neurons were increased, in agreement with increased number of PV puncta on reprogrammed CPNs. Taken together, the identity of cortical pyramidal neurons is critical in establishing local circuit inhibition.

Disclosures: Z. Ye: None. **M.A. Mostajo-Radji:** None. **J.R. Brown:** None. **C. Rouaux:** None. **G. Srubek Tomassy:** None. **T.K. Hensch:** None. **P. Arlotta:** None.

Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 778.01/A26

Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: UC Davis RISE Award (AKM)

NIHM Grant MH088879 (AKM)

NINDS Grant NS060125 (AKM)

Dennis Weatherstone Predoctoral Fellowship (MLE)

UC Davis Graduate Research Mentorship Fellowship (MLE)

Title: Interleukin-1β alters cortical connectivity in development and disease through regulating synaptic localization of IL-1β receptors

Authors: *M. ESTES[1], A. MCALLISTER[2];
[1]UC Davis, Davis, CA; [2]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: M.L. ESTES, A. K. MCALLISTER; Univ. of California Davis, Davis, CA Abstract: Autism spectrum disorder (ASD) and schizophrenia (SZ) appear to be caused by both genetic mutations and environmental factors, many of which alter synaptic function and/or the immune response. The pro-inflammatory cytokine IL-1β, in particular, has been implicated in both disorders. IL-1 is elevated in the plasma and cerebrospinal fluid in ASD and polymorphisms in the IL-1 gene cluster, as well as mutations in components of the IL-1β receptor complex, including IL1RAPL1, are linked to ASD and SZ. During brain development, IL1RAPL1 and another IL-1β receptor, IL-1RAcP, function as central synaptic organizers through trans-synaptic interactions with PTPδ. Recently, our lab discovered that IL-1 levels are altered in the postnatal brain of offspring following maternal immune activation (MIA), which is the most compelling environmental risk factor for both ASD and SZ. These data suggest that IL-1 signaling might mediate the effects of a maternal peripheral immune response in altering connectivity and leading to ASD- and SZ-like behaviors in offspring. We have started to test this hypothesis by assessing the effects of MIA and exogenous IL-1 on IL-1 receptor composition and synapse density in dissociated cortical cultures. IL-1β and its receptors are present in the healthy, developing brain where it bi-directionally regulates cortical connectivity in a dose-dependent manner. At elevated concentrations, as seen in the brains of MIA offspring, IL-1β decreases synapse density through altering the synaptic localization of immune receptors that double as trans-synaptic adhesion molecules. In contrast, physiological concentrations of IL-1β promote synapse formation through an alternative mechanism. Similar to the effects of MIA, elevated concentrations of IL-1β that decrease glutamatergic synapse density also increase MHCI

expression on neurons, which itself causes synapse elimination. We are currently determining the relative roles for, and potential interactions between, IL-1β and MHCI in mediating the effects of MIA on cortical development. Together, these results suggest that environmental factors that alter IL-1 levels, and genetic mutations in IL-1 receptors themselves, converge on the synaptic localization of IL-1 receptors as a central mechanism of altering cortical connectivity in ASD and SZ.

Disclosures: M. Estes: None. **A. McAllister:** None.

Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: MRC UK G0800498

The Royal Society UIE131294

Title: Dissecting the structural role of GABA(A) receptors in synapse formation

Authors: L. E. BROWN, J. E. BENILLOUCHE-ARAMA, M. W. NICHOLSON, F. A. STEPHENSON, A. M. THOMSON, *J. N. JOVANOVIC;
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Abstract: The formation of inhibitory synapses is a precisely controlled process that is initiated by the establishment of specific cell-cell contacts between the presynaptic GABAergic neurons and their postsynaptic targets. Although the interactions between cell surface molecules are known to be the key regulators of this process, the molecular and functional characterization of these interactions remains sparse. Our recent experiments have indicated that GABA(A) receptors, themselves the main postsynaptic functional components of these synapses, play an important regulatory role (*Fuchs C, et al. (2013) Eur J Neurosci **38**, 3146*). Our aim is to characterize further the structural role of GABA(A) receptors in synapse formation. We have developed a heterologous co-culture model system, which incorporates the embryonic medium spiny neurons (MSNs) and HEK293 cells expressing various combinations of GABA(A) receptor subunits: α1/β2, α1/β2/γ2, α1/β2/δ, α1/β3 or α1/β3/γ2. After 24 h in co-culture, synapse formation was analyzed by immunolabelling with antibodies specific for the vesicular gamma-

aminobutyric acid transporter, as a presynaptic marker, and GABA(A) receptor subunits as postsynaptic markers. The activity of synapses was assessed using FM4-64 uptake. Synapse formation was quantified using Image J software. Our results indicate that the γ2 subunit promotes synapse formation. However, the magnitude of the effect of the γ2 subunit is influenced by the type of the β subunit, with the α1/β2/γ2 combination being significantly more effective than the α1/β3/γ2 combination. In comparison, minimal, if any contacts, formed between the MSNs and control HEK293 cells or those expressing the α1/β2/δ combination. The synaptogenic effects of GABA(A) receptors may be mediated by interactions between their large N-terminal extracellular domains (ECDs) and the proteins residing in the synaptic cleft. To investigate this, we have cloned, expressed and purified the N-terminal ECDs of α1, α2, β2 and γ2 subunits using a baculovirus/Sf9 cell system. The controls were prepared using the extracts of untransfected Sf9 cells which were taken through the same purification protocol. Synapse formation was reduced in the presence of the exogenous ECDs, probably due to competition in binding with the endogenous ECDs of GABA(A) receptors. These findings further support the structural role of GABA(A) receptors in synapse formation.

Disclosures: L.E. Brown: None. **J.N. Jovanovic:** None. **J.E. Benillouche-Arama:** None. **M.W. Nicholson:** None. **F.A. Stephenson:** None. **A.M. Thomson:** None.

Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant T32 HD007505

NIH Grant R01 NS070005

Title: Receptors and signaling that distinctly mediate excitatory and inhibitory presynaptic differentiation induced by the presynaptic organizers FGF22 and FGF7

Authors: *A. K. DABROWSKI[1,3], A. TERAUCHI[3,2], C. STRONG[1], H. UMEMORI[3,2]; [1]MBNI, [2]Mbni, Univ. of Michigan, Ann Arbor, MI; [3]F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA

Abstract: Formation of synaptic connections between appropriate partner neurons is crucial to proper brain development. During early stages of synapse development, molecules exchanged between complementary neurons facilitate differentiation of nascent synapses. We have shown that two target-derived fibroblast growth factors (FGFs), FGF22 and FGF7, are secreted from dendrites of CA3 pyramidal neuron and differentially promote excitatory and inhibitory presynaptic development in the CA3 of the hippocampus (Terauchi et al. 2010). Here we ask the signaling mechanisms through which FGF22 and FGF7 promote specific differentiation of excitatory and inhibitory presynaptic terminals, respectively. For this, we performed a series of *in vivo* and *in vitro* experiments in mice constitutively or conditionally lacking putative FGF receptors (FGFRs). We found that mice lacking FGFR2b or FGFR1b have excitatory presynaptic deficits phenocopying loss of FGF22, and mice lacking FGFR2b have inhibitory presynaptic deficits phenocopying loss of FGF7 in hippocampal CA3 *in vivo*. We then used an *in vitro* responsiveness assay and found that neurons lacking FGFR2b or FGFR1b do not respond to FGF22, and neurons lacking FGFR2b do not respond to FGF7. Since FGF22 requires both FGFR2b and FGFR1b, while FGF7 only requires FGFR2b, we propose that FGFR2b is a general signal to promote presynaptic differentiation, while FGFR1b provides specificity to the FGF22 response. We further asked whether FGFRs are functioning presynaptically in response to FGF22, and which signaling pathways are required downstream of FGFRs. To do this, we selectively knocked out FGFR2 and FGFR1 from the presynaptic cells, the dentate granule cells (DGCs), *in vitro*, and assayed their responsiveness to FGF22. We found that both FGFR2 and FGFR1 are required for DGCs to respond to FGF22. Furthermore, we created FGFR2b constructs that are kinase dead, FRS2 binding deficient, PI3K binding deficient, and PLCg binding deficient, which we selectively overexpressed in DGCs. We found that loss of kinase activity, or FRS2 or PI3K binding blocked response to FGF22, suggesting that the FRS2/AKT pathways are required for FGF22 responsiveness in the presynaptic cell. Together, our results suggest a model in which FGFR2b and FGFR1b function in the presynaptic cell to respond to target-derived FGF22, activating signaling pathways downstream of FRS2 and AKT to organize excitatory presynaptic terminals, while FGFR2b alone responds to FGF7 to promote inhibitory presynaptic differentiation. Our results provide insight into signaling mechanisms of how FGF22 and FGF7 selectively promote excitatory and inhibitory synapse differentiation.

Disclosures: A.K. Dabrowski: None. **A. Terauchi:** None. **C. Strong:** None. **H. Umemori:** None.

Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

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Dana Foundation

Title: Ephrin-B3 regulates excitatory synapse density through cell-cell competition

Authors: N. T. HENDERSON, S. LEMARCHAND, *M. HRUSKA, M. DALVA;
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Abstract: Synaptic communication is fundamental to the function of the nervous system, providing a means for rapid signaling and computation within neural networks. The number of synaptic inputs received by individual neurons varies across different brain regions and cell types from thousands to hundreds of thousands, yet the mechanisms by which this heterogeneity in synapse density is determined remain largely unknown. Eph receptors and their ligands, the ephrins, function as trans-synaptic cell adhesion molecules that play important roles in synapse development and function. Previous work has shown that ephrin-B3 functions postsynaptically to regulate excitatory synapse density in cortical pyramidal neurons through direct binding to Erk1/2, inhibiting MAPK signaling. Synapse density in neurons from ephrin-B3 knockout (KO) mice remains unchanged with respect to wild-type controls both *in vitro* and *in vivo*, indicating that ephrin-B3 is not required for synapse formation. However, in mixed cultures of wild-type and ephrin-B3 KO neurons, synapse density is reduced in ephrin-B3 KO neurons and increased in wild-type neurons, suggesting that relative levels of ephrin-B3 determine synapse density by a mechanism involving cell-cell competition. To directly test this hypothesis, we studied isolated neurons or pairs of neurons cultured on microislands and asked whether ephrin-B3 regulates synapse density through direct competition between individual neurons. In single-neuron microislands, knockdown of ephrin-B3 did not affect synapse density when compared to control-transfected neurons. In two neuron microislands, knockdown of ephrin-B3 in one neuron resulted in a decrease in synapse density compared to the untransfected neuron, while synapse density was increased in the untransfected neuron. Interestingly, the overall number of synapses in these microislands remained the same. Taken together, these data provide strong evidence for a model in which ephrin-B3-mediated cell-cell competition regulates the density of excitatory synaptic inputs received by individual neurons.

Disclosures: N.T. Henderson: None. **M. Hruska:** None. **S. LeMarchand:** None. **M. Dalva:** None.

Poster

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Title: EphB tyrosine kinase signaling in living dendritic filopodia

Authors: Y. MAO, J. ZHU, K. HANAMURA, *M. B. DALVA;
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Abstract: The formation of synapses is induced at sites of contact between axons and dendrites of neurons by specific protein-protein interactions. Contact between axons and dendrites are driven by motile axonal and dendritic filopodia while a number of proteins including EphBs and ephrin-Bs can induce synapse development. EphB kinases are required for the movement of dendritic filopodia and excitatory synapse formation. However, whether EphB localize in filopodia and what the local signaling by EphBs is to mediate filopodia motility and synaptogenesis are not well understood. Here we demonstrate that EphB2 proteins are localized to the tips of certain types of dendritic filopodia that are thought to be important for synapse formation. In order to study the activity of Eph tyrosine kinases at cellular level in living cells, we have developed a set of dual color genetically-encoded Eph indicators. These tools enable the visualization of the local signaling by specific Eph receptors (both EphA4 and EphB2) in HEK cells and neurons. In cells, we find that EphA4 and EphB2 kinases have different rates of deactivation consistent with differences in signaling by these receptors. In cultured cortical neurons, EphB signaling is higher within stable dendritic filopodia that contact axons than in moving filopodia. These findings suggest that EphB signaling is tightly linked the generation of stable contacts and consistent with previous work indicating the role of EphBs in the induction of synapse formation. Our indicator tools demonstrate that tyrosine kinase signaling by EphBs within living cells can be restricted to small sub-cellular domains. The design of these tools should enable visualization of tyrosine kinase activity at high resolution and should allow us to determine the relationship between kinase activity and cell behavior.

Disclosures: Y. Mao: None. **M.B. Dalva:** None. **J. Zhu:** None. **K. Hanamura:** None.

Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: HHMI

NIH grant 1K99NS084988-01

Title: Transcriptional coordination of synaptogenesis and neurotransmitter signaling in *C. elegans* motor neurons

Authors: *P. KRATSIOS[1], B. PINAN-LUCARRÉ[2], S. KERK[1], J.-L. BESSEREAU[2], O. HOBERT[1];
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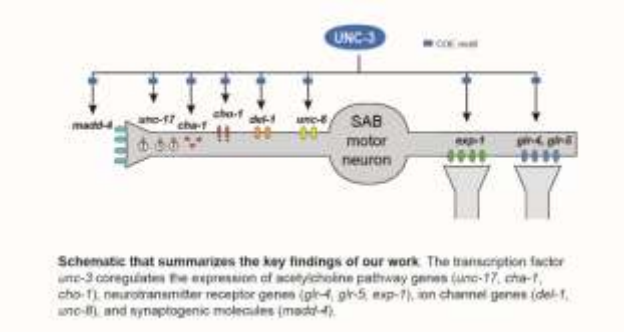
Abstract: How the process of synaptogenesis is coordinated with the adoption of other neuron type-specific identity features during neuronal differentiation is a largely unexplored question in developmental neuroscience. Synaptogenic events could be controlled in a manner that is independent of the adoption of, for example, neuronal signaling features of a specific neuron type, such as the choice which neurotransmitter system to use to communicate with postsynaptic partners (“neurotransmitter identity”), or they could be tightly coupled. We describe here two distinct mutant *C.elegans* strains, retrieved from a genetic screen for mutants in which the SAB head motor neurons (MNs), a previously little studied class of MNs in the *C. elegans* nervous system, fail to form synapses with their muscle targets. One mutant strain harbors a loss of function allele of the Collier/Olf/Ebf (COE)-type transcription factor UNC-3, the other carries a mutation in the ADAMTS-like extracellular matrix protein MADD-4/Ce-punctin, a presynaptically secreted synapse organizing molecule that clusters postsynaptic receptors. We demonstrate that *madd-4* expression is controlled by UNC-3 via an UNC-3-binding site in the *madd-4* locus. In addition to postsynaptic receptor clustering defects, *unc-3* mutants also fail to assemble presynaptic specializations. Apart from being required to assemble synaptic specialization, UNC-3 directly controls, via UNC-3 binding sites, the coordinated expression of enzymes and transporters that define the cholinergic neurotransmitter phenotype of the SAB neurons. The coupling of neurotransmitter choice and synaptogenesis appears to be a common theme in MN differentiation, as we find that *unc-3* coregulates cholinergic neurotransmitter pathway genes and *madd-4* in other, distinct types of cholinergic MNs in *C. elegans*. These findings show how neuron type-specific signaling features of a neuron are hardwired together

through coordinated transcriptional control, and provide a simple and robust mechanism for the development and maintenance of a functional motor circuit.



Schematic that summarizes the key findings of our work. The transcription factor unc-3 coregulates the expression of acetylcholine pathway genes (unc-17, cha-1, cho-1), neurotransmitter receptor genes (glr-4, glr-5, exp-1), ion channel genes (del-1, unc-8), and synaptogenic molecules (madd-4).

Disclosures: P. Kratsios: None. **B. Pinan-Lucarré:** None. **S. Kerk:** None. **J. Bessereau:** None. **O. Hobert:** None.

Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: March of Dimes Grant FY11456

Title: The gamma-protocadherins interact physically and functionally with the neurexin-neuroligin adhesion complex

Authors: M. J. MOLUMBY[1], D. J. NEWBOLD[2], D. SCHREINER[4], N. K. KOBLESKY[2], A. M. GARRETT[5], J. J. RADLEY[3], *J. A. WEINER[2];
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Abstract: The γ-Protocadherins (γ-Pcdhs) are cadherin superfamily adhesion molecules encoded by the Pcdhg gene cluster. The cluster consists of 22 “variable” exons--each encoding most of a single isoform--and three small “constant” exons encoding a shared C-terminal domain. The γ-Pcdhs are combinatorially expressed, with each neuron estimated to express ~5-7. We showed that the γ-Pcdhs promiscuously form cis-tetramers that interact homophilically in

trans, which indicates that this family could generate over 10,000 distinct adhesive interfaces. Depending on the neuronal cell type, the γ-Pcdhs play critical roles in synaptogenesis, dendrite arborization, and developmental apoptosis. Their far-from-exclusively synaptic localization suggests that they may regulate synapse number indirectly, through modifying the function of other synaptogenic molecules. Here we report a physical and functional interaction between the γ-Pcdhs and the neurexin-neuroligin synapse adhesion complex. Multiple tagged γ-Pcdh isoforms (including members of the γA, γB, and γC subfamilies) co-immunoprecipitate with neuroligin-1, -2, and -3 in transfected cells. This physical interaction occurs via the extracellular domains, as it persists when constructs lack cytoplasmic domains. To identify a function for this interaction, we used an assay measuring binding of a soluble neurexin-Fc protein to HEK cells transfected with neuroligin. Co-expression of a γ-Pcdh in the HEK cells disrupts neurexin-Fc binding, suggesting that the γ-Pcdhs somehow block the interaction between neurexin and neuroligins. Using the "artificial synapse" assay in which neurons are co-cultured with COS cells, we show that the presence of γ-Pcdhs along with neuroligins reduces the clustering of synaptic vesicle proteins at sites of COS cell contact, compared to that on cells expressing neuroligins alone. Together, these data suggest that the γ-Pcdhs bind postsynaptic neuroligins in cis and prevent them from promoting the formation or stabilization of synapses through binding their presynaptic ligands, the neurexins. One possibility is that homophilic trans-interactions between matched γ-Pcdh tetramers free neuroligins to bind to neurexins and promote synapse development. To test this hypothesis, we are performing artificial synapse assays in which neuronal γ-Pcdh complements are manipulated to match, or not match, that of the neuroligin-expressing COS cells. Consistent with these *in vitro* results, loss of the γ-Pcdhs in the cortex *in vivo* results in dendritic spine alterations; further analysis of mice overexpressing individual γ-Pcdhs, and thus increasing homophilic matching, are in progress.

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Poster

778. Synapse Formation: Trans-Synaptic Mechanisms

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

Support: NSF IOS1256114

NIH R21 RR026074

Title: Stepping out of the neurogenic shadow: Role of basic helix loop helix protein, daughterless, in post-mitotic neurons in *Drosophila* melanogaster

Authors: *M. D'ROZARIO[1], F. LIEBL[2], D. MARENDA[1];
[1]Drexel Univ., Philadelphia, PA; [2]Southern Illinois Univ. - Edwardsville, Edwardsville, IL

Abstract: Neurogenesis, the production of neuronal and glial lineages from undifferentiated precursor cells, is a critical step for embryonic neurodevelopment. Proneural proteins of class I/II Basic helix-loop-helix (bHLH) proteins are a large family of evolutionarily conserved transcription factors that have well-established roles in neurogenesis and neural differentiation across multiple species. However, their role in post-mitotic neurons remains less clear. Here, we report that the bHLH transcription factor Daughterless (Da, Tcf4 in mammals) is expressed in post-mitotic, differentiated motor neurons where it is required to restrict synaptic growth and axonal arborization at the *Drosophila* Neuromuscular Junction (NMJ). We have used bioinformatic tools to identify candidate genes whose transcription is regulated by Da and have identified and validated a small number of specific target genes required for Da-mediated restriction of of synaptic growth.

Disclosures: M. D'Rozario: None. **F. Liebl:** None. **D. Marenda:** None.

Poster

779. Development of Motor Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 779.01/A34

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Decreased number of muscle synergy during gait is associated with neural dysfunction and motor function in cerebral palsy

Authors: *Y. HASHIGUCHI[1], K. OHATA[2], R. KITATANI[2], N. YAMAKAMI[3], S. OSAKO[2], Y. AGA[2], S. YAMADA[2];
[1]Shogoin, Kyoto Univ. Human Hlth. Sci., Kyoto, Japan; [2]Kyoto Univ., Kyoto, Japan; [3]Fujita Hlth. Univ. Hosp., Aichi, Japan

Abstract: INTRODUCTION Synergy is defined as the grouping of a neural output to simplify motor control. Non-negative matrix factorization (NNMF) was recently used to assess the

characteristics of synergy during a reaching task and during gait. Clark et al. showed that synergies during gait are altered after stroke. And the number of synergies is associated with severity and gait speed. It is reported that children with cerebral palsy (CP) exhibit a lowered number of synergies during gait compared with healthy adults. However, the relationship between the number of synergies and neural dysfunction or motor function in children with CP has not been reported. The objective of this study is to clarify the relationship between the number of synergies and clinical or gait parameters. METHODS Sixteen children with CP were enrolled. Electromyography (EMG) data were recorded from eight muscles on an impaired lower limb during gait. The EMG data were decomposed using NNMF into an activation pattern of each synergy and a weight ratio of muscles for each synergy. Variability accounted for was used to determine the number of synergies in each child. Further, children were classified into two groups. The “patterned group” comprised children who had two or fewer synergy patterns, whereas the “isolated group” comprised children who had three or more synergy patterns. The modified Ashworth scale (MAS) and the modified Trost selective motor scale (mTSMC) were used to assess the spasticity and selective motor control. We measured gait speed and foot pressure using an FDM system (Zebris Inc.) during gait. The foot pressure data were used to calculate the average displacement of the center of pressure (dCOP) during stance phase. We used the Mann-Whitney U test and Student’s t test to assess significance of differences between the two groups. RESULTS Six children had two synergy patterns, eight children had three synergy patterns, and two children had four synergy patterns. Significant differences were found in mTSMC ($p = 0.036$) and dCOP ($p < 0.001$). DISCUSSION mTSMC can detect the neural disorder and a decrease in the number of synergy patterns. dCOP is a biomechanical factor and influences the number of synergies. In a previous study, a smaller number of synergies during gait was demonstrated in children with CP than in a typical toddler, which showed that healthy toddlers have four synergy patterns. Similarly, Neptune et al. showed that the number of synergies corresponds to the biomechanical function during gait in healthy adults. Therefore, results of the present study show that the number of synergies determined using NNMF reflects the neural and biomechanical function in children with CP.

Disclosures: **Y. Hashiguchi:** A. Employment/Salary (full or part-time):; Kyoto University. **K. Ohata:** None. **R. Kitatani:** None. **N. Yamakami:** None. **S. Osako:** None. **Y. Aga:** None. **S. Yamada:** None.

Poster

779. Development of Motor Systems

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Program#/Poster#: 779.02/A35

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: The role of GAD67 and VGAT in the functional and morphological development of motor neurons

Authors: *M. J. FOGARTY, R. KANJHAN, M. C. BELLINGHAM, P. G. NOAKES;
Sch. of Biomed. Sci., Univ. of Queensland, Brisbane, Australia

Abstract: We have investigated developmental roles of glycinergic and GABAergic neurotransmitter deficiency on motor neuron (MN) spontaneous synaptic activity and dendritic morphology at embryonic day 18/birth. To achieve this, we have used mice lacking GABAergic (67 kDa isoform of glutamate decarboxylase, GAD67 knockout) and mice lacking both glycinergic and GABAergic transmission (vesicular inhibitory amino acid transporter, VGAT knockout) to study the development of hypoglossal (XII) MNs. Brainstem slices (300μm) from wild type (n=18) and mutant mice (GAD67: n=17; VGAT: n=22) at E18.5 were used to record spontaneous synaptic currents from XII MNs that were filled with Neurobiotin post recording. These slices were then immuno-stained for the distribution of excitatory glutamatergic and inhibitory GABAergic neurochemical synapses on the dye-filled cell within confocal z-stacks. Data was analyzed by one-way ANOVA, with all reported increases compared to wild type controls having $P<0.05$. In mice lacking GABA or GABA and glycine neurotransmission, XII MNs responded by increasing the length (GAD67: +50%; VGAT: +104%) and complexity of their dendritic branches (GAD67: +35%; VGAT: +45%), and the number of somatic (GAD67: +95%; VGAT: +210%) and dendritic spine density (GAD67: +195%; VGAT: +290%). We also observed that excitatory glutamatergic neurochemical synapses were increased (GAD67: +55%; VGAT: +90%), but inhibitory neurochemical synapses were unaltered. Spontaneous synaptic current analyses showed increased frequency of excitatory post-synaptic currents (GAD67: +35%; VGAT: +65%). However, we observed no compensatory increases in inhibitory spontaneous synaptic transmission in either mutant. We speculate that before switching to their mature inhibitory activity, deficiency in GABAergic and/or glycinergic synaptic activity during embryogenesis may be compensated for by increases in glutamatergic neurotransmission. Our results suggest that glycinergic and GABAergic synaptic activity play vital roles in regulating XII MN morphology and network properties during late embryonic development.

Disclosures: M.J. Fogarty: None. **R. Kanjhan:** None. **M.C. Bellingham:** None. **P.G. Noakes:** None.

Poster

779. Development of Motor Systems

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Program#/Poster#: 779.03/A36

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: IRCSET Grant

Title: The differential effects of prenatal LPS challenge on neurobehavioral outcomes in affected offspring depend on gestational age

Authors: *M. STRALEY[1,2,3], S. J. CRAMPTON[1,2,3], J. F. CRYAN[1,3], S. O'MANONY[1,3], G. W. O'KEEFFE[1,2,3];
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Abstract: During development the nervous system is assembled and sculpted by an orchestrated series of neurodevelopmental events that ultimately generate and refine the neural circuitry that governs all facets of human behavior. Many clinical and epidemiological studies have now shown that perturbations in this developmental program, such as those caused by prenatal maternal infection, can increase the risk in affected offspring for a number of neurodevelopmental and neuropsychiatric disorders, including autism, ADHD and schizophrenia. However, the cellular and molecular basis of this increased risk remains to be fully elucidated, in particular the effects of gestational age on neurobehavioral outcome. In this study we aimed to assess the impact of maternal inflammation at different stages of pregnancy on offspring neurobehavioral outcome, especially those related to the dopaminergic system, using the well-established lipopolysaccharide (LPS) rat model of maternal infection. Pregnant rats were administered a 50μg/kg dose of LPS or saline via i.p. injection on E12, E14, E16 or E18 and fetal brain samples were microdissected 48h post. RT-PCR showed an increase in mRNA abundance for IL-1β and TNF at each age, indicating a fetal inflammatory response in the developing brain. To determine if the timing of maternal infection altered neurobehavioral outcome, depending on gestational age, we performed an array of behavioral tests including righting reflex, geotaxis test, ultrasonic vocalization, and open field on P9 rat offspring, following similar maternal immune activation. Preliminary ultrasonic vocalization analysis revealed that animals whose mothers received LPS on E12, E14, E16 and E18 resulted in offspring who made more calls for longer duration when separated from the mother compared to controls. These data suggest an anxiety-like phenotype associated with *in utero* LPS exposure. Currently, we have expanded the study and are analyzing behaviors in adolescent as well as adult offspring following *in utero* LPS exposure. Using the validated LPS rat model of maternal infection, this study describes the differential effects of maternal inflammation at different gestational ages and shows that the timing of exposure can have profound consequences for fetal

neurobehavioral outcome and normal cognitive development. These data suggest that understanding how and why the developing brain is more susceptible to the effects of maternal infection at different gestational ages will be important for developing effective strategies for neuroprotection to prevent against the adverse consequences of maternal infection-induced fetal programming.

Disclosures: **M. Straley:** None. **S.J. Crampton:** None. **G.W. O'Keeffe:** None. **J.F. Cryan:** None. **S. O'Manony:** None.

Poster

779. Development of Motor Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 779.04/A37

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Experience-dependent regulation of singing-driven gene expression in the song system during the critical period for vocal learning

Authors: ***S. HAYASE**, E. OHGUSHI, M. KOBAYASHI, K. WADA;
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Abstract: Vocal learning in human and other species requires coordination of sensory and motor experiences in order to mimic a set of previously perceived models. Vocal learning has the critical period as a time when performances improve efficiently. However, the neural mechanisms underlying the critical period for vocal learning are not well understood. For vocal learning and production, songbirds possess specific neural circuits consisting of several brain nuclei, so called the song system, where many genes are regulated during singing. A male zebra finch develops its syllable acoustic features dramatically in the critical period; especially in morning time during juvenile stage. The singing-driven immediate early genes (IEGs) are strongly induced during juvenile stage, suggesting that the singing-driven IEGs may be regulated in a daily basis and contribute to modification of syllable acoustic features. Thus, we examined expression of the neuroplasticity-related IEGs (Arc; activity-regulated cytoskeleton associated gene, Egr1; Early growth response 1 and c-fos; FBJ murine osteosarcoma viral oncogene homolog) in juvenile and adult birds, which sang at different time points during the day. We found that glutamatergic projection neurons in the pallial nuclei RA and NIf were selectively regulated with their induction of IEGs matching syllable acoustic changes. These data suggest a potential relationship between changes of syllable acoustic features and strong induction of IEGs

in RA and NIf during song learning. In addition, the resulting IEG induction rates in RA and NIf were dependent on singing experience both in a short term daily way and a long term mechanism during the critical period. These results indicate a singing amount-dependent mechanism for regulation of IEG expressionn in a brain region- and critical period- specific manners.

Disclosures: **S. Hayase:** None. **E. Ohgushi:** None. **M. Kobayashi:** None. **K. Wada:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: MMF Grant 4090-9224-12

NIH Grant UL1TR000114

Title: Optimizing inhibitory effect in primary motor cortex by low-frequency repetitive transcranial magnetic stimulation

Authors: ***M. CHEN**, H. DENG, R. SCHMIDT, T. KIMBERLEY;
Physical Med. and Rehabil., Univ. of Minnesota, Minneapolis, MN

Abstract: Introduction Low-frequency repetitive transcranial magnetic stimulation (rTMS) generally inhibits cortical excitability. The primary motor cortex (M1) can be inhibited by low-frequency rTMS applied to: 1) M1 directly or 2) premotor cortex (PMC). The comparison between these two methods has been reported by previous studies with inconsistent results. This work compared inhibition to M1, PMC and a PMC-blocked method which first inhibits PMC then M1 (PMC-M1). The purpose of this work was to determine the most effective method of inhibition. We hypothesized that PMC-M1 method will demonstrate greater inhibitory increase than equal dose of PMC method or M1 method. Method 10 young healthy subjects (3 males; mean age: 25.2 ± 5.4y) were recruited for a randomized cross-over design with a wash-out (1 week) between visits. Each visit consisted of a pre-test, an rTMS intervention and a post-test. Measures included short interval intracortical inhibition (SICI), intracortical facilitation (ICF) and cortical silent period (CSP). Subjects received one of the three interventions at each visit including: M1, PMC and PMC-M1. Change scores of all of the measures were calculated and tested by t-test for pre-post change effect. For the intervention effect, one-way ANOVA was

used with post-hoc testing as appropriate (Bonferroni adjusted pairwise comparisons). Results All excitability measures showed different levels of inhibition after each intervention. For SICI, there was no significant difference between the 3 interventions although there was a trend that M1 decreased inhibition more than the other two (P=0.0581); for ICF and CSP, the PMC-M1 method showed significantly greater inhibition than the other two (ICF: P=0.0006 and CSP: P<0.0001). Discussion PMC-M1 method may have demonstrated greater inhibitory effect because the excitatory projection from PMC to M1 was "blocked" first. Thus, M1 may have been inhibited indirectly without the influence from PMC. Conclusion The PMC-M1 stimulation pattern could be used to probe the relationship between PMC to M1 in individuals with neurological disorders to help elucidate the pathophysiology or may be a more effective inhibitory protocol.

Disclosures: **M. Chen:** None. **H. Deng:** None. **R. Schmidt:** None. **T. Kimberley:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Center for Human Growth Development fNIRS Pilot Study Grant

University of Michigan Rackham Graduate Student Research Award

Title: Emergence of motor cortex activity as infants develop functional motor skills

Authors: *R. NISHIYORI[1,2], S. BISCONTI[2], B. ULRICH[1,2];
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Abstract: The neural changes underlying the emergence of reaching and stepping, two important milestones during infancy have yet to be determined. Functional near-infrared spectroscopy (fNIRS) has the potential to address some limitations presented by using fMRI and EEG and offer a unique insight into the neural changes associated with the emergence of these motor skills early in life. The purpose of this study was to use fNIRS to determine the changes in motor cortical activity during reaching and stepping in infants. We employed a cross-sectional design to compare motor cortical activity of typically developing infants at 6 months and 12 months during reaching and stepping. We chose these ages as reaching emerges around 4 to 5

months, while walking emerges around 11 months of age. All infants performed reaching and stepping tasks while recording fNIRS. For the reaching task, infants were secured into a customized infant seat. We presented a toy within reach at mid-chest level for the infants. We only included successful reaches and grasp of the toy. For the stepping task, an experimenter held the infant under the arms over a pediatric treadmill. Infants were only positioned over the belt once the belt started to move. We only included steps with the moving belt. Four emitters and 8 detectors (for a total of 12 recording channels) were centered over Cz and extended bilaterally to the C3 and C4 electrode locations (International 10-20 system) to cover the primary motor cortex. We used Homer2, a pre-processing software (Huppert et al., 2009) to apply a wavelet motion correction algorithm (Brigadoi et al., 2013), band-pass filters, and averaging trials. All successful trials were grouped to obtain an average response per channel. We compared rest with task to detect any significant changes in hemodynamic activity during task performance. Preliminary results (6 younger and 6 older infants) show that when compared to rest, younger infants while reaching showed activation in 7 channels distributed bilaterally out of 12 channels, whereas the older infants showed 4 channels unilaterally. During stepping, younger infants showed activation in 8 channels distributed bilaterally out of 12 channels, whereas the older infants showed 6 channels bilaterally out of the 12 channels. These results show that younger infants demonstrate a dispersed area of activation within the motor cortex during both reaching and stepping when compared to older infants. The results support the idea that infants explore options achieving their motor goal and learn, with self-initiated goal-directed movements to refine the neural activity as well as the behavioral outcomes.

Disclosures: **R. Nishiyori:** None. **S. Bisconti:** None. **B. Ulrich:** None.

Poster

779. Development of Motor Systems

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Program#/Poster#: 779.07/A40

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH R01NS079569

Title: Postnatal development of the corticospinal system examined in awake animals using indwelling stimulation and recording techniques

Authors: E. SINOPOULOU[1], P. T. WILLIAMS[1], *J. H. MARTIN[2];
[1]Physiol, Pharmacol & Neurosci, The City Col. of the City Univ. of NY, New York, NY;
[2]Physiol, Pharmacol & Neurosci, The City Col. of the City Univ. of NY, NEW YORK, NY

Abstract: Development of the corticospinal (CS) system in animals has been investigated by taking single snapshots of its anatomical and physiological organization at different postnatal time points. Using this approach, studies in the cat support a sequence of early exuberant CS tract (CST) spinal projections and refinement to achieve the mature projection pattern, followed by development of the motor cortex (M1) motor map and further strengthening of stable projections. Two critical unanswered questions for understanding the functional development of the CS system are: 1) when does the corticospinal system begin to participate in the control of skilled movements, and 2) when does the system become mature? The single snapshot approach of acute investigation is limited in answering these questions because of the affects of anesthesia and that different animals mature at somewhat different rates. Rather, it is essential to study motor development in awake animals and follow individual animals during development. In this study we used chronic stimulation and recording techniques to monitor changes in the strength of M1 to muscle connectivity in awake kittens from postnatal week (PW) 4-15 and in adult cats. Animals were instrumented with epidural stimulating electrodes over the forelimb area of M1, bilateral forelimb EMG electrodes (biceps, ECR), and free-floating microwire recording electrodes within M1. Individual animals were followed for several weeks to months, depending on implantation age (n=3, PW 4-8; n=2, PW 9-15; n=2 Adult). Here, we focus on M1 stimulation in awake animals at rest. We compared EMG responses at different ages and computed EMG recruitment curves (response to M1 stimulation at multiples of threshold; tested mostly in older kittens and adults) across sessions. Between PW 4-5, M1 stimulation evoked contralateral forelimb movements and EMG responses (biceps or ECR), but became less effective by the end of this period. Beginning at about PWs 5-6 stimulation became progressively more effective and consistent in evoking responses. Responses evoked by PW 10-15 were similar to adults, suggesting maturation of the CS projection. Our studies are the first to use chronic stimulation/recording approaches to monitor changes in M1-to-muscle connection strength. Our findings suggest multiple developmental phases. The phase beginning at PW 5-6, which is before the onset of M1 map development, may mark the start of a period of continuous and rapid ramping of connection strength that gives M1 access to spinal motor circuits for movement control.

Disclosures: E. Sinopoulou: None. **J.H. Martin:** None. **P.T. Williams:** None.

Poster

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Program#/Poster#: 779.08/A41

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Development of corticospinal projection and its difference between cervical and lumbar targets

Authors: *H. KAMEDA[1], T. KAMIYAMA[1], N. MURABE[1], S. FUKUDA[1], N. YOSHIOKA[1], H. MIZUKAMI[2], K. OZAWA[2], M. SAKURAI[1];
[1]Dept. of Physiol., Teikyo Univ. Sch. of Med., Tokyo, Japan; [2]Div. of Genet. Therapeutics, Ctr. for Mol. Med., Jichi Med. Univ., Tochigi, Japan

Abstract: The corticospinal (CS) tract is essential for voluntary movement, but what we know about the organization and development of the CS tract remains limited. To determine the total cortical area innervating the 7th cervical (C7) and 4th lumbar (L4) spinal cord segments, we injected retrograde tracers (fluorescent microspheres) into C7 and/or L4 such that it would spread widely within the unilateral gray matter (to > 80%), but not to the ventralmost dorsal column (CS tract). Subsequent detection of the tracer showed that, in both infant and adult mice, neurons distributed over an unexpectedly broad area of the rostral two-thirds of the cerebral cortex converge to C7. This included cortical areas controlling the hindlimb. With aging from the infant to the adult, the cell densities greatly declined, mainly due to axon branch elimination. Labeling of axons and presynaptic structures through cotransfection of DsRed and synaptophysin-EGFP expression plasmids using exo utero electroporation suggest that at least more than 80% of CS axons present in the gray matter at this period make synaptic connections with spinal neurons. Whole cell recordings from spinal cord neurons evoked by selective optogenetic stimulation of CS axons also revealed that the neurons received CS inputs widely distributed in the gray matter. These implicate C7 neuronal circuits incorporated the CS innervation are largely reorganized during development. By contrast, the cortical areas innervating L4 are limited to the conventional hindlimb area, and the cell distribution and density showed little or no change during development. To investigate the developmental changes of axonal distribution of CS neurons located in M1/S1, M2 and S2, we injected replication-incompetent adeno-associated virus serotype 1 vectors expressing fluorescent proteins (XFPs) of different colors fused with channelrhodopsin-2 (ChR2) into the cortical areas separately at P0. ChR2-XFPs labeled axons clearly without gaps down to their terminals because ChR2 is the transmembrane protein, and the tagged XFPs are located just beneath the plasma membranes of axons. In addition, these neurons can be activated by photostimulation. The mice underwent the viral injection were fixed at P7, P14 and P21. We analyzed the axonal distribution in the C7 and found that the axon terminals derived from M1/S1 and S2 in the spinal cord were differentially distributed. CS axons from S2 were predominantly distributed at the dorsal part of spinal cord, and those from M1/S1 were observed at both dorsal and ventral parts.

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Poster

779. Development of Motor Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NSERC Grant 238569-2011

Title: Differential expression of nachrs at zebrafish red and white fiber nmjs during development

Authors: *K. T. AHMED, D. W. ALI;
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Abstract: Nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels that are highly expressed at the vertebrate neuromuscular junction (NMJ), where they are required for muscle fiber activation. Developmental regulation of the NMJ ensures proper muscle development and fine control of muscular movement, whereas improper development can lead to poor fiber recruitment and weak muscle contraction. Therefore, understanding the factors that underlie NMJ development is critical for a complete understanding of muscle function. Embryonic and larval zebrafish express two morphologically and functionally distinct muscle fibers: white twitch fibers and red tonic fibers. As functionally different muscle fibers require different mechanisms to control muscle activation, we examined the properties of nAChRs that are expressed by red and white muscle fibers during development, from 30 hours post fertilization (hpf) to 5 days post fertilization (dpf). Miniature endplate currents (mEPCs) recorded by whole cell patch clamp electrophysiology exhibited significant differences between the red and white muscle fibers in embryonic and larval fish. Most mEPCs from 36 hpf white fibers decayed relatively slowly, with a single exponential component. By 2 and 3 days post fertilization the mEPC kinetics sped up, and decayed with a double exponential component. By 5 dpf the exponential decay was further sped up and consisted of a single component. However, mEPCs from red fibers exhibited a different developmental profile with respect to their decay kinetics. In 36 hpf embryos, most mEPCs decayed with a single, slow exponential time course, which sped up and switched to a double exponential time course by 2 dpf. By 5 dpf mEPC kinetics were slightly faster but remained as a double exponential component in contrast to white fibers. We recorded single channel activity of nAChRs via the outside-out patch clamp configuration and identified three major conductance levels (39-46 pS, 59-62 pS, and 72-81 pS)

and two open time constants (0.38-0.52 ms, 2.14-4.75 ms), expressed in both red and white fibers at 36 hpf, 2 dpf and 5 dpf. Channels with long open time constants constituted 30% of single channel activity analyzed from red fibers, whereas they were less than 1% of the channel activity obtained from white. Single channel mean open times were significantly smaller from white fibers compared to red fibers at all ages. Finally, there was a trend towards smaller mean open times of nAChRs obtained from red and white fibers of older animals. Our findings reveal previously unknown differences between the nAChRs expressed at NMJs of red and white fibers in embryonic and larval zebrafish.

Disclosures: K.T. Ahmed: None. **D.W. Ali:** None.

Poster

779. Development of Motor Systems

Location: Halls A-C

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R01 NS28421

NIH Grant R01 NS045195

Title: Timing is everything: hormonal control of the developing SNB in mice

Authors: *A. M. WELSCH, C. L. JORDAN, S. M. BREEDLOVE;
Neurosci., Michigan State Univ., East Lansing, MI

Abstract: In rats there is a critical period for the organizational masculinization of soma size in motoneurons of the spinal nucleus of the bulbocavernosus (SNB) that extends beyond the critical period for masculinization of SNB cell number. This finding demonstrates that critical periods of hormone-dependent development do not all occur within the same window. The current study aimed to assess similar critical periods in the mouse. Female mice were exposed to testosterone propionate (TP) or vehicle during one of three potential critical periods (embryonic days 16-18), early postnatal (PN 1, 3, 5), or late postnatal (PN 7, 9, 11)). Testosterone (T) was then given in adulthood via Silastic capsules to half of the animals in each group to serve as “activational” background from which organizational effects of the perinatal hormones could be detected. Early postnatal TP treatment significantly increased the number of SNB motoneurons in female mice by doubling their number (control females, mean = 21.6 ± 4.63 (SEM) motoneurons compared to

early postnatal TP-treated females, 53.3 ± 4.26; $p < 0.01$). Early postnatal TP treatment also increased the size of SNB somas (481.1 ± 17.12 μm^2) compared to control females (395.2 ± 18.61 μm^2; $p = 0.001$). This perinatal effect was also observed in the size of SNB nuclei (control females: 125.3 ± 6.68 vs. early postnatal TP mice, 147.4 ± 6.15; $p = 0.001$). Further, adult T treatment significantly increased SNB soma size (blank capsules: 395.7 ± 13.04 μm^2 compared to 491.6 ± 13.33μm^2 in T-treated; $p < 0.001$) and the size of nuclei in SNB motoneurons (control females w/ blank: 124.5 ± 4.69 μm^2. T treated: 151.9 ± 4.79μm^2; $p < 0.01$). There was no significant interaction of perinatal and adult hormone treatment on cell number, soma size, or nuclear size. These results begin to define the critical periods in which androgens masculinize the SNB system in mice where genetic tools are available to perturb, and thus understand, mechanisms of androgen action on SNB development.

Disclosures: A.M. Welsch: None. **C.L. Jordan:** None. **S.M. Breedlove:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Whitehall Foundation Grant 2010-05-42

Title: Spontaneous network activity in the embryonic spinal cord is regulated by presynaptic nicotinic modulation of GABAergic transmission

Authors: *C. E. GONZALEZ-ISLAS[1], M. GARCIA-BEREGUIAIN[2], P. WENNER[2]; [2]Physiol., [1]Emory Univ. Sch. Med., Atlanta, GA

Abstract: Developing neural circuits display episodic patterned spontaneous activity due to a recurrently connected network in which both GABA- and glutamate-mediated neurotransmission are excitatory. In the embryonic spinal cord, a form of this activity known as spontaneous network activity (SNA) is involved in multiple aspects of motor maturation, such as muscle and joint development, axonal pathfinding and regulation of the strength of synaptic inputs to motoneurons. SNA is a network-driven phenomenon that depends on the chemical nature of the synapses between spinal neurons. Nevertheless, the neurotransmitter receptors that support SNA change during development. In early stages (embryonic day 4 to 6, E4-6) nicotinic cholinergic receptors appear to be most important, but excitatory GABAA receptors also contribute. Later in

development (E8-12), glutamatergic receptors become increasingly important, and nicotinic receptor influence decreases, however, nicotinic modulators still influence the frequency of SNA. We hypothesized that nicotinic transmission influences SNA frequency through postsynaptic phasic activation of nicotinic receptors. However, we found little evidence supporting the activation of postsynaptic nicotinic currents. Rather, it became clear that nicotinic modulators influenced presynaptic release of GABAergic vesicles. We found that nicotinic antagonists decreased GABAergic mPSC frequency and evoked GABAergic responses, while nicotine increased GABAergic mPSC frequency and evoked GABAergic responses. Furthermore, we demonstrate that this response is mediated through the activation of presynaptic non-α7 nACh-Rs. We propose that the nicotinic modulation of SNA frequency occurs through a presynaptic control of GABA release.

Disclosures: C.E. Gonzalez-Islas: None. **M. Garcia-Bereguiain:** None. **P. Wenner:** None.

Poster

779. Development of Motor Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH grant R21NS080103-02

Title: Differential gene expression in purified populations of mouse sympathetic neurons that express neuropeptide Y

Authors: *J. P. HORN, K. M. SIKORA, P. H. M. KULLMANN;
Dept of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Neuropeptide Y (NPY) is selectively expressed by 50-70% of paravertebral sympathetic neurons, most of which are involved in modulation of cardiovascular function. Using NPY as a marker, the goal of this study was to discover new genes that are differentially expressed in NPY-positive and NPY-negative neurons. We used a transgenic reporter mouse in which humanized Renilla green fluorescent protein expression is driven by a bacterial artificial chromosome that contains large upstream and downstream sequences surrounding the NPY peptide coding sequence (Jackson Laboratory [strain B6.FVB-Tg(Npy-hrGFP)1Lowl/J]; J Neurosci 29:462239 (2009)). Antibody staining of the superior cervical ganglion (SCG) confirmed that all neurons with bright GFP fluorescence were immunoreactive for NPY, but

revealed some cells with weak GFP expression were ambiguous in their NPY immunoreactivity. For gene screening, SCGs were removed from 15 - 28 week old male mice, enzymatically dissociated and plated on glass coverslips. Within 2.5 hours of harvesting the ganglia, groups of 10 single cells were manually sorted using coarse glass micropipettes on the basis being fluorescent or non-fluorescent. Cells were deposited into an oligonucleotide extraction buffer and then processed to amplify mRNA and convert it to cDNA (Nugen Ovation One-Direct System). cDNA samples were then tested for the presence of mRNA for NPY using qPCR. Taking this approach enabled us to focus on paired samples in which the differential expression of NPY was very high. Samples meeting this criterion were then run on Illumina mouse WG-6 BeadChip microarrays, which contain 45,200 target sequences. Paired cell samples from 5 mice were then compared using NIH BRB Array tools software (R Simon & BRB-ArrayTools Development Team). The microarray data indicated a 17-fold higher expression of NPY mRNA in samples that had been selected using GFP fluorescence and qPCR validation. Statistical analysis of the microarray data identified 244 genes that fall into many functional categories. These include G-protein coupled receptors, kinases, axon guidance molecules, cytoskeletal proteins, transcription factors and ion channels. We are now studying these genes to identify potential new molecular tools for targeting the specialized sympathetic neurons that control cardiovascular and non-cardiovascular functions.

Disclosures: J.P. Horn: None. **K.M. Sikora:** None. **P.H.M. Kullmann:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Division of Natural and Computational Sciences, Benedictine University at Springfield

Title: Expression of membrane-targeted Arch in the spinal cord, dorsal root ganglia and peripheral nerves of embryonic chick

Authors: *A. A. SHARP[1], H. L. DEAL[2], S. FROMHERZ[1];
[1]Dept Anat, SIU Sch. Med., CARBONDALE, IL; [2]Benedictine Univ., Springfield, IL

Abstract: The ability to control the membrane potential of neurons during development would allow for a better understanding of how excitability contributes to the process of proper

development. Polarity and excitability are known to contribute to cell migration, neuronal pathfinding, neuronal survival and the generation of patterned embryonic behaviors. However, traditional electrophysiological and pharmacological method for manipulating membrane potential are particularly challenging in the embryonic environment. Given the relative transparency of thin embryonic tissues to light, optogenetic manipulation of membrane potential is a feasible approach to this problem. We have been utilizing an electroporation-based transposon system to introduce genes encoding light-activated ion channels into the neurons of the spinal cord and dorsal root ganglia (DRGs) of embryonic chick. This system allows for stable transformation of precursor cells such that our genes of interest can be expressed throughout the lifetime of all subsequently derived cells. In prior work, we have demonstrated the ability to establish persistent expression of channelrhodopsin, halorhodopsin and archaerhodopsin in neurons of the spinal cord and DRGs. Both channelrhodopsin and halorhodopsin are primarily expressed in the membranes of neuronal somata as well as axonal and dendritic processes. Peripheral expression of channelrhodopsin is sufficient that illumination of peripheral nerves with a blue LED is sufficient to both evoke leg movement and modulate spontaneous motility. Halorhodopsin activation in transformed embryos produces subtle modulation of motility that varies significantly between embryos. Many investigators have reported difficulties in getting robust inhibition of neuronal activity with halorhodopsin, and archaerhodopsin often proves a better choice. Unfortunately, our original archaerhodopsin protein product was found primarily in the cytoplasm and did not allow for behavioral manipulation of embryos. Therefore, we have now inserted the coding sequence for a modified archaerhodopsin, eArch3.0, that contains membrane-targeting sequences (Mattis et al., Nature Methods, 2012). This form of archaerhodopsin is expressed largely in the membranes of neurons both centrally and peripherally. Experiments are currently in progress to determine if peripheral activation of this archaerhodopsin can be used to regulate embryonic behaviors.

Disclosures: **A.A. Sharp:** None. **H.L. Deal:** None. **S. Fromherz:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: A role for gap junction coupling in segregating axial and appendicular spinal locomotor networks in metamorphosing Xenopus laevis tadpoles

Authors: M. WAGNER, *K. T. SILLAR;
Sch. of Psychology and Neurosci., Univ. St Andrews, St Andrews, United Kingdom

Abstract: The spinal locomotor networks in Xenopus laevis frog tadpoles undergo a remarkable transformation during metamorphosis to accommodate a switch from axial-based swimming to a limb-based locomotor strategy. For a brief period during ontogeny, both the axial- and limb-based systems are present and functional, but reliant on different central pattern generators (CPGs) that are simultaneously active (Combes et al., (2004) J. Physiol. 559, 17-24). However, the mechanisms controlling the functional coupling both within and between these locomotor networks is unknown. At metamorphosing larval stages (53-58), retrograde motoneuron (MN) backfilling from both axial (13th-14th post-otic segments) and hindlimb muscles with fluorescent rhodamine- and fluorescein-conjugated dextran dyes revealed two discrete MN populations (axial and appendicular) within the spinal cord. We found evidence that the two MN pools in the lumbar enlargement may be coupled by gap junctions (GJs), since backfills revealed a number of MNs which were clearly co-labelled (n=6; 13.67±6.83 co-labelled cells). This co-labelling between axial and appendicular MN pools was absent when the GJ blocker carbenoxolone (CBNX) was applied (200μM; n=8; 0±0 co-labelled cells). Preliminary functional electrophysiological data from animals at the same larval stages suggest that axial CPG activity is modified and its output desynchronised by GJ blockers (CBNX 200-400μM, n=9; 18-β-glycyrrhetinic acid 100μM, n=8). For spontaneous fictive locomotor bouts, the number of episodes initially increased, but then sharply decreased from control levels ~15-20 minutes after drug application. The number of swim cycles in spontaneous and electrically evoked episodes increased and remained elevated. Additionally, burst durations increased and became less synchronised, again for both spontaneous and evoked swimming, effects that usually reversed in the wash. These data suggest that axial CPG output partly regulated by GJs. Our anatomical data also suggest that GJs exist between the axial and limb MN pools and may be involved in the initial cycle-by-cycle coupling of the two CPG outputs. Thus the rhythm of the appendicular CPG is initially highly dependent on that of the axial CPG, which in turn is dependent on its own GJ connections within the spinal circuit. It appears that the immature limb MN pools in the lumbar spinal cord possess functional connections via electrical synapses to the axial locomotor system, which are presumably “pruned” as metamorphosis proceeds, and eventually lost completely by the adult stages when the limb network has become completely independent.

Disclosures: M. Wagner: None. **K.T. Sillar:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.01/A48

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Gene expression programs for retinal ganglion cell type development

Authors: *T. C. BADEA, S. SAJGO, M. G. GHINIA, M. BROOKS;
Retinal Circuits Develop. and Genetics/N-NRL/DIR/NEI/NIH, Natl. Eye Inst., Bethesda, MD

Abstract: The mammalian retina consists of about 50 distinct neuronal cell types, which participate in a variety of circuits, resulting in about 20 channels, conveyed by specific Retinal Ganglion Cell (RGCs) types to the brain. Whereas we have some understanding of the development and functions of RGCs as a class, the morphology, physiology, circuit function, and development of distinct RGC types are active areas of investigation. At the molecular level, it is believed that individual cell types may be defined by the unique expression pattern of sets of molecular markers, which convey specific developmental and functional properties. We have been focusing on the transcriptional code that defines the generation of RGC types. Previously we had we uncovered combinatorial expression and distinct functions of the three Brn3 transcription factors in the definition of RGC types. Using conditional reporter knock-in alleles we had generated for Brn3a, Brn3b and Brn3c. We now report immuno-magnetic affinity purification of Brn3aAP/WT, Brn3aAP/KO, Brn3bAP/WT and Brn3bAP/KO RGCs, coupled with deep sequencing of RNA and whole genome expression profiling of these subpopulations of RGCs at specific times during development. We report combinatorial expression of multiple gene families of transcription factors, adhesion molecules and cytoskeletal adaptors which have the potential to participate in a complex combinatorial code of neuronal cell type specification.

Disclosures: T.C. Badea: None. **S. Sajgo:** None. **M.G. Ghinia:** None. **M. Brooks:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: CIHR Grant 102653

NSERC Grant 7660

NSERC grant 298167

Title: Darkness does not restore visual or neural plasticity in the central visual pathways of adult cats

Authors: K. HOLMAN, K. R. DUFFY, *D. E. MITCHELL;
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Abstract: In a recent study (Duffy & Mitchell, Curr. Biol. 23: 383-386, 2013) it was shown that a 10 day period of total darkness could rejuvenate plasticity in the visual cortex and promote fast and complete recovery of vision in kittens with experimentally induced amblyopia. These kittens were about 3 months of age when placed in darkness and so were still within the critical period of ocular dominance plasticity in the visual cortex that extends to between 6 and 8 months age. In order to establish whether the beneficial effects of darkness were confined to an early period of plasticity, we conducted four studies to examine the ability of the same10 day period of darkness to restore either visual or anatomical plasticity in adult cats that were about 1 year of age. The ability of darkness to restore plasticity was examined in terms of its ability to either promote recovery from the effects of an early period of monocular deprivation (MD) or to re-establish susceptibility to MD in adults. The behavioural studies of the extent of visual recovery were conducted on 3 cats that had received a period of MD from postnatal day (PD) 30 to either PD37 or PD43 after which longitudinal measurements of the grating acuity of each eye were made by use of a jumping stand. Animals were placed in darkness for 10 days at PD383 to PD412. An additional 2 animals were reared normally until PD482 at which time they both received a 14 day period of MD. For one animal the period of MD was immediately preceded (at PD472) by 10 days of darkness. Longitudinal measurements of visual acuity revealed no evidence of a reduction in the vision of the deprived eye in either cat, suggestive that darkness did not restore susceptibility to the effects of MD. Two anatomical studies of the potential benefits of darkness imposed in adulthood were conducted in parallel to the behavioural studies. In the first we studied the ability of darkness imposed in adulthood to promote recovery of soma size and immunolabeling for neurofilament in the dorsal lateral geniculate nucleus (dLGN) of 7 cats that had received a period of MD from PD30 - PD365. The second anatomical study imposed a 7 day period of MD on 5 cats at PD375 that followed 10 days of darkness and examined the effects on the soma size and neurofilament immunolablling in the dLGN. Neither anatomical study revealed any evidence of a change in the dLGN that could be attributed to the period of darkness. On the basis of the behavioural and anatomical studies we conclude that the dramatic and beneficial effects of a 10 day period of darkness observed in kittens are not observed in adulthood suggesting that the former effects are limited to an as yet undetermined critical period.

Disclosures: K. Holman: None. **D.E. Mitchell:** None. **K.R. Duffy:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R01 EY012736

NIH Grant T32 EY013933

Title: Eye-specific organization of retinal ganglion cell axons between intermediate and thalamic targets of the mouse retinogeniculate pathway

Authors: *A. A. SITKO[1], T. KUWAJIMA[2], C. A. MASON[3];
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Abstract: Growing axons must navigate a complex series of guidance cues to reach and innervate their appropriate targets in the brain. The molecular and spatiotemporal specificity of such cues that establishes accurate neural circuitry in the growing brain have been studied at intermediate targets and locally in final targets in various systems. However, axon organization and its molecular mechanisms in tracts between intermediate and final targets remain less well studied, and may be a crucial step in the neural circuit formation. We are investigating how cohorts of axons are organized in the developing axon tract of the mouse binocular visual system. Retinal ganglion cells (RGCs) extend axons either ipsi- or contralaterally at the optic chiasm (OC), the intermediate target of the retinogeniculate system. RGC axons then travel in the optic tract (OT) to their thalamic target, the dorsal lateral geniculate nucleus (dLGN), where they terminate in eye-specific zones (i.e., ipsi- or contralateral). We aim to characterize the eye-specific RGC axon organization in the developing OT and distinguish various axon-intrinsic and extrinsic factors mediating that organization. We show that ipsi- and contralateral RGC axons are segregated in the developing mouse OT, where ipsilateral axons are situated in the lateral OT surrounded by contralateral axons. This order is evident early in development starting around E16, when it appears as though early-growing contralateral axons avoid the lateral OT, where ipsilateral axons later grow. Previous studies indicate that axon position within the OT reflects retinal topography and age of outgrowth. Here we examine other possible mechanisms, previously unexplored, for the eye-specific axon organization we observe in the OT: axon cohort position at the OC exit; position in the OT of ipsi- and contralateral axons originating from the

same topographic retinal region; the relationship between eye-specific RGC axons and glia in the OT; and selective fasciculation of each cohort of axons. We explore these factors *in vivo* from E15 to early postnatal ages and we use an *in vitro* retinal explant co-culture system to further investigate the suggestion that ipsilateral RGC axons have a greater tendency to fasciculate than do their contralateral counterparts. Understanding these aspects of eye-specific organization in the OT will set the stage for probing the role axon organization in the tract plays in establishing the appropriate synaptic connections in the target.

Disclosures: **A.A. Sitko:** None. **T. Kuwajima:** None. **C.A. Mason:** None.

Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NEI/NIH R01EY020517

Title: Development of face perception following the onset of sight in congenitally blind humans

Authors: ***T. K. GANDHI**[1,2], P. SWAMI[3], A. KALIA[2], V. MAHAJAN[4], S. GORLIN[5], M. MENG[6], S. GANESH[7], H. MAHAJAN[4], S. GABRIELI[2], P. SINHA[2];
[1]Defence Inst. of Physiol. & Allied Sci. (DIPAS), New Delhi, India; [2]Brain and Cognitive Sci., MIT, Cambridge, MA; [3]Biomed. Engin., Indian Inst. of Technol., Delhi, India; [4]Radiology, Mahajan Imaging Ctr., New Delhi, India; [5]Choicestream, Boston, MA; [6]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [7]Opthalmology, Shroff Charity Eye Hosp., New Delhi, India

Abstract: Can individuals who have been blind for the first several years of life acquire the ability to visually distinguish faces from non-faces after sight restoring surgery? What kinds of neural changes underlie the very initial stages of such development? Can localized functional specialization develop late in life, and if so, how rapidly does it emerge? Is it spatially and topographically similar in organization to that found in the normally developed brain? We report here results from an unusual opportunity to address these questions by studying congenitally blind individuals whose sight we were able to restore post-adolescence. Our behavioral tests reveal the development of face/non-face discrimination skills over the span of a few months after sight onset. We complemented these assessments with fMRI studies. In order to examine the

development of category-specific response regions in the ventral stream, images of several distinct categories - faces, objects, places, and scrambled patterns - were shown to three subjects in a block paradigm. The experiment was conducted as close to the surgery date as possible and repeated several times in the subsequent months, depending on each subject's availability. We find strong evidence of brain plasticity in these subjects. There is a rapid emergence of spatially localized, functionally specific responses in higher visual cortical areas after surgery. Taken together, these findings have important implications for our understanding of brain plasticity as well as the development of object representations in the brain.

Disclosures: **T.K. Gandhi:** A. Employment/Salary (full or part-time):; INSPIRE Faculty, Govt. of India. **P. Swami:** None. **A. Kalia:** None. **V. Mahajan:** None. **S. Gorlin:** None. **M. Meng:** None. **S. Ganesh:** None. **H. Mahajan:** None. **S. Gabrieli:** None. **P. Sinha:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: P30GM32128

Title: A novel class of fast spiking neurons identified in the Xenopus tadpole optic tectum

Authors: A. S. HAMODI[1], C. M. CIARLEGLIO[2], C. D. AIZENMAN[3], *K. G. PRATT[1]; [1]Zoology and Physiol., Univ. of Wyoming, Laramie, WY; [2]Dept. of Neurosci., [3]Brown Univ., Providence, RI

Abstract: The Xenopus laevis tadpole optic tectum is the primary visual structure in the tadpole brain. It receives direct input from the contralateral retina. The immature tectum has only two principal layers: The innermost layer is adjacent to the middle ventricle and contains the majority of tectal cell somata, while the outermost layer encompasses the neuropil. Electrophysiological characterization of neurons in the developing tectum remains incomplete, since most studies have focused on whole cell electrophysiological recordings from principal neurons closest to the ventricular surface in the innermost layer, as these are the most accessible. As a result nothing is known about the physiology of neurons situated within or close to the neuropil. To remedy this, we developed a preparation that allows for the visualization and recording of neurons from across all layers of the tectum. This preparation involves making a horizontal cut through the

most dorsal third of the tectum, then orienting the brain so that the horizontal sliced side is facing up in the recording chamber. Using this preparation, we have identified a novel population of neurons in the outer layer that exhibit distinct physiology compared to neurons of the innermost layer, including faster excitatory synaptic currents and larger voltage-gated sodium and potassium currents. These cells are also able to fire more action potentials in response to current injection and, unlike typical neurons in the innermost layer, display less spike accommodation. They also display a distinct spike form that features a fast and robust repolarization, similar to action potentials of mammalian GABAergic neurons. To further characterize these neurons, we are constructing a profile linking information regarding the physiology, morphology, and neurotransmitter phenotype of these cells.

Disclosures: **A.S. Hamodi:** None. **C.M. Ciarleglio:** None. **C.D. Aizenman:** None. **K.G. Pratt:** None.

Poster

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Support: General Researcher Program (#2013058415) of National Research Foundation of Korea

Future Systems Healthcare Project of KAIST

Title: Local repulsive interactions in retinal mosaic generate long-range order and consistent periodicity

Authors: ***J. JANG**, P. SAILAMUL, S.-B. PAIK;
Bio and Brain Eng, KAIST, Daejeon, Korea, Republic of

Abstract: Orientation map is of great interest among functional maps in visual cortex, but its developmental mechanism has been controversial. Recently, a theoretical model suggested that a moiré interference pattern between ON and OFF Retinal Ganglion Cell (RGC) mosaics can develop a quasi-periodic orientation map (Paik and Ringach, 2011), but it remained a question how this interference pattern can generate consistent spatial periodicity. Here we suggest a developmental model that a homotypic local interaction in ON/OFF RGCs can develop a long-

range ordered hexagonal lattice mosaics and a heterotypic local interaction can control the alignment between ON/OFF mosaics, which provides an answer to above question about the development of a consistent periodicity in orientation maps. First, we examined how a local repulsive interaction can generate a long-range ordered structure in RGC mosaics. Previously, it was reported that the pairwise interaction point process (PIPP) model, where cell positions are determined by a birth-and-death procedure that only considers a local interaction (Eglen et al., 2005), could not develop a long-range ordered structure in the mosaic (Hore et al., 2012). We modified this model, assuming that cell positions can be gradually shifted by a local repulsive interaction between the nearby cells, and performed computer simulations on the development of model RGC mosaics. We confirmed that this local interaction model in monotypic layer can develop a long-range order, well fitted to a hexagonal lattice pattern. Next, we assumed that there also exists a heterotypic repulsive interaction between ON/OFF mosaics, and examined how this can affect the alignment between two mosaics. By simultaneously simulating the development of both mosaics, we found the heterotypic interaction significantly alters the alignment between two layers. In addition, when the distance between two layers varied, we observed that the alignment angle between two layers was restricted, at a certain range of inter-layer distance, which is required by the moiré interference model of orientation map development. Our result suggests that a simple local interaction between RGCs can develop a long-ranged structure in the mosaic, and that when ON and OFF mosaics are developed with heterotypic repulsive interaction with certain range of distance between the layers, the alignment angle between ON and OFF mosaics can be restricted and this leads to a consistent spatial periodicity of orientation map. Therefore, our model provides a complementary explanation of how the cortical orientation maps can be developed from the moiré interference in the retinal mosaics structure.

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Poster

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Title: The role of the minor spliceosome in acute and chronic stress response in the mouse retina

Authors: *M. BAUMGARTNER;
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Abstract: Splicing, the removal of non-coding introns from pre-mRNA transcripts, is essential for proper eukaryotic gene expression. Splicing begins with the recognition of conserved exon-intron boundary sequences, which occurs through the base-pairing of the small nuclear RNAs (snRNAs) U1 and U2; the following steps require the snRNAs U4, U5, and U6 to excise the intron and fuse its flanking exons. Together, these five snRNAs and other associated proteins comprise the spliceosome. While most exon/intron boundaries have the consensus sequence AG/GT, a minority of eukaryotic introns (<1%) contain the divergent boundary sequence AT/AC, to which U1 and U2 cannot bind to initiate splicing. This necessitates the existence of distinct snRNAs that can base-pair with this unique boundary sequence. These snRNAs include U11, U12, U4atac, and U6atac, which, together with U5, constitute the minor spliceosome, named due to its processing of a minority of introns. Minor introns are present and conserved in many eukaryotes, including plants, insects, and mammals. In humans, dysfunction of minor splicing causes microcephalic osteodysplastic primordial dwarfism type I (MOPD1), a severe autosomal recessive developmental disorder that normally results in death within 1 year. This indicates that minor intron splicing is crucial for normal development. However, the specific role that minor intron-containing genes (MIGs) play during development has not been investigated. Our goal was to understand the evolutionary pressures that drove these specific genes to maintain minor introns and how they are expressed during development. To do this, we mined deep sequencing data generated from two time points in mouse retinal development: embryonic day (E) 16 and postnatal day (P) 0. The data were then analyzed using a novel pipeline to extract biological functions associated with the MIGs expressed in the retina. This analysis revealed that MIGs expressed in the developing retina were linked to cellular stress response. To study the role of minor splicing in the retinal stress response, we examined the expression kinetics of the minor snRNAs during both acute stress, caused by UV radiation, and chronic stress, using the FVB/NJ retinal degeneration model. qRT-PCR analysis revealed that U12, a key snRNA component of the minor spliceosome, was upregulated in both acute and chronic stress.

Disclosures: M. Baumgartner: None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH/NIDCD Grant K18DC012527

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Title: Postnatal changes of expression patterns of VGLUT1, VGLUT2 and VGAT1 in mouse visual cortex and thalamus

Authors: *T. TAKAHATA[1], T. A. HACKETT[1,2];
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Abstract: Mammalian brains experience dynamic changes in gene expression and neurochemical components during neonatal development. Glutamate transport to presynaptic axon terminals is mediated by vesicular glutamate transporters (VGLUTs). Three subtypes of VGLUTs, VGLUT1, VGLUT2 and VGLUT3 are known, and the first two forms are strongly expressed in the mouse brain. Interestingly, their mRNA expression patterns are mostly complimentary with each other in adult mice: VGLUT1 is abundant in the cortex and scarce in the thalamus, while it is converse for VGLUT2. It has also been reported that their physiological functions are different from each other. Considered with importance of glutamate transmission in the normal brain development and behavior, their expression patterns may be distinct during postnatal development. We studied here the mRNA and protein expression of VGLUT1 and VGLUT2 in postnatal day (P) 7, P11, P14 and P21, as well as adult, in mouse visual cortex and dorsal lateral geniculate nucleus (dLGN). We also examined the expression of vesicular GABA transporter 1 (VGAT1), which is expressed in most of the GABAergic neurons, for comparison. Overall expression pattern of VGLUT1 mRNA was constant throughout the developmental period, but it was transiently more robust at P11 and P14 compared to other ages. In contrast, the immunohistochemistry showed a relatively steady increase in staining intensity from P7 to adult. While the expression of VGLUT2 mRNA was almost exclusively confined to subcortical regions in adult, it was abundant in layers 2-4 of the visual cortex in postnatal animals. There were no noticeable changes in VGAT1 mRNA expression. These results indicate that VGLUT1 and VGLUT2 continue to mature during the first 3 postnatal weeks, while the transporter of GABAergic system is relatively constant.

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Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Wellcome Trust grant 083205

University of Strasbourg Institute for Advanced Studies

Title: Measuring order in retinotopic projections: Assessing technical and biological sources of variability

Authors: *J. HJORTH[1], E. SAVIER[2], D. C. STERRATT[3], M. REBER[2], S. J. EGLEN[1];
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Abstract: The mouse retinotopic map is a model system for studying self-organisation during neurodevelopment. Gradients of Eph receptors and ephrin ligands in the retina and the superior colliculus, together with waves of retinal activity, specify retinal ganglion cell axons organise into a topographic projection on the superior colliculus. These maps can be quantified by focal tracer injections, labelling small numbers of axons in the retina and visualising their termination zones in the superior colliculus. Prior to dissection, a nasal cut is made in the eye at the level of the nictitans membrane, indicating the nasal pole of the retina the labeled retina is then dissected and flattened by 4 orthogonal cuts (nasal, ventral, temporal and dorsal).To define the nasotemporal axis of the retina, two opposite lines are drawn, connecting the corners of the nasal-ventral/nasal-dorsal (line A) and temporal-ventral/temporal-dorsal (line B) leaflets, A third line connecting the center of line A to line B via the optic disk defines the nasal-temporal axis of the retina. This transformation from a curved axis in a 3D structure to a line in a 2D plane introduces errors. A recently developed software package "Retistruct" allows the image of a flattened retina to be folded back onto a sphere, enabling us to work in the native 3D coordinates of the retina. The uncertainty in our assessment comes from two sources, technical variability and biological variability. By using two different methods to analyse the data we can get an estimate of the technical variability. To address the biological variability we use one method, but either repeat our experiments or resample the experimental data. The new analysis methods enables us to better assess phenomena such as the collapse point in the heterozygous Isl2-EphA3 mutant. Acknowledgements JJJH, DCS, SJE are supported by the Wellcome Trust (grant number 083205). ES, MR and SJE are supported by the USIAS (University of Strasbourg Institute for Advanced Studies).

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Poster

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Augsburg URGO

Augsburg College

Dean Sundquist

Title: A study of the daphnia magna hedgehog gene and its role in eye development

Authors: M. GRAFELMAN, *M. L. BECKMAN;
Biol., Augsburg Col., MINNEAPOLIS, MN

Abstract: Early in the development of a *Daphnia magna* embryo two distinct eye spots are observed within the eye field. As the embryo continues to develop the two eye spots grow larger and fuse, resulting in a central cyclopean eye. No details regarding the developmental genetic basis of this eye fusion event are yet known. The present study focused on cloning a candidate gene, *Hedgehog*, and characterizing the developmental time-course of its expression using RT-PCR and *in situ* hybridization. The homologous human gene, *Sonic hedgehog*, when mutated, is associated with holoprosencephaly (HPE), a disorder that can be manifested in a variety of midline defects, including cyclopia. This study sought to determine if similarities exist between *D. magna hedgehog* and the mutated *H. sapiens Sonic hedgehog*, which may explain the comparable phenotypes. We compared the known human Sonic hedgehog amino acid sequence alterations associated with HPE to the wild-type *D. magna* Hedghog protein sequence and found no obvious amino acid changes that might be associated with cyclopia in this protein. Amino acid sequence analysis revealed high identities between the *D. magna* Hedgehog protein and human Sonic hedgehog sequences in the N-terminal domain (79.6%), but only 35.1% identity in the C-terminal domain. A similar pattern of sequence identity was observed between the *D. magna* Hedgehog and the *M. musculus* Sonic hedgehog (80.3% in the N-term and 35.3% in the C-term), while a moderate sequence identity was found between the *D. magna* Hedgehog and the

D. melanogaster Hedgehog in both regions (68.8% in the N-term and 46.9% in the C-term). Preliminary expression studies in *D. magna* reveal *hedgehog* mRNA transcripts are present throughout early embryogenesis (RT-PCR) and in the anterior midline of the developing animal (*in situ* hybridization). Preliminary studies of the Hedgehog signaling pathway using pharmacological methods show that when cholesterol synthesis is inhibited with U18666a, *Daphnia* eye development is altered. Further studies will focus on establishing a high-resolution time-course and spatial map of *hedgehog* gene expression using *in situ* hybridization and RT-PCR. In addition, we will pursue the study of additional cholesterol synthesis inhibitors to further determine what role Hedgehog plays in *Daphnia magna* cyclopean eye development.

Disclosures: M. Grafelman: None. **M.L. Beckman:** None.

Poster

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Title: Binocular deprivation reveals developmental zif268 expression central-to-peripheral gradient in cat area 17, but not in area 18

Authors: *K. BURNAT[1], K. LASKOWSKA-MACIOS[1,2], T.-T. HU[2], M. KOSSUT[1], L. ARCKENS[2];
[1]Nencki Inst., Warsaw, Poland; [2]Neuroplasticity and Neuroproteomics Biol., KU Leuven, Leuven, Belgium

Abstract: Although the development of the retina proceeds along a central-to-peripheral gradient a possible cortical counterpart has not yet been identified. The timing and duration of the maturation of the central and peripheral visual field representations in cat primary visual areas 17 and 18 remains unknown. In cats binocular deprived from birth from pattern vision (BD), a model for congenital cataract, motion perception impairments co-occur with anatomical modifications in the temporal Y-type alpha retinal ganglion cells (Burnat et al. 2012). BD might

thus disrupt the normal development of the motion-sensitive cortex driven by Y-type input including area 18 and peripheral area 17. The impact of early BD from eye opening for 2, 4 or 6 months, and late onset BD upon 2 months of normal vision, on cortex maturation was measured using the expression pattern of the visually-driven activity reporter gene zif268 as readout. Decreasing zif268 mRNA levels between month 2 and 4 characterized the normal maturation of the (supra)granular layers of the central and peripheral visual field representations in area 17 and 18. In general, all BD animals had higher than normal zif268 levels. In area 17, early BD showed a central-to-peripheral developmental gradient based on a delayed decrease in zif268 signal. In contrast in area 18, the decrease occurred between month 2 and 4 in both central and peripheral visual field representations. A parallel functional proteomics investigation for central and peripheral area 17 further revealed age- and region specific protein expression changes for normal and BD kittens. Lack of pattern vision stimulation during the first 4 months of life therefore has a different impact on the development of central and peripheral regions of area 17.

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Poster

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S10RR021110

Title: The normal development of resting state functional connectivity in infant macaque monkeys

Authors: *J. L. VINCENT, K. SRIHASAM, M. S. LIVINGSTONE;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Spontaneous Blood Oxygenation Level-Dependent (BOLD) fluctuations are correlated within distributed functional brain networks in the adult brain. These so-called "resting-state" networks correspond to anatomical networks identified using anatomical tracers, as well as to functional networks identified using task paradigms. To explore in the normal development of these networks, we collected 30-45 minutes of fMRI data in two newborn monkeys while they rested quietly with their eyes closed. To enhance signal-to-noise, the monkeys were injected with 12 mg/kg of a Monocrystalline Iron Oxide Nanoparticle contrast agent before each scanning session. In these two infant monkeys (B5: 10-days-old and B4: 18-days-old), we observed interhemispheric functional correlations using seed regions placed in primary motor cortex, primary visual cortex, and several other regions in the frontal, parietal, and temporal lobes. In the 10-day-old infant, seeds in the oculomotor network (FEF or LIP) showed robust interhemispheric correlations, and weak frontal-parietal correlations. However, in the 18-day-old infant, there were robust frontal-parietal correlations within the oculomotor network. In the default network, correlations were observed between posterior cingulate and medial prefrontal cortex in both the 10- and 18-day-old infants. These results suggest that coherent resting state networks are present early in development, which is consistent with resting-state functional connectivity studies of human infants.

Disclosures: J.L. Vincent: None. **K. Srihasam:** None. **M.S. Livingstone:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.13/A60

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R01EY020560

Title: Loss of Hgs in developing retinas causes defective photoreceptor outer segment development

Authors: *T. THEIN, L. JIANG, S. BLACKSHAW;
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Abstract: Hepatocyte Growth Factor-Regulated Tyrosine Kinase Substrate (Hgs/Hrs) is an evolutionarily conserved component of the endosomal sorting complex required for transport (ESCRT) machinery that regulate protein trafficking from endosomes to lysosomes. Recent

studies suggest that some ESCRT components play an important role in eye development and diseases. However, the role of Hgs, an ECCRT-0, during eye development is not well-characterized. Hgs is strongly and selectively expressed in developing photoreceptors of early postnatal mice. In order to determine whether Hgs plays a role in photoreceptor development, we used *in vivo* electroporation to deliver shRNA targeting Hgs to retinal progenitor cells of newborn mice. We have found that shRNA electroporated photoreceptors failed to develop proper outer segments. This defect is rescued by co-electroporation of shRNA-resistant Hgs constructs. Thus, our findings suggest that Hgs is important for proper development of photoreceptor outer segments.

Disclosures: T. Thein: None. **L. Jiang:** None. **S. Blackshaw:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.14/A61

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NSC

Title: Synaptotagmin I in RGCs regulates the patterned spontaneous activity in the developing rat retina

Authors: *C.-C. YANG, C.-T. WANG;
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Abstract: During a critical period of the developing vertebrate visual system, the patterned spontaneous activity is required for visual circuit refinement, termed retinal waves. Retinal waves in mammals can be classified into three stages that are mediated by various mechanisms. The stage II waves, appearing from postnatal day P0 to P9 in rats, are initiated by starburst amacrine cells (SACs) undergoing Ca2+-regulated exocytosis, thus releasing neurotransmitters onto other SACs and retinal ganglion cells (RGCs). Our previous study showed that in the developing SACs, a Ca2+ sensor protein, Synaptotagmin I (Syt I), can regulate the patterns of retinal waves via Ca2+ binding to its Ca2+-binding domains, C2A and C2B. Although Syt I is also expressed in the developing RGCs, it remains unclear whether Syt I in RGCs may involve in regulating the properties of retinal waves. To address this question, we expressed Syt I or its C2A mutant (D230S, designated Syt I-C2A*) in RGCs by utilizing a RGC-specific promoter

(pBrn3b). Live Ca2+ imaging was subsequently performed to monitor the wave-associated Ca2+ transients after pBrn3b-driven expression. We found that Syt I significantly increased the frequency of Ca2+ transients compared to control, but Syt I-C2A* significantly decreased the Ca2+ transient frequency compared to control and Syt I. Moreover, Syt I reduced the amplitude of Ca2+ transients compared to control, whereas Syt I-C2A* increased the amplitude and duration of Ca2+ transients compared to control and Syt I. Therefore, our results suggest that Syt I in RGCs may regulate the properties of retinal waves via Ca2+ binding to its C2A domain.

Disclosures: C. Yang: None. **C. Wang:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.15/A62

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH T-32 DE007057

Title: Functions of the GFL/Ret signaling pathway in the development of the peripheral taste system

Authors: *C. R. DONNELLY, B. A. PIERCHALA;
Biologic and Materials Sci., Univ. of Michigan - Sch. of Dent., Ann Arbor, MI

Abstract: During the development of the peripheral nervous system, target-derived neurotrophic factors aid in the establishment of synaptic connections and govern neuronal survival and differentiation. In the peripheral taste system, neurotrophin-4 (NT-4) and brain-derived neurotrophic factor (BDNF), signaling through the TrkB receptor, are important mediators of axon guidance and survival of chemosensory geniculate neurons projecting to the anterior tongue. While the functions of the neurotrophins in the development of the peripheral taste system have been extensively explored, it is unknown whether additional families of neurotrophic factors are involved in this process. Furthermore, it is unknown whether distinct subpopulations of geniculate neurons exist that can be delineated based on their dependence on different neurotrophic factors, as in other sensory populations. In this study, we provide evidence that the glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs), signaling through the receptor tyrosine kinase, Ret, have an important role in the development of the peripheral taste system. Utilizing a recently developed Ret reporter line, we observed that Ret is

expressed in a subset of taste receptor cells in the circumvallate and fungiform papillae, a subset of chemosensory axons innervating fungiform taste buds, and a subset of somatosensory axons innervating fungiform and filiform papillae. Additionally, we observed distinct subgroups of Ret+/TrkB-, Ret+/TrkB+, and Ret-/TrkB+ geniculate ganglion neurons. Analysis of both Ret germline knockout mice and neuron-specific Ret conditional knockout mice (using the Synapsin-Cre driver), we observed a loss of fungiform taste buds, but not a loss of fungiform papillae. Furthermore, genetic deletion of Ret resulted in innervation defects by E18.5. Collectively, these data indicate that Ret is required for the development of a subset of geniculate ganglion neurons, and may represent a novel subpopulation with unique functional properties. Ongoing experiments are analyzing the expression of Ret at additional developmental time points, identifying the spatiotemporal expression of the GFLs, and examining whether geniculate and/or trigeminal ganglion neurons are lost in Ret knockout mice.

Disclosures: C.R. Donnelly: None. **B.A. Pierchala:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: R01 DC00407

Title: Postnatal reorganization of primary gustatory afferent terminal fields in the mouse brainstem is altered by prenatal dietary sodium history

Authors: *S. ZHENG, C. SUN, D. L. HILL;
Univ. Virginia, CHARLOTTESVLE, VA

Abstract: Age-related decreases in terminal field sizes occur in a variety of sensory systems at various neural levels. In rat, we have shown that the terminal fields of the greater superficial petrosal (GSP), chorda tympani (CT), and glossopharyngeal (IX) nerves decrease in the amount of spread in the nucleus of the solitary tract (NTS). Moreover, offspring of pregnant rats fed a sodium-restricted diet when they were embryonic day 3 (E3) to E12 results in a lack of postnatal pruning. In fact, the amount of terminal spread for all three nerves in the NTS increases dramatically after postnatal 35. Thus, a very early period of dietary sodium restriction leads to a late-onset expansion of terminal fields in the rat NTS. We also demonstrated earlier that terminal

field size in E3-E12 sodium restricted mice, as measured by amount of terminal field label, is at least doubled at adulthood compared to mice fed normal lab chow throughout development. Here, we examined the normal postnatal development of the three terminal fields and their overlapping fields in the NTS and compared this development to that of E3–E12 sodium restricted mice. Our preliminary findings show that the terminal field volume of each of the three nerves decreases from postnatal day 15 (P15) to P60 in control mice, albeit at different rates. In contrast, the terminal fields of the three nerves in E3-E12 sodium restricted mice are similar to that in controls at young ages; however instead of pruning, the terminal fields of the GSP, CT, and IX all enlarge after P30 so that the terminal field volumes are at least double the size of controls at adulthood. Establishing these developmental patterns in mice now allow us to ask more mechanistic questions about how and why terminal fields decrease with age in normally fed mice and what factors have a role in the late plasticity period that expresses itself when mice are restricted of dietary sodium at a very early stage of embryonic development.

Disclosures: S. zheng: None. **C. Sun:** None. **D.L. Hill:** None.

Poster

780. Development of Sensory Systems

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.17/A64

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Barrel Cortex Function

Swiss Foundation for Excellence in Biomedical Research

Title: Postnatal development of sensory-evoked neuronal population activity in mouse barrel cortex

Authors: *A. VAN DER BOURG, F. HELMCHEN;
Brain Res. Inst., Zürich, Switzerland

Abstract: The mammalian neocortex is essential for high-level sensory processing, motor control and higher cognitive functions, yet the principles of neocortical circuit operation and their implementation in the developing brain remain poorly understood. The rodent whisker system is a widely adopted model for sensorimotor integration. However, the studies of developmental changes in the neuronal activity in somatosensory cortex S1 ('barrel cortex') are

scarce. Here, we investigated spontaneous and sensory-evoked neuronal population dynamics in layer 2/3 (L2/3) of the developing mouse barrel cortex (between postnatal day P10 and P30). Population activity was measured in anesthetized mice using *in vivo* two-photon calcium imaging following bolus-loading of the calcium indicator Oregon Green BAPTA-1. For precise stimulation of the principal whisker we used a newly developed multidimensional galvanometric stimulation system capable of moving single or multiple whiskers along their longitudinal and transversal axes at high temporal and spatial resolution, either simultaneously or independently. Longitudinal force exertion on the whisker follicle ('tapping') presumably is an important aspect of touch events but has been rarely employed so far. Between P10 to P30 spontaneous L2/3 activity markedly changed, showing increased sparseness, larger heterogeneity and progressive desynchronization. For principal whisker-evoked responses (either longitudinal, transversal, or both) we discovered a clear shift from highly synchronized unselective responses to sparse and stimulus-selective activity patterns. Whereas longitudinal and transversal stimulation activated the same sub-populations of neurons in young mice, two distinct groups could be identified in young adult animals implicating the emergence of tuning to specific whisker forces in L2/3. Stimulus-selective neurons emerged around P13 when overall neuronal population activity was starting to decrease significantly. At around P16 neuronal network activity already showed a highly selective and sparsified activity pattern comparable with evoked activity in young adult animals. Various mechanisms may underlie this functional maturation of L2/3 cortical circuitry, including changes in intrinsic excitability, formation of lateral connectivity, and maturation of the inhibitory network, warranting further examination. In the future, our findings may help to understand how maturation of sensory neocortical networks relates to emerging behaviors of young mice.

Disclosures: **A. Van Der Bourg:** None. **F. Helmchen:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: BBSRC Grant

Title: Early postnatal development of synaptic input and intrinsic excitability in barrel cortex

Authors: *L. MURRAY, M. C. ASHBY;
Bristol Univ., Bristol, United Kingdom

Abstract: Cortical development is controlled by a combination of genetic factors, spontaneous activity and experience dependent plasticity. In mouse barrel cortex, by postnatal day (P) 4, the genetically encoded somatotopic whisker map in layer IV has been formed and marks the end of the critical period for gross structural plasticity. This coincides with the inside-out formation of cortical layers, with layer VI cells differentiating and arriving first in the cortex and subsequent layers migrating through deeper layers in turn. Migration of cells is complete by the end of the first postnatal week. However, the formation of functional cortical circuits undergoes a critical period of development between P4 and P10 that is primarily regulated by sensory experience and neuronal activity and does not follow the same structural maturation pattern. Using a combination of immunohistochemistry and whole-cell patch clamp electrophysiology, we have compared the development of synaptic input and intrinsic excitability of layer IV stellate and layer V pyramidal cells within barrel cortex slices throughout the functional critical period from P3 to P12. Current observations indicate a rapid maturation in action potentials and excitability, as well as significant changes in passive membrane properties across both layers. Postnatal development of intrinsic properties of neurons appears to be interrelated with synaptic modification, including a decrease in NMDA to AMPA receptor ratio. Interestingly, we observe that layer IV and V cells differ in their developmental profile, but all neurons appear tuned to respond to developmentally regulated synaptic inputs. Our results demonstrate some of the vast physiological changes which occur early in development, particularly during critical periods. Defects which alter the careful balance of synaptic and intrinsic properties early in postnatal growth may have long lasting effects of cortical processing.

Disclosures: L. Murray: None. **M.C. Ashby:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: March of Dimes 5-FY11-570

NIDCD RO1, DC013157

Title: Developmental profiles of the intrinsic properties and synaptic function of auditory neurons in preterm and term baboon neonates

Authors: S. KIM[1], S. LEE[1], C. BLANCO[2], *J. KIM[1];
[1]Physiol., Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX; [2]Pediatrics, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: The human fetus starts to hear and undergoes major developmental changes in the auditory system during the third trimester of pregnancy. Although there are significant data regarding development of the auditory system in rodents, changes in intrinsic properties and synaptic function of auditory neurons in developing primate brain at hearing onset are poorly understood. We performed whole-cell patch clamp recordings of principal neurons in the medial nucleus of trapezoid body (MNTB) in preterm and term baboon brainstem slices to study the structural and functional maturation of auditory synapses. Each MNTB principal neuron received an excitatory input from a single calyx of Held terminal, and this one-to-one pattern of innervation was already formed in preterm baboons delivered at 67% of normal gestation. There was no difference in frequency and amplitude of spontaneous excitatory postsynaptic synaptic currents (PSCs) between preterm and term MNTB neurons. In contrast, the frequency of spontaneous GABAA/glycine receptor-mediated inhibitory PSCs, which were prevalent in preterm MNTB neurons, was significantly reduced in term MNTB neurons. Preterm MNTB neurons had a higher input resistance than term neurons and fired in bursts whereas term MNTB neurons fired a single action potential in response to suprathreshold current injection. The maturation of intrinsic properties and dominance of excitatory inputs in the MNTB allow it to take on its mature role as a fast and reliable relay synapse in which each presynaptic action potential results in a single and reliable postsynaptic action potential in the primate auditory system.

Disclosures: S. Kim: None. **J. Kim:** None. **C. Blanco:** None. **S. Lee:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Royal Society Newton International Fellowship

Title: A genetic fate map of the avian brainstem auditory nuclei

Authors: *M. LIPOVSEK, D. FRANCHINI, R. WINGATE;
MRC Ctr. For Developmental Neurobio., London, United Kingdom

Abstract: The auditory systems of birds and mammals have undergone separate evolution since these lineages shared their last common ancestor 320 million years ago. While the common origin of the auditory end organ is well established, there is no consensus as to whether the brainstem auditory nuclei of birds and mammals are developmentally homologous. However, both in birds and mammals auditory space coordinates on the horizontal plane are processed in similar ways. In both clades this is based on computing interaural time differences (ITD) using a similar circuit comprising brainstem auditory nuclei that nevertheless appear to arise from different axial developmental origins. The axial and cell lineage origin of the neurons involved has recently been elucidated for the mice nuclei. Here, we present data on the fate mapping of the analogous nuclei of chicken. We performed in ovo electroporations of enhancer-driven Cre-recombinase expression constructs, together with a Flox-pA-EGFP and -mCherry constructs, to label rhombic lip derived Atoh1+, or ventricular zone derived Ptf1a+ lineages. Electroporations were performed at embryonic day 2 (E2). Eggs were further incubated until E9-10. Hindbrains were dissected, fixed and processed for cryosectioning and confocal imaging. We used a combination of cell position and axon projections to identify different cell populations. Atoh1 expressing progenitors from the rhombic lip give rise to the glutamatergic neurons of the nucleus magnocellularis and at least two neuronal types of the nucleus angularis (radiate and vertical cells). The inhibitory neurons of the superior olivary nucleus originate from Ptf1a+ progenitors. The progenitors for the neurons that compose the nucleus laminaris, the coincidence detector for ITD, could not be identified within these genetic lineages. This suggests a divergence in evolutionary origins of the ITD circuit between mammals and birds.

Disclosures: M. Lipovsek: None. **D. Franchini:** None. **R. Wingate:** None.

Poster

780. Development of Sensory Systems

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Program#/Poster#: 780.21/A68

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Dicer knockout affects the expression of vGluT1 in type Ia afferents

Authors: *M. M. FERRER SOCORRO, S. M. O'TOOLE, S. B. NELSON;
Nelson Lab., Brandeis Univ., Waltham, MA

Abstract: Proprioceptive feedback from type Ia sensory afferents is crucial for normal locomotion. Various transcription factors and signaling molecules are involved in the development and maintenance of these neurons and the targets they innervate. However much less is known about the role of post-transcriptional regulation by microRNAs (miRNAs) within type IA sensory neurons. miRNA biogenesis is dependent on the endoribonuclease Dicer. By conditionally ablating Dicer using a parvalbumin driven Cre we were able to impair miRNA biogenesis within type IA sensory neurons. Conditional knockout mice displayed severe ataxia, characterized by an uncoordinated gait and hyperextension of the limbs. Our behavioral analysis demonstrated that this deficit began at the middle of the third post-natal week. Immunohistochemical inspection of the muscle spindles revealed that intrafusal fibers were preserved and had intact overall morphology. Furthermore, Type IA sensory neurons continued to innervate the spindles as evidenced by the presence of characterstic annulospiral endings. However, we observed a near complete absence of vGluT1 staining, a marker of the spindle associated sensory endings (SSEs) within the peripheral sensory afferents. This observed drop in SSEs was statistically significant within both the gastrocnemius($p=5.41*10-5$) and tibialis anterior($p=0.03$) at post natal day 30 (p30). However, at p5 SSEs were largely intact when compared between knockout and control animals. Our findings place Dicer in a pathway that is vital for the maintenance of the type IA sensory neurons. Ongoing experiments will address the extent to which the innervation pattern and vGluT1 expression is affected in the spinal cord of Pvalb-cre Dicer condtional knockout mice.

Disclosures: M.M. Ferrer Socorro: None. **S.M. O'Toole:** None. **S.B. Nelson:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant SC1HD068129

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City University of New York Seed Grant 93348-14-01

Title: Coordinated changes in neuronal activity and vascular network properties in Wistar rat auditory brainstem during postnatal development

Authors: L. SHI[1], Q. BROWN[1], B. FU[2], *A. RODRIGUEZ-CONTRERAS[1];
[1]Biol., CCNY, CUNY, NEW YORK, NY; [2]Biomed. Engin., CCNY, CUNY, New York, NY

Abstract: Neurovascular coupling reflects the functional metabolic and signaling relationship between the blood vasculature and neural tissue. However, little is known about the developmental origins of neurovascular coupling. Recently, we found that the first postnatal week is important for cell proliferation and generation of astrocytes in the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem of rats (Saliu et al., 2104. J Comp Neurol 522:971-985). Astrocytes are good candidates to modulate the development of neurovascular coupling because they interact simultaneously with synapses and blood vessels, but there is a paucity of data on the development of neuronal and vascular network properties in the MNTB. In this study we examined developmental changes in neuronal ensemble activity and vascular network structure and function in the MNTB of Wistar rat pups. All values reported here represent mean ± SD. To measure changes in the activity of MNTB neuronal ensembles we performed multi-electrode recordings in anesthetized pups. Mulit-unit activity (in spikes/s) increased from 1.1 ± 3.0 in the first postnatal week (n=9 pups) to 12.1 ± 20.2 in the second postnatal week (n=5 pups). To examine the development of the vascular network we used IB4 histochemistry to label blood vessels in 80 μm thick horizontal brainstem sections and obtained laser scanning confocal microscope z-stacks (512x512 pixels per image frame, 1 pixel = 0.581 μm). We performed semi-automated digital analysis of confocal stacks normalized to 10 μm thickness and found that vasculature density in the MNTB (in μm^3) increased from 30,199 ± 8313 in the first postnatal week (n=2 pups) to 47,690 ± 18,050 in the second postnatal week (n=2 pups). There was a similar trend in the number of branches per image stack from 7.5 ± 2.3 in the first postnatal week to 17.2 ± 8.8 in the second postnatal week. Finally, to quantify vascular function we injected the fluorescent solute TRITC-dextran (155 kD) via the carotid artery and quantified its permeability in individual capillaries or post-capillary venules in the MNTB of postnatal rats *in vivo* using two-photon microscopy. Our preliminary results show a decrease in the permeability of microvessels (in cm/s) from $2.9 \pm 1.1 \times 10^{-7}$ in one-week old rats (n=11 vessels) to $1.6 \pm 0.6 \times 10^{-7}$ in two-week old rats (n=5 vessels). Based on these preliminary results we propose that the first week of postnatal life could be important for coordinated changes in astrocytes, neurons, and the vascular network that might lead to the establishment of neurovascular coupling in neonate rats.

Disclosures: L. Shi: None. **A. Rodriguez-Contreras:** None. **B. Fu:** None. **Q. Brown:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NSERC #312015

NSERC #3595

Title: c-Fos immunohistochemical study of vestibular activity in developing opossums, Monodelphis domestica

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

Abstract: Even if they are born very immature, the newborn marsupials crawl, unaided, from the mother's birth canal to a nipple where they attach to pursue their development. In most species, the crawling is effected against gravity; the vestibular system is thus one of the sense organs thought to be involved in the guidance of the newborn to the nipple. Behavioral studies in which newborns were placed head down showed that they could revert themselves head up, but it was not ascertained if the vestibular system played a role in this reflex, their immobile hindquarters possibly acting as dead weight imposing the change in body orientation. Anatomical studies in opossum and wallaby have shown that even if the labyrinth is very immature, the macula (perceiving gravity) may be functional and innervated by vestibular afferents which distribute centrally in the vestibular nuclei. We recently showed that low intensity electrical stimulations of the brainstem where vestibulospinal cells are located induce forelimb movements in *in vitro* preparations of newborn opossums Monodelphis domestica. To additionnally test if the vestibular system may influence motor behaviors *in vivo*, immobilized but conscious opossums from the day of birth (P0) to P18 were subjected for 60 minutes either to sinusoidal acceleration along the three planes or to vertical angular displacement. Control animals were subjected to the same conditions, but without stimulation. The animals were then anesthetized by hypothermia, decapitated and the heads were fixed by immersion in paraformaldehyde to be sectioned. Sections were processed immunochemically to reveal c-Fos, a protein expressed in the karyon following sustained stimulation and used as a marker of neuronal activity. From P0 onwards, c-Fos labeled neurons were observed in different parts of the brainstem, such as the reticular formation or the solitary nuclei, but not in the vestibular labyrinth or nuclei. Labeling in the vestibular nuclei appeared at P15 and was more abundant at P18. No significant labeling was found in the vestibular sensory organs at any age examined. These results do not support an influence of the vestibular system in movement control of newborn opossums. However, c-Fos labeling in the vestibular nuclei appears slightly before the age at

which opossums start detaching from the mother and begin to use quadrupedal locomotion, albeit often returning to the nipple for several more weeks. It is likely that similar results would be obtained in other marsupial species.

Disclosures: F. Lanthier: None. **T. Cabana:** None. **J. Pflieger:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: JSPS, Japan (No. 23592485)

NIDCD 1R01DC010154

Title: The developmental expression of PTEN in the spiral limbus of the mouse cochlea

Authors: Y. DONG[1,2], L. SUI[2], M. TOKUDA[2], *B. HU[1];
[1]Ctr. Hearing & Deafness, State Univ. Buffalo, Buffalo, NY; [2]Kagawa Univ. Fac. of Med., Kagawa, Japan

Abstract: Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that regulates diverse cell processes including proliferation, growth and synaptogenesis, as well as neural and glioma stem/progenitor cell renewal. PTEN also regulates the proliferation and differentiation of sensory cells in the developing inner ear. However, it is not clear whether PTEN plays a role in the development of the spiral limbus (SL). In this study, we examined the expression pattern of PTEN during the development of the SL in the mouse cochlea using RT-PCR, Western blotting, and immunohistochemistry. PTEN starts to express in the fibrocytes of the SL from the postnatal day (P) 4. At this time point, the expression also appears in the interdental cells (IDCs) when these cells begin to differentiate. Upon the maturation of the inner ear (P21), the expression of PTEN becomes undetectable in fibrocytes. However, PTEN expression persists in IDCs. Collectively, these results reveal a dynamic change in PTEN expression during inner ear development. Thus, our study suggests that PTEN plays a role in the differentiation of the SL in mouse cochlea.

Disclosures: Y. Dong: None. **L. Sui:** None. **M. Tokuda:** None. **B. Hu:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: RO1EY022720

Title: Crossmodal plasticity of intracortical connections in auditory cortex

Authors: *X. MENG, P. KANOLD;
Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: Sensory systems do not work in isolation and interactions between auditory and visual systems have been shown during sensory loss. For example, cross-modal compensation in blind individuals leads to functional enhancement of the remaining senses, including enhanced frequency discriminations. However, the underlying mechanism for the compensation is not clear. Since our prior studies show that visual deprivations leads to improved frequency selectivity of neurons in primary auditory cortex (A1), we hypothesized that changes in visual experience can cause changes in the synaptic connections within A1. We investigated this hypothesis by comparing intracortical connections to Layer 2/3 neurons in dark-exposed mice to those from normally reared mice. We study the spatial pattern of excitatory and inhibition connections in acute slices of A1 using laser-scanning photostimulation (LSPS). We find that excitatory and inhibitory circuits related to layer 2/3 neurons are different in dark exposed mice than those from normally reared mice. The differences are characterized by the amount of input from different layers to L2/3 neurons and averaged maps. Our results show that visual experience can change circuits within A1 and these changes might contribute to enhanced auditory processing after visual deprivations.

Disclosures: X. Meng: None. **P. Kanold:** None.

Poster

780. Development of Sensory Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Sir Henry Wellcome Postdoctoral Fellowship (EG)

Wellcome Trust Career Development Fellowship (MSG)

Title: Developmental origin of axon initial segment-possessing olfactory bulb dopaminergic cells

Authors: *E. GALLIANO, A. N. CHAND, M. S. GRUBB;
MRC Ctr. For Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: Dopaminergic periglomerular neurons (DNs) in the olfactory bulb (OB) act at a crucial point in the early olfactory pathway, modulating information processing in OB glomeruli. DNs are extremely plastic cells, capable of regenerating throughout life, regulating their dopamine production levels in an exquisitely activity-dependent manner and undergoing structural plasticity of their dendrites. Previous work from our group has shown in an *in vitro* assay that only a subset of OB DNs has a specialised sub-compartment of the axon defined by local enrichment of the scaffolding protein ankyrin-G and voltage-gated sodium channels - an axon initial segment (AIS). Moreover, the AIS in OB DNs showed a surprisingly high degree of plasticity upon chronic changes in neuronal activity. In order to investigate if such plasticity also occurs *in vivo*, we firstly addressed the issue of which subset of DNs, if any, possesses an AIS in the intact organism. From a neurogenesis point of view DA neurons do not constitute a homogeneous population: while postnatally generated cells derive solely from precursors in the subventricular zone (SVZ), developmentally generated DNs are of different lineages. The majority of embryonically born DNs derive from neurogenetic areas outside the bulb such as the SVZ, the lateral ganglionic eminence (LGE) and the septum. Conversely, it has been recently shown that a small portion of DNs derives from a population of local bulbar precursors that, from as early as E13.5, generates very large DNs residing mostly at the border of glomerular and the external plexiform layers. Our preliminary results obtained in perinatally electroporated animals suggest that only this population of DA neurons derived prenatally from local precursors have an AIS, while the vast majority of small developmentally and postnatally born DNs do not possess this axonal specialisation. Future experiments will address whether these prenatally generated, AIS-possessing large DNs are the only source of OB short-axon cells, defined by their extensively ramifying axons that contact multiple glomeruli.

Disclosures: E. Galliano: None. **A.N. Chand:** None. **M.S. Grubb:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.27/B6

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Wellcome Trust Career Development Fellowship

Title: Axon initial segment plasticity in olfactory bulb dopaminergic interneurons *in vitro* and *in vivo*

Authors: *A. N. CHAND, E. GALLIANO, T. RYAN, M. S. GRUBB;
MRC Ctr. for Dev. Neurobio., King's Col. London, London, United Kingdom

Abstract: The axon initial segment (AIS) is a specialised structure near the start of the axon that is a site of neuronal plasticity. Changes in activity levels can produce structural AIS changes that are linked to alterations in cellular excitability, which has previously been described in excitatory neurons. In the olfactory bulb (OB) inhibitory dopaminergic (DA) interneurons are particularly plastic, undergoing constitutive turnover throughout life, and regulating tyrosine hydroxylase (TH) expression in an activity-dependent manner. We investigated activity-dependent AIS plasticity in these inhibitory interneurons *in vitro* and *in vivo*. Dissociated cultures of rat embryonic day 18 and mouse postnatal day 3 (P3) OBs were immunolabelled for TH and for the AIS scaffolding protein Ankyrin-G. A +10mM K+ stimulus for 24 h produced an inward movement of the AIS in DA cells (~6 μm towards the soma) and an increase in AIS length. These counterintuitive changes, in the opposite direction to those previously reported in excitatory neurons, were not seen in rat OB principal cells, were fully reversed upon reinstatement of control conditions, and were dependent upon L-type calcium channels. To investigate the physiological effects of this 'reverse' AIS plasticity we cultured mouse OB cells from P3 TH:tdTomato mouse pups. Whole-cell electrophysiological recordings from tdT-positive DA cells suggested that inward AIS movement and increased AIS length were associated with increased excitability. Could these activity-dependent AIS changes contribute to homeostasis at OB circuit level? To address this question we manipulated OB activity in 4 week-old mice using 24 h unilateral naris occlusion or olfactory odour enrichment. In preliminary data a decrease in AIS length was observed in OBs deprived of sensory input using naris occlusion. This form of AIS plasticity in DA inhibitory neurons, which is the reverse of previously

observed AIS plasticity in excitatory cells, may therefore contribute to circuit-level adaptation in the OB allowing for consistent detection of odorant stimuli.

Disclosures: **A.N. Chand:** None. **E. Galliano:** None. **M.S. Grubb:** None. **T. Ryan:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.28/B7

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant R03 DC012130-02

NIH Grant R01 DC012441

Title: Developing excitation in olfactory bulb projection neurons

Authors: ***A. S. MOBLEY**[1], M. A. TUSTY[2], C. A. GREER[2];
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Abstract: Mitral cells, the olfactory bulb projection neurons, are born between embryonic (E) days 9-13, peaking at E11. Mitral cell dendrites receive odor input from olfactory sensory neurons that is then relayed by axon projection to higher cortical areas of the brain. We previously showed that the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel in olfactory sensory neurons contributes to the specificity of axon targeting to the glomerular neuropil where they make the first synapse with mitral cell dendrites. To extend our analyses of the role(s) of HCN channels in the development of the olfactory bulb, we examined the expression of the four HCN channel subunits in developing mitral cells. The HCN1 and HCN2 subunits were widely expressed at E11 and colocalized with the mitral cell fate transcription factor Tbr1. Staining with the early progenitor marker Pax6 showed colocalization with HCN1 and HCN2. Such early expression suggests potential roles for HCN1 and HCN2 in fate choice, cue response or excitation in developing mitral cells. HCN3 and HCN4 expression was not observed until E15 when the developing layer of mitral cells is 5-6 cell bodies thick, co-localizing well with the later-onset mitral cell transcription factor Tbr2. By postnatal (P) day 0, when mitral cell layer development is complete (1-2 cell bodies thick), the subcellular localization of HCN1 shifted from the mitral cell somata to the dendrites which are located in the

glomerular layer. Colocalization of HCN1 and HCN2 with the mitral cell-specific marker vesicular glutamate transporter-1 indicates a postsynaptic role. HCN2 expression is also prominent in the glomerular layer, similar to HCN1. To test a functional role for HCN channels we transfected primary cultured mitral cells with shRNA targeting HCN2 and observed increased dendrite length. However, transfection with shRNA that targeted both HCN1 and HCN2 decreased dendrite length. These data suggest a role for HCN1 in neurite extension that is tempered by the presence of HCN2 with its slower kinetics and activation requirement of greater hyperpolarization. Thus far, the early onset of expression and subsequent distribution to sites of synaptic transmission suggest a role in developing mitral cell excitation and a functional contribution to developing morphology.

Disclosures: **A.S. Mobley:** None. **M.A. Tusty:** None. **C.A. Greer:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.29/B8

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH DC007395

Title: Lamin B1 is required for olfactory sensory neuron maturation

Authors: ***C. M. WALL**[1,2], S. G. YOUNG[3], Y. ZHENG[2], H. ZHAO[1];
[1]Biol., Johns Hopkins Univ., Baltimore, MD; [2]Embryology, Carnegie Inst. of Washington, Baltimore, MD; [3]Med. and Human Genet., UCLA, Los Angeles, CA

Abstract: Disruption of the nuclear lamina, a protein network underlying the nuclear envelope, is linked to several human diseases; however, it is unclear how disruptions in a ubiquitous structure could produce a wide range of cell type-specific dysfunctions. The nervous system appears to be particularly sensitive to mutations in B-type lamins, major structural components of the nuclear lamina. To understand the role of B-type lamins in neuronal development, we investigated the differentiation of olfactory sensory neurons in the mouse olfactory epithelium, where local populations of progenitors are responsible for continuous replacement of neurons throughout adulthood. We used mouse genetics to knock out B-type lamins in an olfactory sensory neuron progenitor population (the horizontal basal cells) and then monitored differentiation after chemically-induced regeneration in adult animals. Lamin B1 mutant lineages

display a decrease in mature neuron markers, with no decrease in immature neuron markers or change in progenitor proliferation. These results suggest that Lamin B1 is not required for early stages of olfactory sensory neuron development, but may be necessary for neuronal maturation. This work aims to uncover a cell type-specific role for the nuclear lamina in the *in vivo* differentiation of a neuronal lineage.

Disclosures: C.M. Wall: None. **S.G. Young:** None. **Y. Zheng:** None. **H. Zhao:** None.

Poster

780. Development of Sensory Systems

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 780.30/B9

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant HD63071

Title: Self-generated movements with "unexpected" sensory consequences

Authors: *A. TIRIAC, C. DEL RIO-BERMUDEZ, M. S. BLUMBERG;
The Univ. of Iowa, Iowa City, IA

Abstract: Effective motor control demands that we distinguish sensations arising from self-generated movements (reafference) from those arising from other-generated movements (exafference). To make this distinction, reafferent signals are compared to motor copies (or corollary discharges). Recent findings in newborn rats demonstrate that myoclonic twitches, limb movements exclusive to REM sleep, trigger sensory responses throughout the brain, whereas wake-related limb movements do not. Here we tested the hypothesis that these differences result from state-dependent modulation of corollary discharge. While recording from the hindlimb region of primary motor cortex (M1), we evoked hindlimb movements using manipulations that differ with respect to their "expectancy" and, therefore, their presumed recruitment of corollary discharge. Only unexpected movements triggered M1 activity, thus supporting the notion that twitches, uniquely among all known self-generated movements, are processed as if they lack corollary discharge. This unique feature may be necessary for twitching to drive activity-dependent development of the sensorimotor system.

Disclosures: A. Tiriac: None. **C. Del Rio-Bermudez:** None. **M.S. Blumberg:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.01/B10

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant HD061543

Title: Biochemical characterization and single particle electron microscopy of AMPA-Rs embedded in lipid nanodiscs

Authors: *T. NAKAGAWA, C. M. AZUMAYA;
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Abstract: Determining the molecular mechanism of AMPA-R modulation by auxiliary subunits remains a challenge. To conduct biochemical, biophysical, and structural studies it is advantageous to have a stable preparation in which the protein complexes are in a near native environment that resembles the cellular membrane. Biochemically isolated complexes made of AMPA-R subunits and auxiliary subunits such as TARPs and CNIHs dissociate by extended exposure to even a relatively mild detergent. To circumvent this problem, we reconstituted detergent solubilized recombinant AMPA-R subunits into lipid bilayers using a lipid nanodisc technology. In this method, purified AMPA-Rs are incubated with phospholipids and membrane scaffold protein (MSP), and subsequently the detergent is removed from the system so that a patch of lipid bilayer is formed within a disc surrounded by the MSP, embedding the AMPA-Rs. Single particle EM images of the reconstituted AMPA-Rs clearly demonstrate larger transmembrane density representing the lipid nanodisc. We have also investigated the global conformational change in response to the ligand glutamate. Particles exposed to glutamate adopt conformations in which the two N-terminal domain dimers were clearly separated, consistent with the previous observation using detergent extracted brain AMPA-Rs. The unliganded particles obtained by using nanodiscs have superior stability over conventional preparation in detergent. Because both the extracellular and cytoplasmic sides of the AMPA-R are accessible when embedded in nanodisc's lipid bilayer, this preparation also offers an opportunity to study molecular interactions and posttranslational modification of the cytoplasmic domain in the context of intact receptor complexes. We are currently extending this approach to incorporate auxiliary subunits into AMPA-R lipid nanodiscs and to investigate other membrane protein complexes in the synapse. Our progress in this area will be presented at this poster.

Disclosures: **T. Nakagawa:** None. **C.M. Azumaya:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.02/B11

Topic: B.02. Ligand-Gated Ion Channels

Support: by NIH/NIMH MH085080 (S.T.)

Title: Porcupine controls stability and composition of hippocampal AMPA receptors

Authors: ***H. YU**[1], N. HARMEL[1,2], T. YAMASAKI[3], S. TOMITA[3], D. S. BREDT[1]; [1]Johnson and Johnson, San Diego, CA; [2]Univ. of Düsseldorf, Düsseldorf, Germany; [3]Dept. of Cell. and Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Neuronal AMPA receptor complexes contain auxiliary subunits that modulate receptor trafficking and gating. In addition to well-studied transmembrane AMPA receptor regulatory proteins (TARPs) and cornichons (CNIH-2/3), recent proteomic studies identified a diverse array of additional AMPA receptor-associated transmembrane and secreted partners. We systematically surveyed this collection and found that PORCN and ABHD6 both increase GluA1 levels in transfected cells. Conversely, knockdown of PORCN in hippocampal neurons, which uniquely express it in high amounts, selectively reduces levels of GluA, TARP γ-2/8 and CNIH-2 proteins. Although PORCN is a membrane-associated O-acyl transferase, regulation of AMPA receptors is independent of its catalytic activity. PORCN knockdown decreases AMPA receptor currents and accelerates desensitization, and this is associated with depletion of TARP γ-8 from AMPA receptor complexes. These studies define novel roles for PORCN in controlling the level and composition of hippocampal AMPA receptor complexes. Hong Yu and Nadine Harmel contributed to this work equally.

Disclosures: **H. Yu:** A. Employment/Salary (full or part-time):; Johnson and Johnson. **N. Harmel:** A. Employment/Salary (full or part-time):; Johnson and Johnson, University of Düsseldorf. **T. Yamasaki:** A. Employment/Salary (full or part-time):; Yale University. **S. Tomita:** A. Employment/Salary (full or part-time):; Yale University. **D.S. Bredt:** A. Employment/Salary (full or part-time):; Johnson and Johnson.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.03/B12

Topic: B.02. Ligand-Gated Ion Channels

Title: CPG2, a regulator of postsynaptic glutamate receptor internalization, directly links components of the endocytic machinery to the spine cytoskeleton

Authors: *S. LOEBRICH, M. BENOIT, J. KONOPKA, J. GIBSON, E. NEDIVI;
Picower Inst. for Learning and Memory, MIT, CAMBRIDGE, MA

Abstract: The nervous system has the remarkable capacity to adapt in response to altered sensory stimulation. Such plasticity is found on various organizational levels, including circuitry, single neurons and individual synapses, and is crucial for learning and memory. One mechanism of adjusting synaptic strength is the regulated display of glutamate receptors (GluRs) in the postsynaptic surface membrane. In response to neuronal activity, AMPA-type GluRs (AMPARs) can get internalized through Clathrin-mediated endocytosis (CME) in a specialized sub-compartment of dendritic spines, the endocytic zone. Candidate plasticity gene 2 (cpg2) is a brain-specific activity-regulated transcript from the Syne1 locus. It encodes a large protein that localizes to the endocytic zone and regulates AMPAR internalization through reversible association with the spine cytoskeleton. We have previously shown that CPG2 protein binds to F-actin through two coiled-coil domains in its C-terminus. Here, we investigate other CPG2 binding partners. Combining co-immunoprecipitation from synaptoneurosome preparations with mass spectrometry analysis we identified CPG2 interactions with multiple components of the endocytic machinery in brain, including Dynamin, Endophilin, the adaptor protein subunits AP2alpha, AP2beta, and the auxiliary endocytic factor HIP1. Using a yeast-2-hybrid approach to interrogate these interactors for direct binding, as well as heterologous expression in HEK cells for a structure-function analysis, we found that CPG2 binds directly to both AP2beta and EndophilinB2 via distinct protein domains. Further, CPG2 anchors components of the CME machinery at the spine cytoskeleton, directly linking the process of CME to F-actin. Our data suggest that CPG2 functions as a postsynaptic bridge that integrates second messenger signaling to instruct GluR internalization by linking components of the endocytic machinery to the spine cytoskeleton. These findings may have direct implications for neuropsychiatric disorder, since the human SYNE1 gene shows genome-wide significant association with Bipolar Disorder in regions homologous to rat cpg2.

Disclosures: **S. Loebrich:** None. **M. Benoit:** None. **J. Konopka:** None. **E. Nedivi:** None. **J. Gibson:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

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Program#/Poster#: 781.04/B13

Topic: B.02. Ligand-Gated Ion Channels

Support: AHA Founder's Affiliate Fellowship

SBU/CSHL Research Alliance

Title: Multiple structural domains influence ampa receptor assembly

Authors: ***Q. GAN**[1,2], C. L. SALUSSOLIA[3], R. KAZI[3], L. P. WOLLMUTH[1];
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Abstract: Ionotropic glutamate receptors (iGluR), including AMPA, kainate and NMDA receptors, are the major mediator of fast excitatory synaptic transmission in the vertebrate central nervous system. These ligand-gated ion channels function as tetrameric complexes consisting of four identical or similar subunits. Each iGluR subunit contains multiple structural domains: an extracellular amino-terminal domain (ATD), a ligand-binding domain (LBD), a transmembrane domain (TMD) consisting of three transmembrane helices (M1, M3 & M4) and a reentrant loop (M2), as well as an intracellular C-terminal domain. The assembly of iGluR subunits into tetrameric complexes is the pre-requisite for forward trafficking and synaptic insertion. To gain insight into the structural mechanisms underlying iGluR assembly, we investigated the contribution of each structural domain in the assembly of homomeric GluA1 AMPA receptors. We have found that while the ATD of GluA1 mediates the formation of dimeric intermediates, it is not required for the formation of a functional tetramer, nor does it affect the stability of the tetramer as assayed by titrating with incremental concentrations of SDS. In contrast, the TMD is the critical driving force behind tetramerization. Isolated TMD of GluA1 lacking both the ATD and the LBD still forms a tetramer. Previously we have identified a series of mutations in the M4 helix that prevent tetramerization in the full-length receptor or in the receptor lacking ATD. Interestingly, these mutations do not disrupt tetramer formation in the isolated TMD construct, although they still affect the stability of the tetramer. These results suggest that the LBD of

GluA1 negatively modulates the receptor tetramerization process. Consistent with this idea, disulfide cross-links introduced into the dimer-dimer interface of the LBD are able to partially rescue the tetramerization deficit of some M4 mutants. Elucidating how exactly the LBD confers the negative effect on AMPAR assembly will aid in the design of novel pharmacological agents that target this process to modulate the number and subunit composition of synaptic AMPARs.

Disclosures: Q. Gan: None. **C.L. Salussolia:** None. **R. Kazi:** None. **L.P. Wollmuth:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.05/B14

Topic: B.07. Synaptic Transmission

Support: JSPS KAKENHI 24300140

Title: Photoinactivation analysis of synaptic AMPA receptors in PSD-95 knockout mice

Authors: *H. KAMIYA;
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Abstract: Various scaffold proteins determine dynamic localization of neurotransmitter receptors within the postsynaptic density (PSD). Among them, PSD-95 is one of the major proteins constituting a macromolecular complex of PSD in the central nervous system. It has been demonstrated that PSD-95 interacts with AMPA receptor subunits and play a key role in stabilizing AMPA receptors within the poststsynaptic membrane. In this study, synaptic delivery of AMPA receptors in PSD-95 knockout mice was examined using photochemical inactivation analysis using a photoreactive AMPA receptor blocker ANQX. Using acute hippocampal slices, field EPSPs were elicited by stimulation of Schaffer collaterals and were recorded from stratum radiatum in the CA1 region. Rate of synaptic delivery of AMPA receptors was evaluated by time course of recovery of EPSPs after photochemical inactivation of synaptic AMPA receptors using ANQX. Since ANQX forms crosslink and irreversibly blocks cell surface AMPA receptors, rate of recovery of EPSPs after photoinactivation is supposed to reflect rate of synaptic delivery of AMPA receptors from the intracellular pools. As described previously, photoinactivation persistently suppressed EPSPs in wild-type mice after a short period of recovery possibly due to washout of unbound photoproduct. In PSD-95 knockout mice, on the other hand, recovery of EPSPs was significantly accelerated than in wild-type. These results suggest that abundant

expression of PSD-95 in acute slice preparation may facilitate immobilization of AMPA receptors on the postsynaptic membranes in intact synapses. Molecular interaction between PSD-95 and AMPA receptors (or auxiliary subunits TARPs) critically limits the mobility of native synaptic AMPA receptors *in situ*.

Disclosures: H. Kamiya: None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.06/B15

Topic: B.02. Ligand-Gated Ion Channels

Title: Acetylation of the AMPA receptor subunits GluR1 and GluR2 and the endoplasmic reticulum chaperone sigma-1 receptor: Implication for abuse of cocaine

Authors: *Y. YASUI, T.-P. SU;
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Abstract: The sigma-1 receptor (Sig-1R) is an endoplasmic reticulum (ER) chaperone whose chaperone activity can be regulated by the ligands such as cocaine. We previously showed that cocaine causes behavioral and neuronal responses in mice by translocating the Sig-1R from the ER-mitochondrion contacts to interact with the potassium channel Kv1.2 on the plasma membrane (PM). Thus, Sig-1R has been implicated in the abuse of cocaine. AMPA receptor (AMPAR) trafficking and incorporation into PM play an important role in cocaine abuse. Post-translational modifications are key steps in the regulation of receptor functions. It has been reported that cocaine affects phosphorylation levels of GluR1 and GluR2 at the specific serine which contribute to the cocaine-induced changes of AMPAR. Acetylation is known to play key roles in the processing, trafficking, stabilization, and recycling of functional proteins. Recently it has been revealed that the PM proteins can also be regulated by acetylation. However, acetylation of AMPAR remains to be explored. In this present study we examined if Sig-1R may affect the AMPAR trafficking with a final goal of providing a potential mechanism on how cocaine may differentially regulate the PM contents of GluR1 and GluR2. We found that the Sig-1R physically associated with GluR1 and GluR2. The Sig-1R associated with p300/CBP-associated factor (PCAF) and GCN5 which are mammalian members of GCN5-related N-acetyltransferases and share about 73% of homology throughout their sequences. GluR1 and GluR2 also interacted with PCAF and GCN5. We found that GluR1 and GluR2 were acetylated

at the N-termini facing an extracellular space or the ER lumen, and that the acetylation levels were regulated by PCAF. Our immunocytochemistry data showed that these interactions occurred at the ER. Interestingly, our data showed that Sig-1R agonists, cocaine or PRE-084, could affect differently the acetylation levels of GluR1 and GluR2. Together, these results suggest that cocaine differentially regulates acetylation of GluR1 and GluR2 at the ER through Sig-1R, which may be a potential new mechanism underlying cocaine-induced dynamics of AMPAR subunits. (supported by the Intramural Research Program, NIDA, NIH/DHHS)

Disclosures: Y. Yasui: None. **T. Su:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.07/B16

Topic: B.07. Synaptic Transmission

Support: Eunice Kennedy Shriver NICHD Intramural Grant

Title: Dissecting the role of AMPAR subunit composition on synapse maturation in CGE-derived hippocampal interneurons

Authors: *G. AKGUL, E. BARKSDALE, K. A. PELKEY, C. J. MCBAIN;
Natl. Inst. of Hlth., Bethesda, MD

Abstract: Disrupting the excitatory synaptic recruitment of GABAergic inhibitory interneurons can promote excitation-inhibition imbalance within cortical circuits that can precipitate neurological diseases such as schizophrenia and epilepsy. In the hippocampus, two major lineages of inhibitory interneurons, derived from caudal ganglionic eminence (CGE) and medial ganglionic eminence (MGE), contribute distinct subgroups, which give rise to microcircuit complexity. The specific biophysical properties of excitatory input onto distinct interneurons strongly correlate with cellular lineage and dictate the circuit recruitment of discrete forms of feedforward and feedback inhibition. CGE-derived interneurons primarily express calcium impermeable AMPARs and exhibit relatively large NMDAR-mediated currents with slow kinetics due to prominent expression of GluN2B subunits throughout development. In contrast MGE-derived interneurons typically express GluA2-lacking calcium permeable AMPARs with smaller NMDAR-mediated currents that transit through a developmental GluN2B-GluN2A subunit switch. To investigate the role of AMPAR-mediated glutamatergic input on the

development of CGE- derived interneurons and maturation of their excitatory input, we created a knockout (KO) mouse line that eliminates the GluA2 subunit expression selectively in CGE-derived interneurons. KO mice have been tested for AMPAR and NMDAR activity in CGE-derived interneurons and compared with the activity in wild type (WT) CGE- and MGE-derived interneurons. KO CGE-derived interneurons display inwardly rectifying AMPAR-mediated EPSCs with fast kinetics that are sensitive to the calcium permeable AMPAR antagonist philanthotoxin confirming successful targeted loss of GluA2 within this interneuron cohort. Interestingly, despite this shift to an MGE-like AMPAR profile, CGE-KO interneuron NMDAR-mediated currents retained properties similar to WT CGE-derived interneurons. Thus, forcing CGE-derived interneurons to express calcium permeable AMPARs did not alter their basal developmental program for expression of NMDARs. Of interest, the frequency of spontaneous excitatory synaptic activity in CGE-KO interneurons is significantly lower than that observed in MGE and CGE-derived WT interneurons revealing a deficit in the circuit integration of KO CGE derived interneurons. We are currently investigating whether this decreased activity results from a change in presynaptic release probability, or a lower number of active synapses.

Disclosures: **G. Akgul:** None. **E. Barksdale:** None. **K.A. Pelkey:** None. **C.J. McBain:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.08/B17

Topic: B.07. Synaptic Transmission

Support: KAKENHI Grant number 241035

Title: Reduced kainate receptors at hippocampal mossy fiber synapse in PSD-95 knockout mice

Authors: *E. SUZUKI[1,2], H. KAMIYA[1];
[1]Dept. of Neurobio., Hokkaido Univ. Grad. Sch. of Med., Sapporo, Hokkaido, Japan; [2] ＪＳＰＳ Res. Fellow, Tokyo, Japan

Abstract: PSD-95 is a major postsynaptic density (PSD) protein, and has been shown to play an important role in stabilizing AMPA receptors (AMPARs) as well as NMDA receptors (NMDARs) within the PSD. It has been reported that PSD-95 directly binds to GluK2 and GluK5 subunits of kainate receptors (KARs). However, it is still unknown whether this interaction regulates synaptic localization of native KARs. In this study, we examined a role of

PSD-95 in synaptic localization of native KARs using PSD-95 knockout mice. Acute hippocampal slices were obtained from wild type and PSD-95 knockout mice. KARs-mediated excitatory postsynaptic currents (EPSCs) were recorded from mossy fiber-CA3 synapses where KARs are densely expressed. EPSCs were evoked by the stimulation of granule cell layer of dentate gyrus. In wild type, 30 μM GYKI 53655, an antagonist of AMPARs, reduced both amplitude and charge transfer of EPSCs, and left small and slow components of EPSCs, as shown in the previous studies (amplitude: to 11.7 ± 0.9% of control, charge transfer: to 27.4 ± 2.5% of control, n = 16). These GYKI-resistant slow components were blocked by co-application of KARs antagonist, 1 μM ACET (amplitude: to 5.3 ± 0.6% of control, charge transfer: to 9.0 ± 1.8% of control). These data confirmed that slow components of EPSCs were mediated by synaptic activation of KARs. In PSD-95 knockout mice, KARs-mediated slow components were significantly reduced than those in wild type mice (amplitude: to 8.7 ± 0.7% of control, $P<0.05$, charge transfer: to 18.9 ± 2.0% of control, $P<0.05$, n = 8). These results suggest that PSD-95 is critical for synaptic localization of not only AMPARs, but also KARs at the hippocampal mossy fiber synapse.

Disclosures: E. Suzuki: None. **H. Kamiya:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.09/B18

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS035812

Title: Genetic dissection of a defined neural circuit reveals the role of kainate and AMPA receptors in taxis behaviors

Authors: P. MALDONADO, *P. J. BROCKIE, J. GARDNER, J. E. MELLEM, D. M. MADSEN, A. V. MARICQ;
Dept. of Biol., Univ. of Utah, SALT LAKE CTY, UT

Abstract: Animals find sparsely distributed resources by navigating along gradients of sensory cues. For example, animals can associate temperature or chemical cues with favorable environmental resources and move towards those cues. The relatively simple nervous system of the nematode *C. elegans* has facilitated the identification of circuits that contribute to distinct

behaviors. For example, navigation along thermal and chemical gradients depends on a neural circuit that includes the RIA interneurons. RIA are the major integrating neurons of this circuit and receive glutamatergic synaptic inputs from several different sensory neurons. We have shown that the RIA neurons express both the AMPA and kainate classes of ionotropic glutamate receptors (iGluRs). Notably, the GLR-3 and GLR-6 kainate iGluR subunits are exclusively expressed in RIA. By coexpressing these subunits in heterologous cells, we have shown that they constitute the components of a heterologous iGluR that can be gated by both glutamate and kainate. To determine the role of GLR-3/GLR-6 iGluRs in taxis behaviors, we generated null mutations in both the *glr-3* and *glr-6* genes. *In vivo* electrophysiological analysis of these mutants showed that GLR-3 and GLR-6 are required for a kinetically distinct component of the excitatory glutamate-gated current in RIA. Furthermore, we determined that the remaining component is mediated by the GLR-1 AMPA-type iGluR. Interestingly, using optogenetic techniques, we have also shown that AMPA and kainate receptors mediate distinct components of the behavioral response to light activation of sensory neurons that provide synaptic inputs to RIA. Together, our results indicate that AMPA and kainate receptors are required for navigation along sensory gradients and together mediate behaviors critical for survival of the animal.

Disclosures: P. Maldonado: None. **P.J. Brockie:** None. **J. Gardner:** None. **J.E. Mellem:** None. **D.M. Madsen:** None. **A.V. Maricq:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.10/B19

Topic: B.02. Ligand-Gated Ion Channels

Support: C.2328.0247

Title: Corticosteroid regulation of AMPA receptor mediated synaptic function

Authors: *H. XIONG[1], F. CASSÉ[2], S. MARTIN[2], M. JOËLS[3], H. KRUGERS[1]; [1]Swammerdam Inst. For Life Sci., Amsterdam, Netherlands; [2]Ctr. Natl. de la Recherche Scientifique, Inst. de Pharmacologie Moléculaire et Cellulaire, Valbonne, France; [3]Rudolf Magnus Inst. of Neurosci., Utrecht, Netherlands

Abstract: Corticosteroid hormones are released from the adrenal glands in large amounts upon exposure to aversive events. These hormones are lipophilic, cross the blood brain barrier and

bind to mineralocorticoid receptors and glucocorticoid receptors. Via these receptors, corticosteroid hormones exert rapid non-genomic and genomic effects on brain function. Behaviorally, corticosteroid hormones promote the consolidation of emotionally arousing information. The underlying mechanisms of these effects remain poorly understood. Mounting evidence suggests that corticosterone induces changes in glutamate neurotransmission in the prefrontal cortex and the hippocampus, which may underlie their effects on cognitive processing. We report that corticosterone regulates (i) AMPA receptor mediated synaptic function in an activity-dependent fashion, (ii) AMPA receptor mobility, (iii) AMPA receptor function via MRs and GRs in a time dependent manner, and (iv) these effects may underlie the effects on emotional memory formation.

Disclosures: H. Xiong: None. **F. Cassé:** None. **S. Martin:** None. **M. Joëls:** None. **H. Krugers:** None.

Poster

781. AMPA Receptor Modifications

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 781.11/B20

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH

Title: Chronic progesterone treatment increases the AMPA receptor-mediated neurotransmission via the activation of progesterone receptors

Authors: *S. JOSHI[1], K. RAJASEKARAN[2], C. PASSMORE[1], J. WILLIAMSON[1], J. KAPUR[1];
[1]Dept of Neurol., Univ. of Virginia, Charlottesville, VA; [2]Dept. of Neurol. & Neurotherapeutics, Univ. of Texas Southwestern Med. Ctr., Dallas TX, TX

Abstract: The effects of progesterone and its neuroactive derivative allopregnanolone on GABAA receptor (GABAR)-mediated inhibitory neurotransmission are well characterized. However the effects of prolonged progesterone treatment on glutamatergic transmission are not known. Chronic increase in progesterone levels could alter the AMPA receptor (AMPAR) expression via homeostatic mechanisms or through the activation of progesterone receptors (PR). Adult female rats were given PMSG (20 IU) followed 48 hrs later with β-HCG (10 IU). A separate set of animals were given a single dose of progesterone (50 mg/kg). The expression of

GluA1 and GluA2 subunits of AMPARs in the hippocampi was determined using standard Western blotting techniques. Surface expression of the GluA1 and GluA2 subunits was indirectly determined using a BS3 cross-linking assay. AMPAR-mediated sEPSCs were recorded from CA1 pyramidal neurons (PNs) of acutely isolated slices by whole-cell patch clamp technique. Progesterone levels increased in the animals treated with PMSG β-HCG (50.2 ± 5.9 ng/ml vs 20 ± 3.2 ng/ml, n=6, $p<0.05$). Chronic increase in serum progesterone enhanced the AMPAR current. AMPAR-mediated sEPSCs recorded from PNs were larger in PMSG β-HCG-treated animals (19.78 ± 3 pA, n=7 cells/3 animals vs 10.7 ± 0.4 pA, n=16 cells/3 animals, $p<0.05$). The expression of GluA1 and GluA2 subunits was also enhanced in the hippocampi of PMSG β-HCG-treated animals (174 ± 39% and 174 ± 24% of that in controls respectively, n=9, $p<0.05$). Further the ratio of intracellular to total expression of GluA1 (0.45 ± 0.17 vs 0.84 ± 0.28, n=10, $p<0.05$) and GluA2 (0.34 ± 0.07 vs 0.72 ± 0.15, n=9, $p<0.05$) subunits in PMSG β-HCG-treated animals was lower than that in controls, suggestive of increased cell surface expression. Progesterone treatment (50 mg/kg) for 2 days also increased the GluA1 and GluA2 subunit expression (134 ± 8% and 149 ± 17%, n=5, $p<0.05$). Preliminary studies found that blocking the PRs with RU-486 (10 mg/kg) prevented the increase in GluA1 and GluA2 subunit expression in progesterone-treated animals. Whether estrus cycle-associated fluctuations in progesterone levels influenced the AMPAR subunit expression was also determined. Using the cytology of vaginal smears obtained daily, the animals were monitored for one estrus cycle and hippocampi were isolated during the next cycle. The expression of GluA1 and GluA2 subunits was higher in the animals in estrus and diestrus stages than that in the animals in proestrus. Thus progesterone appears to increase AMPAR-mediated excitatory neurotransmission through the activation of PRs.

Disclosures: S. Joshi: None. **J. Kapur:** None. **C. Passmore:** None. **J. Williamson:** None. **K. Rajasekaran:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

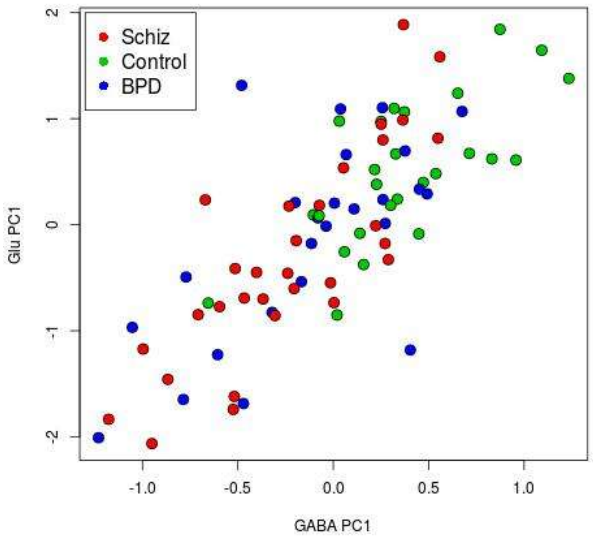
Program#/Poster#: 782.02/B22

Topic: B.02. Ligand-Gated Ion Channels

Title: Glutamate and GABA-A receptor mRNA levels in human brain co-vary homeostatically and low values characterize psychosis

Authors: *M. REIMERS, X. CHEN;
Virginia Inst. of Psychiatric and Behavioral Genet., RICHMOND, VA

Abstract: Inhibitory neurotransmission is crucial for brain function, and GABA-A receptors are thought to be critical for maintaining the balance between excitation and inhibition in the brain. Nevertheless an investigation into four human brain gene expression data sets (Kang et al, Nature, 2011, Gibbs et al PLoS Genetics, 2011, and two unpublished data sets) reveals consistently very large (at least 20-fold) differences across neurologically normal human brains in expression of the GABR genes coding for the main components of the GABA-A receptors. A comparative study of these four data sets indicates highly consistent estimates of GABR mRNA level variability, and highly consistent correlations among expression of different receptor components. Receptor components of the same type co-vary positively across samples, indicating that they cannot be substituting for one another to conserve function. Glutamate receptors vary less (at most ten-fold); nevertheless in all data sets the first PC of levels of Glutamate receptor mRNAs are highly correlated (>70%) with the first PC of GABR expression levels. In the Stanley Brain Series, aggregate GABR expression scores of more than half of the cortical samples from schizophrenic individuals were in the lowest 5% of the normal range (see Figure). Bipolar individuals also more commonly had lower scores. Similar results were obtained in an independent sample from hippocampus. These results confirms a variety of investigations suggesting lower inhibitory activity in psychosis.



Disclosures: **M. Reimers:** None. **X. Chen:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.03/B23

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NICMHHD MD00232

NSF HBCU-UP

Title: A methyoxypiperidine differentially interacts at αβ and αβγ GABAA receptors

Authors: *D. B. WILLIAMS, J. P. CLAVIJO, D. T. PARKER, J. J. KEITH;
Dept Life Sci., Winston-Salem St Univ., WINSTON SALEM, NC

Abstract: We previously reported a novel di-fluoride methoxypiperidine, WSS-2, that showed strong agonistic effects at α1β2γ2s GABAA receptors. At that isoform, WSS-2 could induce near maximal (1 mM GABA) current with nM EC50's in a two site model. We investigated if these properties would be affected by substituting the α1 subunit with the α2. We chose α2 because we were interested in treatments for cocaine addiction, and α2 may be important in such as it is highly expressed in the nucleus accumbens. We expressed α2β2γ2s receptors in Xenopus oocytes and measured GABA or WSS-2 induced currents using two-electrode voltage clamp at -60 mV. Compounds were applied for 30 s (or until peak current seen) and washed out with buffer for 3-5 min. At the α2β2γ2s isoform, WSS-2 also demonstrated nM affinity and a large maximal current in a two site model. The two site models of both the α1 and α2 isoforms gave extremely low Hill numbers; since the subunit mRNA was injected at a 1:1:1 ratio, we could have had a mixture of αβ and αβγ receptors. Therefore the dose response curves were repeated for both α1 and α2 containing forms, injecting α and β 1:1, and in different cells α, β, γ in a 1:1:5 ratio. At both α1β2 and α2β2 isoforms, WSS-2 induced a one site dose response curve, with EC50's near 5 nM. Maximal currents were about 99% for α1β2 and 68% for α2β2. Hill numbers were 2.27 and 2.64 respectively, more in line with reported piperidine responses. However, when the γ subunit was added at 5x the α or β, a biphasic effect was seen. The first effect was stimulatory, with EC50's approximately 1 nM, maximal currents estimated at 80%, and Hill numbers of 1.4 for both α1β2γ2s and α2β2γ2s. The affinity and maximal effect are stronger than seen with most GABA-acting piperidines. The second effect of WSS-2 at these 1:1:5 receptors was inhibitory; higher concentrations of WSS-2, such as 50 nM and 100 nM, caused the WSS-2 current to disappear, with IC50's of 52 and 14 nM for α1 and α2 containing receptors respectively. In general, WSS-2

had slightly higher affinities at α2 containing forms than α1 isoforms when γ was present. WSS-2 does seem to act on at least two sites, with the presence of a γ subunit causing WSS-2 to act antagonistically at one of those sites. Based in the differences in affinity with α1 and α2, and the antagonistic effect seen in receptors injected with 1:1:5 α:β:γ, we speculate that one of the sites WSS-2 binds could be the benzodiazepine site. A compound based in WSS-2 could form the basis of a high affinity GABA ligand, either a probe or drug, one that could target αβ receptors, or be used to differentiate αβ and αβγ receptors. It cannot yet be used as a treatment for drug addiction, due to these mixed GABA effects, and high affinity for the DAT.

Disclosures: D.B. Williams: None. **J.P. Clavijo:** None. **D.T. Parker:** None. **J.J. Keith:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.04/B24

Topic: B.02. Ligand-Gated Ion Channels

Support: CONACYT 131778 to JMA

Title: Molecular cloning of a GABA receptor subunit from crayfish and voltage-clamp analysis of an homo-oligomeric receptor expressed in HEK-293 cells

Authors: E. N. JIMÉNEZ-VÁZQUEZ[1], C. E. DÍAZ-VELÁSQUEZ[2], *J. M. ARIAS[2], U. GARCÍA[1];
[1]Fisiología, Biofísica y Neurociencias, Ctr. de Investigación y Estudios Avanzados - IPN, México, Mexico; [2]Programa de Neurociencias - UIICSE, FES Iztacala -UNAM, Tlalnepantla De Baz, Mexico

Abstract: In vertebrate central nervous system, GABA acts as inhibitory neurotransmitter by binding to two general classes of receptors: chloride ligand-gated ion channels complex GABAARs and metabotropic GABABRs that are linked via trimeric G-proteins to potassium channels. However, in invertebrates GABA can activate inward cationic currents through an homo-oligomeric receptor as has been described in the nematode *C. elegans*, where the central ionic conduction pore corresponds to an amino acidic sequence that permits the cationic flux. Another kind of cation-selective heteromultimeric receptor formed by the combination of two ligand-gated channel subunits (LCCH3 and GRD) was described in the fruit fly D. melanogaster. Similarly, in the x-organ neurons of the crayfish Procambarus clarkii we have identified two

GABA-gated currents: an early transient inward current depending on the extracellular sodium concentration, and other one generated by chloride ions. Both currents are activated by muscimol and blocked by picrotoxin, while cis-aminocrotonic acid only activates the chloride current. A cDNA encoding an ionotropic GABAR subunit was isolated from these neurons and transiently transfected into HEK 239T cells. Pharmacologically this subunit forms an anionic ligand-gated GABAR activated by muscimol and cis-aminocrotonic acid, and blocked by picrotoxin but not by bicuculline. Clearly, it becomes necessary to find out which type of subunit could help us to explain the cation-dependent current.

Disclosures: **E.N. Jiménez-Vázquez:** None. **C.E. Díaz-Velásquez:** None. **U. García:** None. **J.M. Arias:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.05/B25

Topic: B.02. Ligand-Gated Ion Channels

Support: Albany Medical College

Title: Modulation of GABA(A) receptors by dopamine

Authors: *P. HOERBELT, M. W. FLECK;
Ctr. for Neuropharm. and Neurosci., Albany Med. Col., Albany, NY

Abstract: GABA(A) receptors are pentameric chloride channels and targets for benzodiazepines, barbiturates and anesthetics. They are made of various combinations of 19 subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, and ρ1-3). Certain GABA(A)R subtypes have been shown *in vitro* to be directly activated or modulated by histamine. Related Cys-loop chloride channels in invertebrates are directly activated by histamine or other biogenic amines, including dopamine, octopamine, serotonin, tyramine, choline and acetylcholine. However, the extent to which biogenic amines can modulate the large class of GABA(A)R subtypes in mammals is underexplored. Based on homology to invertebrate receptors, we hypothesized that biogenic amines would directly activate or modulate GABA(A)R. We used whole-cell recording and fast perfusion in HEK 293 cells to study the effects of biogenic amines on common and uncommon assemblies of recombinant rat GABA(A)R subunits. Dopamine directly activated GABA(A)R currents (EC50 ≈ 700 μM), an effect that was concentration-dependent and blocked by the

GABA(A)R antagonist, bicuculline. Dopamine currents were consistently much smaller than GABA-evoked currents in the same cells, suggesting low efficacy. Dopamine activation was strictly linked to the presence of γ subunits, and was also seen in cells containing only β/γ subunits (which lacked α). Other tested biogenic amines (including epinephrine, norepinephrine, tyramine, choline and acetylcholine) did not activate GABA(A)R. In conclusion, dopamine directly and selectively activates γ subunit-containing mammalian GABA(A)R with low potency and efficacy. Whether such effects are present in brain receptors, are more pronounced with other biogenic amines, or are linked to mammalian behaviors, remain to be determined. Our results indicate that drug discovery and development efforts would benefit by considering GABA(A)R as potential targets for novel and selective dopamine-like drugs. The therapeutic potential includes treatment for epilepsy, anxiety, insomnia, Parkinson's disease, depression, schizophrenia, or other motivational/movement disorders.

Disclosures: P. Hoerbelt: None. **M.W. Fleck:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NINDS Grant NS075245

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Veterans Affairs Medical Research Funds

Z.P. and X.T. contributed equally to this work.

Title: Ectopic expression of GABAA receptor subunits increases tonic inhibition in somatostatin neurons in the hilus of the dentate gyrus

Authors: Z. PENG[1], X. TONG[1], M. WALLNER[2], N. ZHANG[1], Y. CETINA[1], T. S. OTIS[1], *C. R. HOUSER[1];

[1]Dept. Neurobiol, David Geffen Sch. of Med. at UCLA, Los Angeles, CA; [2]Dept. Mol. and Med. Pharm, UCLA, Los Angeles, CA

Abstract: Somatostatin (SOM) neurons in the dentate hilus are highly vulnerable to excitotoxic damage, but the reasons for such vulnerability remain unclear. Our recent immunohistochemical studies have shown that hilar SOM neurons exhibit very little labeling for the δ subunit of the GABAA receptor (GABAAR), and subsequent electrophysiological studies have demonstrated that tonic inhibition is also very low in these neurons. As tonic inhibition is recognized as a key regulator of neuronal excitability, these observations have led to the suggestion that tonic inhibition could play a role in protecting such neurons from excitotoxic damage. The current studies tested the hypothesis that expressing exogenous GABAAR subunits, either the α6 subunit that is normally absent from the forebrain but mediates tonic inhibition in cerebellar granule cells or the δ subunit that mediates tonic inhibition in other regions of the dentate gyrus, could form functional receptors and increase tonic inhibition in hilar SOM interneurons. Utilizing SOM-Cre mice and Cre-dependent adeno-associated virus (AAV) vectors, we were able to deliver ectopic GABAARs α6 or δ subunit selectively to SOM neurons in the hilus. Cre-recombinase inducible GFP tagged GABAAR subunits (α6 and δ) were cloned into AAV vectors (AAV-DIO-GABRα6- or δ-EGFP) and assembled into AAV particles (Stanford vector core). One month following stereotaxic injections into the hilar region of SOM-Cre mice, confocal and electron microscopy demonstrated robust expression of GFP in hilar SOM neurons. Immunohistochemical studies with subunit-specific GABAAR antisera demonstrated strong labeling of either α6 or δ subunits in GFP-labeled hilar neurons. Tonic currents were measured in transfected hilar interneurons at 3 months of age (one month post transfection). We found a robust increase of tonic inhibition in both α6-Cre GFP and δ-Cre GFP transfected SOM hilar neurons (51.5 ± 7.9 pA, n=15; 27.8 ± 4.9 pA, n=16 respectively) compared to control hilar neurons (5.1 ± 1.5 pA, n=7 in non-injected SOM-Cre Ai9 mice; 3.0 ± 0.4 pA, n=13 in GFP virus delivered SOM-Cre mice, $p < 0.01$). Furthermore, we tested sensitivity to the δ subunit-selective agonist THIP (1 μM) and found that it induced larger inward current in both α6 and δ subunit transfected SOM hilar neurons relative to control (α6: -30.1 ± 4.3 pA, n=7; δ: -20 ± 2.4 pA, n=7; GFP: -4.2 ± 0.6 pA, n=6). Further experiments will determine how ectopic α6 and δ subunits of GABAARs in SOM hilar neurons affect hippocampal neuronal circuits and test the hypothesis that increased tonic inhibition might protect vulnerable neuronal subtypes that are lost during severe seizure activity or ischemic insult.

Disclosures: Z. Peng: None. **X. Tong:** None. **M. Wallner:** None. **N. Zhang:** None. **Y. Cetina:** None. **T.S. Otis:** None. **C.R. Houser:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.07/B27

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR

NSERC

Title: Input-specific expression of the alpha5 GABAA receptor subunit in hippocampal interneurons

Authors: E. MAGNIN, O. CAMIRE, *L. TOPOLNIK;
CRIUSMQ, Laval Univ., Quebec, QC, Canada

Abstract: Synaptic expression of the alpha 5 GABAA receptor subunit (α5-GABAAR) has been reported in hippocampal CA1 inhibitory interneurons. However, the types of synapses that express α5-GABAAR and its functional role have not been investigated. Using a combination of whole-cell patch-clamp recordings, optogenetics and immunohistochemistry, we examined the synapse-specific expression of the α5-GABAAR and its modifications in the animal model of temporal lobe epilepsy (TLE). Our data showed that both the α5-GABAAR and its anchoring protein radixin exhibit a strong colocalisation with a vesicular GABA transporter in hippocampal CA1 oriens-alveus (O/A) interneurons. Moreover, inhibitory postsynaptic currents evoked in interneurons by selective activation of calretinin-positive (CR+) interneuron-specific cells in CR-Cre mice were decreased in the presence of the α5-GABAAR inverse agonists. Synaptic expression of the α5-GABAAR was revealed in different interneuron types, including oriens-lacunosum moleculare cells, bistratified cells, basket cells and oriens-oriens interneurons. Finally, a rapid decrease in the α5-GABAAR and radixin expression was observed in pilocarpine model of TLE, pointing to a significant disinhibition of O/A interneurons. Our data showed that the α5-GABAAR is expressed at inhibitory synapses formed at different types of interneurons by the local inhibitory input from CR+ cells. Moreover, the rapid decline of the α5-GABAAR in hippocampal interneurons during TLE may contribute to their disinhibition and hyperexcitability with consequences for network activity.

Disclosures: E. Magnin: None. **L. Topolnik:** None. **O. Camire:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.08/B28

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01NS038752

Title: RNF34 interacts with and promotes GABAA receptor degradation via ubiquitination of the γ2 subunit

Authors: *H. JIN, T.-T. CHIOU, C. P. MIRALLES, A. L. DE BLAS;
Univ. of Connecticut, Storrs, CT

Abstract: $GABA_A$ receptors ($GABA_A$Rs) mediate the majority of fast synaptic inhibitory neurotransmission in the brain. The efficacy of GABAergic synaptic inhibition depends on the number of functional $GABA_A$Rs localized in the postsynaptic membrane. It has been shown that GABAergic synaptic inhibition can be regulated by ubiqutination of the β3 or γ2 subunits in an activity-dependent manner (Saliba et al., 2007; Arancibia-Cárcamo et al., 2009). However, the specific E3 ubiquitin ligase(s) that mediate(s) this post-translational modification remains to be identified. We have found that RNF34, which is a RING-domain E3 ubiquitin ligase, specifically interacts with the γ2 subunit of the $GABA_A$Rs in the rat brain. The interaction occurs between the C-terminal RING domain of RNF34 and the large intracellular loop of the γ2 $GABA_A$R subunit (γ2IL), as shown by yeast-two-hybrid and *in vitro* pull-down assays. In brain extracts, RNF34 co-immunoprecipitates with assembled $GABA_A$Rs. A feature of the RING-domain E3 ubiquitin ligases is that they bind to the substrates that they ubiquitinate. Therefore, we investigated if RNF34 ubiquitinates and regulates the expression of the γ2 subunit and γ2-containing $GABA_A$Rs. In co-transfected HEK293 cells, RNF34 reduces the expression of the γ2 $GABA_A$R subunit by increasing the ratio of ubiquitinated/non-ubiquitinated γ2. Mutating several lysines of the γ2IL into arginines (γ2 8KR, 9KR, or 10KR) makes the γ2 subunit resistant to RNF34-induced degradation. RNF34 also reduces the expression of the γ2 subunit when the α1 and β3 subunits are co-assembled with γ2. This effect is partially reversed by Leupeptin or MG132, indicating that both the lysosomal and proteasomal degradation pathways are involved. Immunofluorescence of hippocampal neurons shows that RNF34 forms clusters and that a subset of these clusters is associated with GABAergic synapses. RNF34 is not expressed until the second postnatal week of rat brain development, being highly expressed in some interneurons. Overexpression of RNF34 in hippocampal neurons decreases both the density of γ2 $GABA_A$R clusters and the number of GABAergic contacts that these neurons receive. Knocking down endogenous RNF34 with shRNA leads to increased γ2 $GABA_A$R cluster density and increased GABAergic innervation. The results indicate that RNF34 i) interacts with and ubiquitinates the γ2 $GABA_A$R subunit promoting $GABA_A$R degradation and ii) regulates postsynaptic γ2

$GABA_AR$ clustering and the stability of GABAergic synapses. **Reference** 1, Arancibia-Cárcamo et al., (2009) Proc Natl Acad Sci USA. 106:17552-17557. 2, Saliba et al., (2007) J Neurosci 27:13341-13351.

Disclosures: H. Jin: None. **T. Chiou:** None. **C.P. Miralles:** None. **A.L. De Blas:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.09/B29

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant MH100561

Title: Synaptic pruning in the male hippocampus during adolescence: Reversal by knock-out of the GABAA receptor α4 subunit

Authors: *J. PARATO, S. SMITH;
SUNY Downstate, Brooklyn, NY

Abstract: Synaptic pruning during adolescence is a well-known phenomenon (Huttenlocher 1979; Yildirim et al., 2008). This decrease in spine density at puberty is thought to be important for normal cognition because it is abnormal in neurodevelopmental disorders such as autism and schizophrenia (van Spronsen and Hoogenrad, 2010). Although the mechanism which underlies this process is unknown, α4βδ $GABA_A$ receptors (GABARs) play a role in pubertal synaptic pruning in the CA1 hippocampus and dentate gyrus of the female mouse. NMDA receptor activation is known to maintain spine stability (Ultanir et al., 2007), and the presence of α4βδ GABARs, which increase expression on the dendritic spine of CA1 pyramidal cells at puberty, inhibits NMDA receptor activation by a shunting inhibition (Shen et al., 2010). Thus, in the current study, we tested the hypothesis that α4βδ GABARs also play a role in synaptic pruning in the CA1 hippocampus and dentate gyrus (DG) of male mice. To this end, we examined spine density at the onset of puberty (Pub, PND 35) compared to post-puberty (Post, PND 56) in male mice. Brains were processed using the Golgi method and spine counts obtained using a Nikon Eclipse Ci-L microscope. Our data show that, in the male mouse, spine density decreases across adolescence in the CA1 hippocampus (Pub, 1.2 spines/υm, Post, 0.6 spines/υm, $P<0.05$) as well as in the dentate gyrus (Pub, 1.7 spines/υm, Post, 1.2 spines/υm, $P<0.05$). Spine density in both regions was significantly greater in the post-pubertal α4-/- (CA1, α4-/-, 1.1 spines/υm, $P<0.05$;

DG, α4-/-, 1.8 spines/υm, P<0.05). These data suggest that α4βδ GABAR expression plays a role in adolescent synaptic pruning in the male as in the female. These findings may have relevance for neurodevelopmental disorders such as autism and schizophrenia where synaptic pruning is abnormal and abnormalities in the α4 gene are reported.

Disclosures: J. Parato: None. **S. Smith:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.10/B30

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM47969

Title: The neurosteroid pregnanolone enhances actions of etomidate as a positive allosteric modulator of α1β2γ2L γ-aminobutyric acid type A receptors

Authors: *G. AKK, P. LI, J. BRACAMONTES, B. MANION, J. STEINBACH, A. EVERS; Dept Anesthesiol Res. Unit, Washington Univ. Sch. Med., St. Louis, MO

Abstract: Many neurosteroids are known to potentiate electrophysiological responses of the γ-aminobutyric acid type A ($GABA_A$) receptor to the transmitter GABA, and to amplify behavioral responses mediated by the $GABA_A$ receptor. We have examined the ability of neurosteroids to modulate electrophysiological and behavioral responses to the allosteric activator etomidate. Whole-cell electrophysiological assays were conducted on rat α1β2γ2L $GABA_A$ receptors expressed in HEK 293 cells. The sedative activity of etomidate was studied in *Xenopus* tadpoles and mice. The effect of neurosteroid on etomidate-elicited inhibition of cortisol synthesis was determined in human adrenocortical cells. The neurosteroid pregnanolone (3α5βP) potentiated peak currents from $GABA_A$ receptors activated by GABA or etomidate. Coapplication of 1 μM 3α5βP shifted the whole-cell macroscopic concentration-response relationship for direct gating by etomidate in the absence of GABA from 218 to 2.8 μM. Further, coapplication of 100 nM 3α5βP reduced the EC_{50} for potentiation by etomidate of currents elicited by 0.5 μM GABA from 2.0 to 0.7 μM. In behavioral studies, 1 mg/kg of 3α5βP reduced the dose of etomidate required to produce loss of righting in mice (ED_{50}) from 0.19 to 0.01 mg/kg. In *Xenopus* tadpoles, exposure to 50 and 100 nM 3α5βP shifted the EC_{50} for loss of righting from 2.1 to 1.0 or 0.4 μM, respectively. Finally, exposure to 3α5βP had no effect on inhibition of cortisol

synthesis by etomidate. We conclude that potentiating neurosteroids act similarly on orthosterically and allosterically activated $GABA_A$ receptors. Coapplication of neurosteroids with etomidate can significantly reduce dosage requirements for the anesthetic, and is a potentially beneficial combination to reduce undesired side effects.

Disclosures: **G. Akk:** None. **P. Li:** None. **J. Bracamontes:** None. **B. Manion:** None. **J. Steinbach:** None. **A. Evers:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. F. Consulting Fees (e.g., advisory boards); Sage Therapeutics.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.11/B31

Topic: B.02. Ligand-Gated Ion Channels

Title: Extracts of Passiflora incarnata modulate the gamma-aminobutyric acid type A receptor

Authors: G. HAMERSKY, M. E. QUACH, T. MCKECHNIE, C. M. INGERSOLL, *J. A. TEISSERE;
Muhlenberg Coll, ALLENTOWN, PA

Abstract: The GABA-induced chloride current (I_{GABA}) of gamma-aminobutyric acid type A receptors ($GABA_ARs$) is sensitive to allosteric potentiation by benzodiazepine binding to a well-characterized high affinity site at the α-γ subunit interface of this receptor. Extracts of passionflower (Passiflora incarnata) provoke anxiolytic and hypnotic behavioral effects in vertebrates that are consistent with benzodiazepine-like allosteric modulation of the $GABA_AR$ and are available commercially as medicinal phytoextracts indicated for anxiety and sleeplessness. However, the specific molecular target(s) of Passiflora extract, as well as the putative identities of its pharmacologically active ingredients, remain unknown. We used two-electrode voltage clamping of Xenopus laevis oocytes expressing α1β2γ2 and α1β2 $GABA_ARs$ to demonstrate directly that Passiflora extracts potentiate I_{GABA} in a dose-dependent fashion. Bath application of the extract in the presence of EC_{15} concentrations of GABA elicited a dramatically potentiated response of I_{GABA} in comparison to GABA alone regardless of whether the γ2 subunit was assembled in the receptor complex. Applications of Passiflora extract in the absence of GABA demonstrated a robust GABA-mimetic ability of these extracts to directly gate the receptor. Co-application with gabazine, a competitive GABA-site antagonist, did not abolish the

ability of Passiflora extracts to directly activate the receptor. Last, gas chromatography-mass spectrometry (GC-MS) of the Passiflora extract demonstrated that the concentration of GABA contained in the extract was not sufficient to explain the robust potentiation of I_{GABA} we observed. Taken together, our results suggest that Passiflora extracts potentiate and directly activate $GABA_A$Rs in a manner distinct from benzodiazepines and GABA.

Disclosures: G. Hamersky: None. **M.E. Quach:** None. **T. McKechnie:** None. **C.M. Ingersoll:** None. **J.A. Teissere:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.12/B32

Topic: B.02. Ligand-Gated Ion Channels

Title: $GABA_A$ receptor agonist activity and anxiolytic effects of organic acids

Authors: *Y. YAMADA[1], H. IZU[2];
[1]Dept of Biotech and Chem Fac of Engin. Kinki Univ., Higashi-Hiroshima, Japan; [2]Natl. Res. Inst. of Brewing, Higashi-Hiroshima, Japan

Abstract: γ-Amino butyric acid receptor ($GABA_A$ receptor) receptors are widely distributed in the central nervous system and were consisted with two α, β, and one of γ subunits. $GABA_A$ receptors can mediate inhibitory neurotransmission by hyperpolarizing the membrane of the postsynaptic neuron, resulting in an inhibitory postsynaptic potential that decreases the probability of firing. The chloride channels can be opened by GABA and are a target for a variety of important drugs such as benzodiazepine, barbiturate, neuroactive steroids, anti-convulsants and anaesthetics. Deficits in the functional expression of $GABA_A$ receptors are critical in epilepsy, anxiety disorders, cognitive deficits schizophrenia, depression and substance abuse. For improving these diseases, it is expected to develop therapeutic agents that are effective to the receptors. We have investigated the effect of some compounds in Japanese sake on the response of $GABA_A$ receptors which were expressed in *Xenopus* oocytes because it was known for long time in Japan that the sake has a relaxation effect. The sakes of six brands were fractionated to four fractions by ion-exchange chromatography. Fractionated samples were lyophilized for removing the alcohol and volatile compounds. One of four fractions (A fraction) contained various organic acids but not GABA showed the agonist activities of $GABA_A$ receptor. A fractions were analyzed by CE-TOFMS (Human Metabolome Technologies, inc., Tsuruoka,

Japan) and 64 compounds were identified. 13 out of 64 components showed $GABA_A$ receptor agonist activities. 3 (lactic acid, pyruvic acid and gluconic acid) out of 13 compounds showed high agonist activities and EC_{50} values of GABA, lactic acid, pyruvic acid, and gluconic acid were 65.1 μM, 16 μM, 210 μM, and 257 μM, respectively. The effects of intraperitoneal injection of $GABA_A$ receptor agonists on anxiety have been investigated by an elevated plus-maze test using mice. In the test, significant anxiolytic effects were observed in the presence of lactic acid, pyruvic acid, and gluconic acid. Our experiments suggest that the anti-anxiety influence of organic acid is in due to the effects of these compounds on $GABA_A$ receptor.

Disclosures: Y. Yamada: None. **H. Izu:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

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Program#/Poster#: 782.13/B33

Topic: B.02. Ligand-Gated Ion Channels

Support: Medical Research Council Grant G1000008

Wellbeing of Women Grant RG1265

Tenovus Scotland Grant

Anonymous Trust Grant

Title: The effects of early-life stress on nucleus accumbens $GABA_A$ receptor function and cocaine-mediated behaviour

Authors: *S. J. MITCHELL[1], E. P. MAGUIRE[1], B. G. GUNN[1], L. CUNNINGHAM[1], M. B. HERD[1], S. L. KING[2], D. STEPHENS[2], J. D. SWINNY[3], D. BELELLI[1], J. J. LAMBERT[1]; [1]Div. of Neurosci., Dundee Univ., Dundee, United Kingdom; [2]Sch. of Psychology, Univ. of Sussex, Brighton, United Kingdom; [3]Sch. of Pharm. and Biomed. Sci., Univ. of Portsmouth, Portsmouth, United Kingdom

Abstract: The nucleus accumbens (NAc) consists primarily of GABAergic neurons, and is an essential integration site within the natural reward-pathway, which is 'hijacked' by drugs of abuse. We have previously shown for accumbal medium spiny neurons (MSNs) that α2-subunit containing $GABA_A$ receptors (α2-$GABA_A$Rs), mediate phasic inhibition and that their genetic

inactivation ($\alpha2^{-/-}$) abolishes behavioural sensitisation to cocaine. Recently, linkage association studies in humans revealed a genetic association of GABRA2 haplotypes with cocaine addiction, which was evident only in individuals who had experienced childhood trauma. Collectively, these studies indicate an association of childhood trauma, drug addiction and the GABARA2 haplotype. Here, we have utilised a mouse model of early life stress (ELS) to investigate the impact of early-life adverse experiences on cocaine-induced behaviours and the putative role of NAc α2-$GABA_A$Rs in this interaction. A fragmented maternal care paradigm was implemented to produce ELS. Adult wild type (WT) control mice received a daily *i.p* injection of cocaine (10 mg/kg) for 10 days, which resulted in behavioural sensitization, manifest as enhanced cocaine-induced increase of locomotor activity *cf.* saline-injected controls. In contrast, adult ELS mice did not sensitise to cocaine, but exhibited a significantly increased acute locomotor response to a single cocaine injection (10 mg/kg) *cf.* WT control. Interestingly, but in contrast to a previous report, both features were exhibited by non-stressed $\alpha2^{-/-}$ mice. Whole-cell voltage-clamp recordings of NAc MSNs of adult mice, previously exposed to the ELS paradigm, revealed a significant reduction in the amplitude and frequency of miniature inhibitory post-synaptic currents (mIPSCs) *cf.* WT controls. Non-stressed $\alpha2^{-/-}$ mice exhibited similar alterations of mIPSCs properties *cf.* WT controls. Complementary immunohistochemical analysis revealed a significant and selective reduction of $GABA_A$R α2, but not α1 subunit staining in the NAc core of adult ELS *cf.* WT control mice, indicating that ELS selectively decreases α2-$GABA_A$R expression. In conclusion, ELS and $\alpha2^{-/-}$ mice share a selective α2-$GABA_A$R mediated reduction of inhibitory phasic transmission, which is accompanied under these experimental conditions for both models by an increased acute cocaine locomotor response and blunting of further behavioural sensitisation to cocaine. Collectively, these findings complement the human studies and suggest that such mouse models may prove useful in permitting a better understanding of the complex association of cocaine abuse, childhood trauma and the GABRA2 gene.

Disclosures: **S.J. Mitchell:** None. **E.P. Maguire:** None. **B.G. Gunn:** None. **L. Cunningham:** None. **M.B. Herd:** None. **S.L. King:** None. **D. Stephens:** None. **J.D. Swinny:** None. **D. Belelli:** None. **J.J. Lambert:** None.

Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 782.14/B34

Topic: B.02. Ligand-Gated Ion Channels

Support: Conacyt 128125

Title: Modulation of spinal motoneuron excitability by extrasynaptic ααalpha4/6 GABAA receptors

Authors: *R. DELGADO-LEZAMA[1], C. ANDRÉS[1], J. AGUILAR[1], R. GONZÁLEZ-RAMÍREZ[2], D. ELÍAS[3], R. FELIX[4];
[1]Dept. of Physiology, Biophysics and Neurosci., CINVESTAV-IPN, Mexico D F, Mexico; [2]Dept. de Biología Mol. e Histocompatibilidad, Hosp. Gen. "Dr. Manuel González", México, Mexico; [3]Dept. de Bioelectrónica, [4]Dept. de Biología Celular, Cinvestav-IPN, México D.F., Mexico

Abstract: Motoneurons are provided with a repertoire of ionotropic and metabotropic receptors as well ion channels that contribute to modulate its excitability. Between the receptors expressed are the GABAA receptors which are known to mediate two types of inhibition in the mature nervous system, phasic and tonic, produced by synaptic and extrasynaptic receptors, respectively. In a previously we have shown that ambient GABA may activate tonically the extrasynaptic receptors in motoneurons producing a persistent inhibitory current. When these receptors were blocked by high concentration of picrotoxin or bicucullin, the EPSPS evoked in motoneurons and the monosynaptic reflex activated by dorsal root stimulation was facilitated. These results suggested that extrasynaptic GABAA receptors might have an important role in motor control. However, functional identification of the α subunits conforming GABAA receptors in spinal motoneurons is missing. In this work we investigated the expression and function of α4/6GABAA receptors in adult turtle motoneurons. By combining electrophysilogical, inmunohistological and molecular biology techniques with pharmacological tools we demonstrate that α4/6 subunit-containing GABAA receptors are expressed in these cells and produce a tonic inhibitory current that modulates their input resistance and excitability.

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Poster

782. GABAA Receptor Pharmacology and Function

Location: Halls A-C

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH (NINDS) Grant NS 40109-06

American Epilepsy Society Post-doctoral fellowship

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The Japan Foundation for Pediatric Research

Manton Center for Orphan Disease Research

Title: Local impermeant anions establish the neuronal chloride concentration

Authors: *J. C. GLYKYS[1], V. DZHALA[1], K. EGAWA[1], T. BALENA[1], Y. SAPONJIAN[1], K. V. KUCHIBHOTLA[3], B. J. BACSKAI[1], K. T. KAHLE[2], T. ZEUTHEN[4], K. J. STALEY[1]; [1]Neurol., Massachusetts Gen. Hosp., CHARLESTOWN, MA; [2]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [3]Skirball Inst. for Biomolecular Med., NYU Sch. of Med., New York, NY; [4]Cell. and Mol. Med., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Neuronal chloride concentration $[Cl^-]_i$ is an important determinant of $GABA_A$ receptor ($GABA_AR$)-mediated inhibition and cytoplasmic volume regulation. Equilibrative cation-chloride cotransporters (CCC) move Cl^- across the membrane, but accumulating evidence suggests CCCs are not the sole determinant of $[Cl^-]_i$. To identify other factors, we tested the effects of pharmacological CCC blockade, osmotic challenge, extracellular matrix disruption and cytotoxic alteration of volume constraints on neuronal $[Cl^-]_i$ using two-photon imaging of brain slices from Clomeleon mice (genetically encoded ratiometric fluorophore sensitive to changes in $[Cl^-]_i$). Our results demonstrated that cytoplasmic impermeant anions ($[A]_i$) and polyanionic extracellular matrix glycoproteins ($[A]_o$) constrain the local $[Cl^-]$. In healthy neurons, CCC inhibition had modest effects on $[Cl^-]_i$ and neuronal volume, but substantial changes were produced by alterations of the balance between $[A]_i$ and $[A]_o$. NKCC1 inhibition reduced $[Cl^-]_i$ in pathological conditions, including seizure activity. In physiological conditions, CCC are important elements of Cl^- homeostasis, but local impermeant anions determine the homeostatic set-point for $[Cl^-]$, and hence, neuronal volume and the polarity of local $GABA_AR$ signaling.

Disclosures: J.C. Glykys: None. **V. Dzhala:** None. **K. Egawa:** None. **T. Balena:** None. **Y. Saponjian:** None. **K.V. Kuchibhotla:** None. **B.J. Bacskai:** None. **K.T. Kahle:** None. **T. Zeuthen:** None. **K.J. Staley:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.01/B36

Topic: B.08. Synaptic Plasticity

Title: Assessing transcriptional and post-transcriptional mechanisms in fast spiking cell homeostatic plasticity

Authors: *S. M. O'TOOLE[1], P. T. TANEJA[2], S. B. NELSON[2];
[1]Brandeis Univ., Waltham, MA; [2]Brandeis, Waltham, MA

Abstract: Cortical neurons respond to changes in activity through multiple cellular and synaptic forms of homeostatic plasticity, and these plasticity mechanisms can operate differently for excitatory and inhibitory neurons. To study homeostatic mechanisms operating in fast-spiking (FS) interneurons, we crossed *Pvalb-IRES-Cre* mice to a cre-dependent reporter strain. We then generated organotypic slice cultures from these mice at post-natal day 9. From equivalent post-natal day 18 to 24 cultures were incubated in tetrodotoxin (TTX) to halt action potential firing across the slice. At this point we assessed the firing properties of the FS cells by measuring FI curves and examined the miniature excitatory postsynaptic currents (mEPSCs). TTX treatment led to a robust increase in mini frequency as well as a decrease in the firing threshold. It's known that homeostatic mechanisms in excitatory neurons rely on both transcriptional and post-transcriptional changes. To determine how fast spiking cells respond at the transcriptional level, labeled FS cells were manually isolated and their RNA was extracted, amplified and used for the construction of deep sequencing libraries. Our examination of the transcriptome within these cells revealed a robust response to activity deprivation (n=8). Amidst the largest expression changes we found that *Crhbp* (FC=-35.6, $p=3.0*10^{-7}$), *NPY* (FC=-7.85,$p=3.4*10^{-6}$) and *VGF* (FC=-7.85,$p=1.8*10^{-9}$) were significantly down regulated in TTX while *Pea15a* (FC=5.82,$p=5.4*10^{-11}$) and *Plk3R1* (FC=3.91,$p=1.6*10^{-9}$) were upregulated. All of these genes have been implicated or closely connected to epileptic disorders supporting the validity of the data set. Furthermore, we verified CRHBP's drop in expression through immunohistochemistry. To begin to address how post transcriptional mechanisms may be involved in the FS cell's response to activity deprivation, we crossed our *Pvalb-IRES-Cre* strain with a $Dicer^{flx/flx}$ line. Upon conditional ablation of Dicer small RNA biogenesis is impaired. We found that both the increase in mEPSC frequency as well the shift in the FI curve were *Dicer* dependent. Ongoing work will attempt to form a connection between the transcriptional and post-transcriptional mechanisms underlying homeostatic plasticity in FS cells.

Disclosures: S.M. O'Toole: None. **P.T. Taneja:** None. **S.B. Nelson:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.02/B37

Topic: B.08. Synaptic Plasticity

Support: NIH F31 NS079124-01A1

Title: Target-cell dependent plasticity of inhibitory synapses in mammalian cortex

Authors: *Y. ESCOBEDO LOZOYA, Q. LEI, S. B. NELSON;
Brandeis Univ., Waltham, MA

Abstract: Homeostatic plasticity mechanisms are thought to promote proper balancing between cortical excitation and inhibition over neuronal circuit development and to maintain stable activity levels. Recruitment of homeostatic plasticity has often been studied after short-term (~24h) activity deprivation and has generally been found to be reversible. However, we were interested in investigating if prolonged activity deprivation (pAD) regimes might trigger irreversible recruitment of plasticity mechanisms which could possibly contribute to circuit disorders such as Epilepsy. Mouse coronal brain slices spanning somatosensory cortex were explanted at postnatal day (P)7-P10, placed on an interface culture chamber and allowed to develop *in vitro*. Previously, our work showed using simultaneous whole-cell recording of pyramidal (Pyr) neuron pairs in layer (L)5 of these slice cultures that 1) network activity spontaneously emerges after an age equivalent to P12; 2) activity is structured into a pattern of slowly alternating episodes (~0.1 Hz) reminiscent of 'Up' states seen in cortex during sleep or anesthesia 3)depriving activity by application of 0.4 μM TTX from EPD12 to 15 or EPD12-17 (pAD) increases average firing during 'Up' states and 'Up'-state frequency. These changes were not reversible within 5 days. Here, we focused on examining synaptic connectivity changes in Pyr and Fast-Spiking Parvalbumin-Expressing Interneurons (FS-I) by using mini excitatory or inhibitory post-synaptic current (mEPSC or mIPSC) recording. We found significant increases in the amplitude and frequency of mEPSCs received by L2/3 and L5 Pyr neurons in slice cultures subjected to pAD. We also found significant decreases in the amplitude and frequency of mIPSCs received by L5 Pyr. This suggests pAD promotes Pyr cell firing both by an increase in Pyr excitatory synaptic drive and a decrease on Pyr inhibitory synaptic drive, which might contribute to post-deprivation hyperactivity (PDHA). Next we investigated if FS-I firing and connectivity were also altered by pAD. Unlike the case for pyramids, FS-I firing frequency during 'Up-states' is not significantly increased after pAD. Most interestingly, mIPSC recordings on FS-I revealed that both mIPSC frequency and amplitude onto FS-I are largely increased by pAD. These results suggest that homeostatic plasticity rules for inhibitory synapses are different

depending on the post-synaptic target and suggest that an increase in inhibitory drive to FS-I might also contribute to PDHA. We are currently using histological methods to investigate if mEPSC and mIPSC frequency and amplitude changes reflect changes in synapse size or abundance.

Disclosures: Y. Escobedo Lozoya: None. **Q. Lei:** None. **S.B. Nelson:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.03/B38

Topic: B.08. Synaptic Plasticity

Title: Reciprocal regulation of gephyrin-mediated clustering of GABA type A receptors by palmitoylation and S-nitrosylation

Authors: *B. DEJANOVIC[1], G. SCHWARZ[2];
[1]Dept. of Chem. and Ctr. for Mol. Med. Cologne, Inst. of Biochem., Cologne, Germany; [2]Inst. of Biochem., Univ. of Cologne, Cologne, Germany

Abstract: Gephyrin, the principal scaffolding protein at inhibitory synapses, is essential for the clustering of glycine and GABA type A receptors (GABAARs). We have recently shown that postsynaptic clustering of gephyrin, an essential process for the formation and homeostasis of GABAergic synapses, is mediated by palmitoylation (Dejanovic et al. PLOS Biology, 2014). Another posttranslational modification that regulates gephyrin function is S-nitrosylation (Dejanovic & Schwarz, J. Neurosci., 2014). Gephyrin association with neuronal nitric oxide synthase (nNOS) and subsequent S-nitrosylation decrease the size of postsynaptic gephyrin clusters. Here we report that gephyrin palmitoylation and S-nitrosylation occur on the same cysteine residues, suggesting reciprocal regulation of gephyrin-mediated clustering of GABAARs. We found that decreased palmitoylation augmented gephyrin S-nitrosylation and vice versa ultimately modifying the size of GABAergic synapses. Thus, we provide a new regulatory mechanism of GABAergic synaptic plasticity mediated by competitive post-translational modifications of gephyrin.

Disclosures: B. Dejanovic: None. **G. Schwarz:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: NCCR Synapsy P28 Schneggenburger

EPFL funds

Title: Spike-timing dependent plasticity of fast-signaling inhibitory synapses in the input layers of auditory cortex

Authors: *E. D. VICKERS, R. SCHNEGGENBURGER;
BMI, EPFL, Lausanne, Switzerland

Abstract: In tonotopically organized auditory cortex, feedforward inhibition in thalamocortical input layers likely plays a critical role in controlling the window of temporal integration, frequency tuning, and gain of responses to sensory inputs. Feedforward inhibition largely relies on fast-spiking interneurons that express parvalbumin (PV-IN). *In vivo*, the delay between the arrival of excitation onto principal cells (PC), driven by glutamatergic thalamic afferents, and feedforward inhibition is only ~2 ms. Such a precise temporal relationship suggests that spike-timing dependent plasticity (STDP) could be present at inhibitory feedforward synapses. To test this idea, we performed paired whole-cell recordings of PV-INs and PCs in the input layers (L3/4) of mouse (P13 - 20) auditory cortex slices. PV-INs were identified by tdTomato fluorescence in PV-IRES-Cre / ROSA26-tdTomato mice. Brief current injection in presynaptic PV-INs triggered action potentials (APs), which evoked fast GABAergic IPSCs in PCs. These IPSCs had rapid rise times; high-frequency trains (10 - 50 Hz) caused depression, but IPSCs were reliably observed toward the end of trains, consistent with the previously described fast and reliable transmission properties of PV-IN output synapses. Connected pairs were subjected to an STDP induction protocol of 50 pre- and post-synaptic AP pairings at 0.2 Hz. We observed a robust STDP with potentiation of IPSCs (~60%) in the post- before pre- sequence, and depression in the pre- before post- sequence (~20%; absolute timing differences of 2 - 20 ms). Experiments with intracellular BAPTA showed that long-term potentiation of IPSCs (iLTP) depended on an increase in post-synaptic Ca^{2+}. Thus, iLTP may involve Ca^{2+} dependent release of a retrograde messenger. We speculate that STDP at inhibitory synapses in the input layers of the auditory cortex might initiate structural changes of inhibitory synapses, and thereby set the balance of excitation and inhibition in a fast feedforward circuit. This plasticity could contribute to sensory-experience dependent wiring decisions during critical periods of auditory cortex development.

Disclosures: **E.D. Vickers:** None. **R. Schneggenburger:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.05/B40

Topic: B.08. Synaptic Plasticity

Title: Compartment-specific regulation of interneuron connectivity

Authors: ***H. VOLKMER**, S. BEUTER, J. WUCHTER, S. KELLER, M. KRIEBEL;
NMI, Reutlingen, Germany

Abstract: Interneurons target different sub-cellular compartments of principal neurons. However, mechanisms that contribute to the compartmental stabilization of inhibitory synapses are poorly understood. In particular, different receptor tyrosine kinases are candidates for compartmental control via regulation of the postsynaptic scaffold protein gephyrin that is a crucial component of GABAA receptor clustering. We applied lentiviral shRNA expression combined with stereotactic injection into the dentate gyrus of adult rats to analyze the contribution of cell surface receptors to compartmental control of gephyrin clustering. Mechanistic insight is provided by Western Blot and histological analyis of hippocampal neurons. We show that EphA7 is implicated in the stabilization of GABAergic synapses in the perisomatic and proximal dendritic compartments of granular cells in the dentate gyrus. By contrast, stabilization of axo-axonic synapses relies on neurofascin that is connected to FGFR1 signaling. EphA7 signaling converges together with other signaling pathways induced by receptor tyrosine kinases on the activation of mTOR that is an interaction partner of gephyrin. Upon mTOR activation, gephyrin dissociates from mTOR and undergoes interaction with the GEF collybistin that is required for gephyrin translocation to the cell surface. In conclusion, compartmental control of GABAergic innervation on principle neurons might rely on the activity of specific receptor protein kinases that confer regulation of gephyrin clustering via mTOR.

Disclosures: **H. Volkmer:** None. **S. Beuter:** None. **J. Wuchter:** None. **S. Keller:** None. **M. Kriebel:** None.

Poster

783. Inhibitory Plasticity

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Program#/Poster#: 783.06/B41

Topic: C.06. Developmental Disorders

Support: Jerome Lejeune Grant 995-CA2012A

Title: Excitatory GABAergic transmission impairs synaptic plasticity and memory in Down syndrome

Authors: *A. CONTESTABILE, G. DEIDDA, M. PARRINI, S. NASKAR, I. FERNANDEZ BOZARTH, L. CANCEDDA;
Neurosci. and Brain Technol., Fondazione Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: Down syndrome (DS) is the most frequent genetic cause of intellectual disability, with DS patients displaying low intelligence quotient, learning deficits, and memory impairment particularly in hippocampus-related functions. Trisomic mouse models of DS reproduce the main cognitive disabilities of the human syndrome. In particular, Ts65Dn mice show impaired synaptic plasticity (i.e., long-term potentiation, LTP) as well as learning and memory deficits. Increased GABAergic transmission through Cl^--permeable $GABA_A$ receptors ($GABA_AR$) largely determines these deficits in DS mice. Indeed, LTP and cognitive impairment can be rescued reducing the magnitude of GABA-mediated signaling by treatment with $GABA_AR$ antagonists. Nevertheless, the efficacy of GABAergic transmission has never been directly assessed in DS. Here, we show that GABAergic signaling is mostly excitatory rather than inhibitory and the reversal potential for $GABA_A$-driven Cl^- currents (E_{Cl}) is depolarized in hippocampi from adult DS mice. Accordingly, expression of cation/Cl^- importer NKCC1 is increased in the hippocampus of trisomic mice and DS patients. Notably, NKCC1 inhibition by the FDA-approved drug bumetanide restores E_{Cl}, synaptic plasticity and hippocampus-dependent learning and memory in DS mice. Our findings demonstrate that GABA is overall excitatory in DS adult mice, and identify a new and safe therapeutic approach to rescue cognitive disabilities of DS patients.

Disclosures: **A. Contestabile:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional Application: US 61/919,195, 2013.. **G. Deidda:** None. **M. Parrini:** None. **S. Naskar:** None. **I. Fernandez Bozarth:** None. **L. Cancedda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional Application: US 61/919,195, 2013..

Poster

783. Inhibitory Plasticity

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Program#/Poster#: 783.07/B42

Topic: B.07. Synaptic Transmission

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IZKF Grant VP I

Werner Reichardt Centre Grant

Title: Depolarizing GABA orchestrates inhibition in developing mouse neocortex *in vivo*

Authors: K. KIRMSE[1], M. KUMMER[1], Y. KOVALCHUK[2], O. W. WITTE[1], O. GARASCHUK[2], *K. HOLTHOFF[1];
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Abstract: Whereas γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain, a large body of *in vitro* evidence suggests that GABAergic transmission is partially excitatory during early development. The latter is supposed to result from a comparatively high intracellular chloride concentration in immature neurons that renders GABA(A)-receptor mediated responses depolarizing. At present, however, the mode of GABA action in the intact developing mammalian brain remains controversial. We here combine two-photon Ca2+ imaging and electrophysiological techniques in spontaneously breathing, head-fixed neonatal mice to address the mode of GABA action *in vivo*. We provide evidence that GABA depolarizes a substantial fraction of immature neurons in the upper cortical plate. Our data further reveal that GABA spatiotemporally constrains the generation of spontaneous network activity in the occipital cortex. Thus, our data identify GABA as a dual depolarizing-inhibitory neurotransmitter in the immature neocortex *in vivo*.

Disclosures: **K. Kirmse:** None. **K. Holthoff:** None. **M. Kummer:** None. **O.W. Witte:** None. **Y. Kovalchuk:** None. **O. Garaschuk:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.08/B43

Topic: B.02. Ligand-Gated Ion Channels

Title: Postsynaptic modulation of GABAergic synaptic transmission by Kainate receptors

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Abstract: Presynaptic Kainate-type glutamate ionotropic receptors (KAR) have been reported to mediate either depression or facilitation of inhibitory transmission. Little attentions have been given to the postsynaptic modulation of GABAergic synaptic transmission by KARs. In the presence of the NMDAR antagonist, AP-5 (50 microM), AMPAR antagonist, GYKI53655 (50 microM), and TTX (1 microM), we tested the effect of the KAR antagonist, ATPA (10 microM), on $GABA_A$ receptor-associated channel-mediated Cl^- currents ($GABA_A$ current) in CA1 pyramidal neurons evoked by local application (puff) of GABA (50 microM). We found that activation of postsynaptic KARs reduced $GABA_A$ currents by desynchronizing $GABA_A$ channel openings and increasing intracellular [Cl^-]. ATPA attenuated desensitization of $GABA_A$ channels and prolonged the rise time and decay of dendritic $GABA_A$ currents. As a result, ATPA induced a sustained opening of dendritic $GABA_A$ channels activated by repetitively applied GABA. During LTP induction, postsynaptic KARs may be activated by tetanic stimulation-evoked synaptic release of glutamate and mediate the depolarizing shift in E_{Cl} and the prolongation of $GABA_A$ channel openings. In such way, the postsynaptic KAR plays a facilitating role in induction of LTP.

Disclosures: **J. Kang:** A. Employment/Salary (full or part-time):; Full. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); None. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

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Program#/Poster#: 783.09/B44

Topic: B.08. Synaptic Plasticity

Support: Korean Ministry of Education, Science and Technology (MEST;2011-0007706)

Title: The GABAergic circuits of the amygdala modulate the fear memory

Authors: *O.-B. KWON, J.-H. LEE, H.-J. KIM, S. LEE, H.-J. JO, J.-H. KIM;
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Abstract: Excessive fear memory is one of major hallmarks of stress and post-traumatic disorder (PTSD). Possible molecular and cellular mechanisms for prevention of excessive fear memory toward non-salient stimuli should be understood to elucidate this fundamental aspect of memory and provide therapeutic intervention for emotion and anxiety disorders. Here we show that the neuronal activity of dorsal intercalated cell (ITCd) in the amygdala could effectively control the fear memory recall. To elucidate physiological features of ITCd, we assessed the synaptic plasticity before and after fear conditioning using *ex vivo* electrophysiology and optogenetics. ITCd neurons yielded long-term depression (LTD) with spike time dependent plasticity (STDP) stimulation protocol after fear conditioning. We also found that activation of dopamine D4 receptor (D4R) was sufficient in producing the physiological outcome that fear conditioning elicits, induction of STDP-LTD in naive mice ITCd. Moreover, selective inhibition of ITCd D4R *in vivo* induced excessive level of fear recall after mild-shock fear conditioning, suggesting that the D4R activation could be necessary for prevention of excessive fear responses and adjustment of emotional behavior in a moderate range. Combined, these data suggest that the fear engram can be modulated by local inhibitory networks and those networks are tightly regulated by dopaminergic signaling in the inhibitory network.

Disclosures: O. Kwon: None. **J. Lee:** None. **H. Kim:** None. **S. Lee:** None. **H. Jo:** None. **J. Kim:** None.

Poster

783. Inhibitory Plasticity

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Program#/Poster#: 783.10/B45

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH082881

Title: Both H1 and H2 receptors are involved in histamine-dependent enhancement of GABAergic transmission in the entorhinal cortex

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Abstract: The entorhinal cortex reportedly expresses histamine (HA) receptors (H1R, H2R, and H3R) and receives input from the tuberomammillary nucleus, the only neuronal source for HA. Receptors for HA are G-protein coupled and enable a neuromodulatory action for HA. We focused on HA's neuromodulatory influence on inhibitory transmission in the EC and found that bath application of HA (30 μM) enhances GABAergic transmission. Whole-cell recordings from EC principal neurons display significant increases in the frequency of spontaneous inhibitory post synaptic currents (sIPSCs) in the presence of HA. A selective antagonist for the H1R, cetirizine, completely abolished the increase in sIPSCs whereas a selective antagonist for the H2R, rantidine, only blunted HA's effect. Experiments using selective agonists for both H1 and H2 receptors reveal that co-activation of both H1 and H2 receptors is required to fully mimic HA's effect. Inclusion of GDP-beta-S in the recording pipette does not block the HA-induced increases in sIPSCs, suggesting HA enhances GABAergic transmission via a presynaptic mechanism. Consistent with this hypothesis, presynaptic recordings demonstrate HA increases excitability of local GABAergic interneurons. HA elicits a moderate (~3-5 mV) depolarization of interneuron resting membrane potential in the presence of TTX. Similar to sIPSC experiments, activation of both H1R and H2R is necessary to induce significant depolarization and combination of both receptor antagonists is necessary to completely block HA-induced depolarization. A significant reduction in the input resistance accompanies HA-induced depolarization, suggesting the opening of a cation channel mediates this effect. Accordingly, replacement of extracellular NaCl with N-methyl-D-glucamine chloride significantly reduces the magnitude of HA-induced depolarization. Taken together, our results suggest that HA-induced increases in GABAergic transmission within the EC occur via presynaptic activation of both H1R and H2R leading to cation influx and depolarization of presynaptic interneurons.

Disclosures: **N.I. Cilz:** None. **S. Lei:** None.

Poster

783. Inhibitory Plasticity

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Program#/Poster#: 783.11/B46

Topic: B.08. Synaptic Plasticity

Support: DOD/USUHS Grant R0751883

Brain & Behavior Research Foundation (NARSAD) Grant

Title: Epigenetic modifications of GABAergic synaptic plasticity and AKAP signaling after early maternal deprivation

Authors: *J. KODANGATTIL NARAYANAN, S. GOUTY, M. E. AUTHEMENT, B. M. COX, F. S. NUGENT;
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Abstract: Adverse early life experiences such as prolonged child neglect and abuse increases the risk of developing mental health disorders including substance abuse and psychiatric disorders in childhood, adolescence and adulthood. Pathological reward-dependent learning within the ventral tegmental area (VTA) and the subsequent dysregulation of dopamine (DA) signaling from the VTA seems to be central to the onset of addiction and stress-related disorders. A single 24h episode of early maternal deprivation (MD) in rodents is widely used as an animal model of severe early life stress. Studies using this model have provided a strong link between the dysregulation of DA signaling and a later propensity to develop stress-related disorders. However it is still unknown how early MD affects the reward learning processes in the VTA. Using immunofluorescence and whole-cell patch clamp recording in rat midbrain slices, we showed that MD per se induced long-term depression (LTD) at GABAergic synapses onto VTA DA neurons and impaired the capability of these GABAergic synapses to exhibit spike timing dependent plasticity (STDP) through epigenetic modifications of the postsynaptic scaffolding A-kinase anchoring protein 79/150 (AKAP79/150) signaling. Moreover, we found that the histone deacetylase (HDAC) inhibitor, sodium butyrate, rescued GABAergic STDP and AKAP signaling in MD animals. Understanding the effects of MD on neurons in the VTA will expand our knowledge of an important but neglected part of the cellular basis of child abuse and neglect. Consequently, we will identify novel mechanisms in the regulation of inhibitory plasticity and memory formation in the VTA that can be selectively targeted in a cell-type and circuit-specific manner in the period immediately following an episode of MD or other severe stress during early development. [Supported by R0751883-DOD/USUHS and Brain & Behavior Research Foundation (NARSAD) grants to FN].

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Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.12/B47

Topic: B.07. Synaptic Transmission

Support: WCI 2009-003 NRF

Title: Synapsin isoforms regulating GABA release from hippocampal interneurons

Authors: ***S.-H. SONG**[1], G. J. AUGUSTINE[1,2];
[1]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technology(KIST), Seoul, Seongbuk-Gu, Korea, Republic of; [2]Lee Kong Chian Sch. of Med., Singapore 637553, Singapore

Abstract: Deletion of the three synapsin genes of mice causes substantial changes in behavior and synaptic transmission. These synaptic defects differ for excitatory and inhibitory synapses, indicating that synapsins have unique functions at different synapse types (J. Neurosci. 24:11368). Synapsin IIa is the main isoform involved in glutamate release and works by accumulating vesicles in a reserve pool (RP; J. Neurosci. 28:1085). Here we identify the synapsin isoform(s) that regulate GABA release by using lenti virus constructs to introduce individual synapsin isoforms into cultured hippocampal interneurons from synapsin triple knockout (TKO) mice. The amplitude of evoked IPSCs (eIPSC) was reduced in TKO neurons, compared to wild-type neurons (TWT) neurons, and could be rescued by introducing any synapsin isoform. During repetitive stimulation (50 Hz, 50 s), the rate of synaptic depression was similar in TKO and TWT neurons. This was also the case for TKO neurons expressing individual synapsin isoforms, aside from a more rapid rate of depression in neurons expressing synapsin llla. As previously reported (Nature Comm. 4:1512), synapsins influence the time course of GABA release: eIPSCs decay more slowly in TKO neurons than in TWT neurons. Deconvolution analysis indicates that this is due to a prolongation of the duration of GABA release following a stimulus. Each of the synapsin isoforms rescued this kinetic phenotype, except for the case of synapsin llla where the duration of transmitter release was still prolonged. Because synapsin llla slowed release kinetics and rescued the defect in IPSC amplitude, there was a 2-fold increase in the number of GABA quanta released in response to a stimulus in TKO neurons expressing synapsin IIIa. This presumably accounts for the more rapid rate of depression

in these neurons. Measurement of the cumulative amount of GABA released during a train of stimuli indicated that the size of the readily releasable pool (RRP) of GABAergic vesicles and the rate of mobilization of these vesicles from the RP to the RRP are unaffected by synapsins. In summary, at GABAergic terminals synapsins are responsible for controlling the synchronization of quantal discharge from the RRP. Synapsin IIIa is unable to synchronize GABA release but does enhance the total number of quanta released. This differs from the case for glutamatergic vesicles, where synapsins control the size of the RP and only one isoform (synapsin IIa) is capable of supporting the RP.

Disclosures: S. Song: None. **G.J. Augustine:** None.

Poster

783. Inhibitory Plasticity

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 783.13/B48

Topic: C.07. Epilepsy

Support: CIHR Grant #119553

Title: Synaptic impairment of frontal cortical fast-spiking basket cells induces cognitive and behavioural deficits in mice with a Cacna1a loss-of-function mutation

Authors: *A. LUPIEN-MEILLEUR[1,2], I. RIEBE[2], L. DAMAJ[1,3], C. VANASSE[1], L. GAGNON[1], A. VAN DEN MAAGDENBERG[4], J.-C. LACAILLE[2], E. ROSSIGNOL[1,2]; [1]CHU Sainte-Justine, Montréal, QC, Canada; [2]Neurosciences, Univ. de Montréal, Montréal, QC, Canada; [3]Pédiatrie, CHRU Rennes, Rennes, France; [4]Human Genet. & Neurol., Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: Loss-of-function mutations in the CACNA1A gene, which encodes the α1 subunit of voltage-gated CaV2.1 channels, result in episodic ataxia (EA2) and epilepsy in humans. These conditions are rarely associated with overt cognitive deficits. We investigated 15 patients from 4 non-consanguinous families carrying different CACNA1A loss-of-function mutations and revealed that the majority of them had moderate to significant neurocognitive impairment, which includes a spectrum of inattention, impulsivity, learning difficulties, working memory deficits, intellectual deficiency and autism. We recently demonstrated that a targeted deletion of Cacna1a causing an ablation of voltage-gated CaV2.1 Ca2+ channels selectively in forebrain GABAergic interneurons (INs) in mice leads to selective synaptic impairment of parvalbumin-positive (PV)

fast-spiking basket cells that is sufficient to induce generalised epilepsy. We therefore, propose that a selective impairment of perisomatic inhibition resulting from PV-INs synaptic dysfunction in neocortical orbitofrontal circuits might contribute to the cognitive deficits observed. To better understand the pathological mechanisms underlying these cognitive deficits, we studied the effects of CaV2.1 channel ablation in PV neurons that are thought to be critical for cognition. To this end, we generated mutant mice carrying a targeted heterozygous Cacna1a deletion restricted to PV neuronal populations (PVcre;Cacna1ac/+), which targets cortical PV-INs post-natally. Using an optogenetic approach (AAV-ChR2) to selectively activate PV-INs while recording inhibitory post-synaptic events (IPSCs) in orbitofrontal pyramidal cells (PC), we demonstrated that this selective mutation significantly impairs perisomatic inhibition of PC in the orbitofrontal cortex. We assessed the behavioural and cognitive abilities of these mutant mice in the Open Field, the Elevated Plus Maze, the T-maze, the Morris Water Maze and the Reversal Learning Task. These investigations revealed that the haploinsufficiency of Cacna1a in PV-INs leads to impulsivity, impaired working memory and reduced cognitive flexibility (impaired reversal learning) in PVcre;Cacna1ac/+ mutant mice. These deficits could be recapitulated by local AAV-Cre injections in the orbitofrontal cortex of Cacna1ac/+ mice. Our results demonstrate that haploinsufficiency of Cacna1a leads to significant cognitive and behavioural impairment in humans and in conditional mutant mice, and that this is partly attributable to disrupted perisomatic inhibition in orbitofrontal circuits.

Disclosures: **A. Lupien-Meilleur:** None. **I. Riebe:** None. **L. Damaj:** None. **C. Vanasse:** None. **L. Gagnon:** None. **A. Van den Maagdenberg:** None. **J. Lacaille:** None. **E. Rossignol:** None.

Poster

783. Inhibitory Plasticity

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Vanderbilt Clinical and Translation Science Award

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Title: Adaptive wildtype GABAA receptor expression, distribution, mobility and seizure activity in Gabrb3+/- mice

Authors: *C.-Q. ZHANG[1,5], W. SHEN[1], C. ZHOU[1], Q. ZHANG[2], E. YANG[1], R. MACDONALD[1,2,3,4], J.-Q. KANG[1,4];
[1]Dept. of Neurology,, [2]Departments of Pharmacol., [3]Mol. Physiol. and Biophysics, [4]Vanderbilt Brain Inst., Vanderbilt Univ. Med. Ctr., Nashville, TN; [5]Dept. of Neurosurg., Third Military Med. Univ., Chongqing, China

Abstract: Gamma-aminobutyric acid type A ($GABA_A$) receptors mediate the majority of rapid inhibitory synaptic transmission in the CNS. Mutations in the $GABA_A$ receptor 3 subunit and deletions of chromosome 15q11-13, which contain the 3 subunit gene *GABRB3* are frequently associated with epilepsy as well as other neurodevelopmental abnormalities like autism and Angelman syndrome. Deletion of 3 subunits in *Gabrb3* knockout mice results in ~90% neonatal lethality, and the surviving *Gabrb3* knock-out (KO) mice have been proposed to be both human Angelman syndrome and autism models due to their neurodevelopmental abnormalities and phenotypic presentations. The $GABA_A$ receptor 3 subunit is essential for pentameric $GABA_A$ receptor assembly and trafficking. Any alteration of 3 subunit expression would change $GABA_A$ receptor assembly, distribution and membrane diffusion in a neuronal milieu of multiple $GABA_A$ receptor subunits coexisting during brain development. We have characterized $GABA_A$ receptor expression, distribution and mobility in *Gabrb3*KO mice. Since all patients reported to harbor 3 subunit mutations are heterozygous, we characterized the expression of $GABA_A$ receptor subunits in both mouse brain and cultured neurons from heterozygous *Gabrb3*KO mice. We used live brain slice surface biotinylation, single Quantal dot labeling and whole cell recording in live hippocampal neurons.We demonstrated that both surface and total expression of 3 subunits were reduced in heterozygous KO mice. Additionally, 2 subunitswere reduced while 2 subunits were upregulated in the heterozygous mice. Using single quantal dot labeling, we found that hippocampal neurons cultured from heterozygous *Gabrb3*KO mice had altered mobility and a larger fraction of extra synaptic $GABA_A$ receptors. Whole cell recordings of hippocampal GABAergic interneurons from both heterozygous and homozygous *Gabrb3*KO mice had reduced current amplitudes and increased zinc inhibition. The heterozygous *Gabrb3*KO mice had absence-like seizures both behaviorally and electrographically. The study suggests that impairment in 3 subunit expression alters expression of other wildtype $GABA_A$ receptor subunits and alters membrane $GABA_A$ receptor distribution and diffusion dynamics during brain development, thus resulting in a complex neurobehavioral phenotype including epilepsy.

Disclosures: C. Zhang: None. **W. Shen:** None. **C. Zhou:** None. **Q. Zhang:** None. **E. Yang:** None. **R. Macdonald:** None. **J. Kang:** None.

Poster

783. Inhibitory Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Title: A critical role for the ADHD associated protein, Cadherin 13, in inhibitory synapse formation and function

Authors: *M. M. SELTEN[1], O. RIVERO[2], S. M. KOLK[3], K.-P. LESCH[2], N. NADIF KASRI[1]; [1]Cognitive Neurosci., Dept. of Cognitive Neurosciences, Donders Ins, Nijmegen, Netherlands; [2]Div. of Mol. Psychiatry, Lab. of Translational Neuroscience, ADHD Clin. Res. Network, Dept. of Psychiatry, Psychosomatics and Psychotherapy, Univ. of Würzburg, Würzburg, Germany; [3]Dept. of Mol. Animal Physiology, Donders Inst. for Brain, Cognition, and Behaviour, Nijmegen Ctr. for Mol. Life Sciences, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Recently, genome-wide association studies have consistently identified the adhesion molecule Cadherin-13 (CDH13) as a risk gene for various neurodevelopmental and psychiatric disorders including ADHD. Although CDH13 has been well-characterized as an atypical adhesion protein in non-neuronal cells, surprisingly little is known about the function of CDH13 in neurons. Similarly, how CDH13 dysregulation impacts ADHD is currently unknown. Recently cadherins have emerged as critical players in the successive steps of synaptogenesis, suggesting a role in the changes in synaptic structure and function that underlie the neuronal circuitry changes observed in ADHD. Here we propose that CDH13, as an atypical member of the cadherin family, plays a critical role in the formation and function of inhibitory synapses onto CA1 hippocampal neurons. Using immunocytochemistry we show that CDH13 is highly expressed in the Stratum Oriens in the hippocampus, where it strongly overlaps with parvalbumin-positive (PV+, fast-spiking) GABAergic interneurons. At the subcellular level we find that CDH13 is specifically localized to the inhibitory pre-synaptic compartment in dissociated hippocampal neurons, suggesting for a pre-synaptic function at inhibitory synapses. Accordingly, using a Cdh13 knock-out mouse model and a knockdown approach we find a decrease in the frequency of miniature inhibitory postsynaptic currents on pyramidal cells in CA1, whereas no changes in excitatory synaptic transmission were observed. These results suggest that CDH13 is essential in the formation and function of synapses formed by PV+ interneurons in Stratum Oriens on excitatory CA1 pyramidal cells.

Disclosures: **M.M. Selten:** None. **O. Rivero:** None. **S.M. Kolk:** None. **N. Nadif Kasri:** None. **K. Lesch:** None.

Poster

783. Inhibitory Plasticity

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Title: Chronic fluoxetine treatment alters the structure, connectivity and plasticity of cortical interneurons

Authors: *J. S. NACHER[1], M. PEREZ-RANDO[2], R. GUIRADO[3], E. CASTREN[3];
[1]Univ. Valencia Esq4618001d, Burjassot, Spain; [2]Cell Biol., Univ. Valencia, Burjassot, Spain; [3]Neurosci., Univ. of Helsinki, Helsinki, Finland

Abstract: Novel hypotheses suggest that antidepressants, such as the selective serotonin reuptake inhibitor Fluoxetine, induce neuronal structural plasticity, resembling that of the juvenile brain, although the underlying mechanisms of this reopening of the critical periods still remain unclear. However, recent studies suggest that inhibitory networks play an important role in this structural plasticity induced by Fluoxetine. For this reason we have analyzed the effects of a chronic Fluoxetine treatment in the hippocampus and medial prefrontal cortex (mPFC) of transgenic mice displaying eGFP labeled interneurons. We have found an increase in the expression of molecules related to critical period plasticity, such as the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), GAD67/65 and synaptophysin, as well as a reduction in the number of parvalbumin expressing interneurons surrounded by perineuronal nets. We have also described alterations in the perisomatic inhibitory puncta on pyramidal neurons and on eGFP interneurons in the mPFC. Finally, we have found that chronic Fluoxetine treatment affects the structure of interneurons in the mPFC, increasing their dendritic spine density. The present study provides evidence indicating that Fluoxetine promotes structural changes in the inhibitory neurons of the adult cerebral cortex, probably through alterations in plasticity-related molecules of neurons or the extracellular matrix surrounding them, which are

present in interneurons and are known to be crucial for the development of the critical periods of plasticity in the juvenile brain.

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Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

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Topic: B.03. G-Protein Linked Receptors

Support: NIH DA0171-88

McKnight Foundation

Title: Somatostatin neurons silence excitatory connections through presynaptic GABAb receptors

Authors: *J. URBAN CIECKO[1], A. L. BARTH[2];
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Abstract: Somatostatin (SOM)-expressing inhibitory neurons represent one of the major sources of inhibition in cortical circuits, synapsing onto the distal dendrites of pyramidal neurons where they regulate excitatory transmission by fast, GABAa-mediated Cl- currents. SOM cells have also been implicated in state-dependent modulation and experience-dependent plasticity, and their spontaneous firing activity is regulated by neuromodulators. Here we provide evidence that SOM neurons regulate excitatory transmission at longer timescales through activation of presynaptic GABAb receptors. We show that spontaneous firing of SOM neurons suppresses excitatory synaptic transmission between connected pairs of L2/3 pyramidal neurons in primary somatosensory cortex. EPSP amplitude is reduced and EPSP failures rates are elevated when SOM neurons are active. Optogenetic suppression of SOM firing is sufficient to enhance EPSP amplitude and reduce failure rates, effects that were fully reversible. Our data indicate that SOM neurons suppress EPSP amplitude and enhance failure rates via presynaptic GABAb receptors, since GABAb antagonists occlude SOM silencing effects. Thus, SOM neurons can rapidly and reversibly modulate neocortical networks through synaptic silencing, and suggest a critical role for these neurons in gating perception and plasticity.

Disclosures: J. Urban Ciecko: None. **A.L. Barth:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

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Topic: B.03. G-Protein Linked Receptors

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National Center for Competences in Research (NCCR) 'Synapsy, Synaptic Bases of Mental Health Disease'

Marie Heim-Vögtlin program PMPDP3-129165

Deutsche Forschungsgemeinschaft SFB 746, TP16, Fa 332/9-1

Title: KCTD12-induced desensitization of G-protein coupled receptor responses under physiological and pathophysiological conditions

Authors: *B. BETTLER[1], R. TURECEK[1], J. SCHWENK[2], T. FRITZIUS[1], K. IVANKOVA[1], G. ZOLLES[2], L. ADELFINGER[1], V. JACQUIER[1], V. BESSEYRIAS[1], M. GASSMANN[1], U. SCHULTE[2], B. FAKLER[2];
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Abstract: G-protein coupled receptors and G-protein regulated ion channels represent important cellular signal transduction systems in the nervous system. Using a proteomics approach we recently identified the KCTD12 protein as an auxiliary subunit of native GABAB receptors (Schwenk et al., Nature 465, 2010). GABAB receptors assembled with KCTD12 subunits generate rapidly desensitizing K+ and Ca2+-channel responses. KCTD12 is a novel regulator of G-protein signaling whose mechanism of action, however, is unknown. We found that KCTD12 induces fast desensitization via a novel mechanism (Turecek et al., in press). This mechanism involves masking of the channel binding-site on the activated G-protein βγ-subunits and their subsequent uncoupling from effector channels. We show that KCTD12 can desensitize the responses of a variety of GPCRs *in vitro*. Quantitative proteomic analysis, however, reveals that virtually all KCTD12 protein in the brain is associated with GABAB receptors. Consistent with the proteomic data we find that neurons of KCTD12 knock-out mice selectively exhibit alterations in the kinetics of GABAB receptor-activated K+-currents. The KCTD12 subunit has

been associated with bipolar disorder, unipolar depression and schizophrenia. Our data predict that a down-regulation of KCTD12 protein will specifically alter GABAB receptor-mediated signaling while an up-regulation of KCTD12 may additionally alter the signaling of other G-protein coupled receptors.

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Poster

784. Serotonin and GABAB Gpcrs

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Topic: B.03. G-Protein Linked Receptors

Title: The inverse agonistic effect of Rimonabant is not mediated by CB1, GABAB, opiod and D2 dopamine receptors

Authors: A. PORCU[1], A. CASTI[1], F. SANNA[1], G. FLORIS[2], M. P. MASCIA[3], P. FOLLESA[2], M. MELIS[1], G. L. GESSA[1], *M. CASTELLI[4];
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Abstract: The selective and potent cannabinoid CB1 antagonist Rimonabant at high micromolar concentrations behaves as an inverse agonist, i.e. decreases [^{35}S]GTPγS binding in rodent and human cerebral cortex and in Chinese hamster ovary (CHO) cells transfected with CB1 receptors. However, Rimonabant inverse agonism is CB1 receptor independent since is produced in CB1 receptor knockout (KO) mouse brain membranes and in CHO not expressing CB1 receptors. The present study was aimed at determining whether Rimonabant inverse agonism is mediated by $GABA_B$, opioid, or dopamine D2 receptors. We found that rimonabant decreased basal [^{35}S]GTPγS binding to cortical membranes of rats, wild type mice, CB1 receptor and GABAB KO mice. Rimonabant induced decrease of basal [^{35}S]GTPγS binding in rat or mouse cortical membranes was not prevented by CB1, $GABA_B$, opioid, and D2 receptor agonist and antagonists. Moreover, rimonabant attenuated the activation of [^{35}S]GTPγS binding to cortical membranes produced by the GABAB agonists baclofen and GABA, the μ agonist morphine, the

D2 agonist quinpirole, but failed to modify the stimulant effect on [^{35}S]GTPγS binding of the D1 receptor agonist SKF81293. Rimonabant suppressed both GABAB and D2 dopamine-activated inwardly rectifying potassium current in VTA dopamine and in CHO cells expressing D2 receptor. Our results suggested that Rimonabant inverse agonism in GTPγS binding is mediated by a multiple action on plurality of G protein coupled receptors (GPCRs) sharing the same Gi/o protein.

Disclosures: **A. Porcu:** None. **A. Casti:** None. **F. Sanna:** None. **G. Floris:** None. **M.P. Mascia:** None. **P. Follesa:** None. **M. Melis:** None. **G.L. Gessa:** None. **M. Castelli:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.04/B55

Topic: B.03. G-Protein Linked Receptors

Support: SNF

Title: K63-linked ubiquitination of GABAB1 sorts GABAB receptors to lysosomal degradation

Authors: ***K. ZEMOURA**, T. CLAUDIA, B. DIETMAR;
Inst. of Pharmacol. and Toxicology, Zurich, Switzerland

Abstract: The G protein-coupled GABAB receptors mediate slow and prolonged neuronal inhibition. The magnitude of GABAB receptor-mediated inhibition essentially depends on the amount of receptors in the plasma membrane. A main factor that determines receptor availability is regulated protein degradation. After internalization, GABAB receptors are recycled to the cell surface or degraded in lysosomes. The signal that sorts GABAB receptors to lysosomes is so far unknown. In present study we analyzed whether ubiquitination is the lysosomal sorting signal for GABAB receptors. We found that inhibition of lysosomal activity in cortical neurons increased total and cell surface GABAB receptors, confirming the constitutive degradation of GABAB receptors in lysosomes. In addition, blocking lysosomal activity considerably increased the amount of K63-linked ubiquitinated GABAB receptors. This observation indicates that K63-linked ubiquitination serves as the sorting signal. Indeed, mutational inactivation of four putative ubiquitination sites in the GABAB1 subunit significantly diminished K63-linked ubiquitination of GABAB1 and prevented lysosomal degradation of the receptors. Overexpressing a dominant-negative mutant of Rab7 to disrupt lysosome function increased the expression level of wildtype

GABAB receptors but not that of receptors containing the GABAB1 mutants. Finally, triggering lysosomal degradation of GABAB receptors by sustained activation of glutamate receptors down-regulated wildtype but not mutant GABAB receptors deficient in GABAB1 K63-linked ubiquitination. These findings indicate that K63-linked ubiquitination of GABAB1 at multiple sites is involved in sorting GABAB receptors to lysosomes for degradation.

Disclosures: K. Zemoura: None. **T. Claudia:** None. **B. Dietmar:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.05/B56

Topic: B.03. G-Protein Linked Receptors

Title: Effect of $GABA_B$ positive allosteric modulators in the DBA/2J mouse audiogenic seizure test: comparison to baclofen and utility as a pharmacodynamic screening model

Authors: *J. W. BROWN[1], S. TURNER[2], V. NIMMRICH[2], L. E. RUETER[1], E. VAN DER KAM[2], M. ZHANG[1];
[1]Abbvie, North Chicago, IL; [2]Abbvie, Ludwigshafen, Germany

Abstract: The GABAB receptor has been indicated as a promising target for multiple CNS-related disorders. Baclofen, a prototypical orthosteric agonist, is used clinically for the treatment of spastic movement disorders, but is associated with unwanted side-effects, such as sedation and motor impairment. Positive allosteric modulators (PAM), which bind to a distinct site apart from the orthosteric binding region, may provide an improved side-effect profile while maintaining baclofen-like efficacy. The identification of a validated preclinical pharmacodynamic assay can be a powerful tool for comparing drugs with related mechanisms of action and to help establish the relationship between target engagement and positive/negative effects. GABA, the major inhibitory neurotransmitter in the CNS, plays an important role in the mechanism and treatment of seizure disorders. Not surprisingly, baclofen is known to produce anticonvulsant effects in the DBA/2J mouse audiogenic seizure test (AGS), suggesting it may be a suitable pharmacodynamic endpoint. Little is known about the effects of GABAB PAMs, however. The studies presented here sought to validate the AGS test as a pharmacodynamic screening model for GABAB PAMs by comparing the profile of reference PAMs against baclofen. Rac-BHFF, GS39783, and CMPPE all produced robust, dose-dependent anticonvulsant effects; a similar profile was observed with baclofen, although it was more potent. Pre-treatment with the GABAB antagonist

SCH50911 completely blocked the anticonvulsant effects of baclofen and CMPPE in the AGS test, indicating such effects are mediated by the GABAB receptor. In addition to the standard anticonvulsant endpoint of the AGS test, video tracking software (SMART) was employed to assess potential drug-induced motoric side-effects during the acclimation period of the test. This analysis was sensitive to detecting drug-induced changes in total distance traveled, which was used to establish efficacy vs. side-effect margins (hypoactivity ED50 / anticonvulsant-tonic ED50) for each compound. Calculated margins for rac-BHFF, GS39783, and CMPPE were 2.51x, 1.76x, and 8.37x, respectively. The calculated efficacy vs. side-effect margin for baclofen was 0. The results presented here suggest the DBA/2J mouse AGS test is a potentially useful screening model for detecting pharmacodynamic effects of GABAB PAMs and can provide an initial read-out on target-related motoric side-effects. Furthermore, improved efficacy vs. side-effect margins were observed for PAMs compared to baclofen, particularly for CMPPE, indicating the PAM approach may be a viable therapeutic alternative to baclofen.

Disclosures: **J.W. Brown:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **S. Turner:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **V. Nimmrich:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **L.E. Rueter:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **E. Van Der Kam:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **M. Zhang:** A. Employment/Salary (full or part-time):; AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

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Program#/Poster#: 784.06/B57

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant NS048900

NSF Grant 0923041

Title: Colocalization of interneuron markers with proteins involved in $GABA_B$ receptor mediated calcium current enhancement in neonatal hippocampus

Authors: D. P. VANDERHOEF, *M. MYNLIEFF;
Marquette Univ., MILWAUKEE, WI

Abstract: Previously, we demonstrated that activation of the G-protein coupled $GABA_B$ receptor ($GABA_BR$) modulates currents through voltage-gated calcium channels with an attenuation of N-Type calcium current mediated by a $G\alpha_{i/o}$ G protein. Our laboratory has also described enhancement of L-type calcium current mediated by a $G\alpha_q$ G protein and the α isoform of protein kinase C (PKCα). This enhancement peaks at postnatal day 7 and is expressed in only 10-20% of cells in the superior region of the hippocampus. Since a large percentage of the cells in the CA1 region are excitatory pyramidal cells, we hypothesized that enhancement of current is confined to one or more inhibitory interneuron subtypes and determining the subtype may provide insight into a physiological role for the L-type current enhancement observed. Studies have reported up to 21 different inhibitory interneuron subtypes. Sloviter et al. (*Neuropharm.*, 38(11), 1707-21, 1999) has demonstrated that in the CA1 region, interneurons expressing the neurochemical markers cholecystokinin (CCK), calbindin, neuropeptide Y, and somatostatin all express $GABA_BR$; making them possible candidates for the neuron(s) in which $GABA_BR$ mediated calcium current enhancement occurs. Interneurons that encompass these parameters are the CCK+ basket cells, Schaffer collateral associated cells, and quadrilaminar cells. In the present study, fluorescent confocal microscopy in the superior hippocampal region from 6-8 day old rats is utilized to determine colocalization of neurochemical markers used to identify interneuron subtypes with different components of the signaling pathway mediating calcium current enhancement. We hypothesized that the neuron of interest is a CCK+ basket cell, due to the morphology of the cell and similar localization as seen in preliminary data. The components of the signaling pathway of interest include $GABA_BR$, $G\alpha_q$, PKC_α and the two L-type calcium channel isoforms expressed in the brain, $Ca_V1.2$ and $Ca_V1.3$. The specific isoform of L-type channel enhanced by $GABA_BR$ activation has not been determined but the developmental expression and colocalization with $GABA_B$ receptors suggest that $Ca_V1.2$ is a component in the pathway. Preliminary data demonstrate a much higher colocalization of calbindin with $Ca_V1.2$ than CCK with $Ca_V1.2$. However, only 1.17% of the total cells counted in the CA1 region express both calbindin and $Ca_V1.2$ and thus, it is likely that enhancement of the calcium current by $GABA_B$ receptor activation occurs in multiple interneuron subtypes.

Disclosures: D.P. VanDerhoef: None. **M. Mynlieff:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.07/B58

Topic: B.03. G-Protein Linked Receptors

Title: Evaluation of multiple doses of GABA(B) ligands on learning and memory

Authors: *C. F. HEANEY, M. M. BOLTON, A. S. MURTISHAW, M. A. LANGHARDT, J. W. KINNEY;
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Abstract: The inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) acts on two main types of receptors, the ionotropic GABAA and the metabotropic GABAB. Evidence indicating a central role of GABAB in regulating oscillatory function suggests a prominent role of this receptor in cognitive processes. However, research has yielded mixed results regarding the extent to which altered GABAB receptor function impairs or enhances learning and memory performance. Previous data from our laboratory indicate that baclofen, a GABAB agonist, impairs learning and memory performance and phaclofen, a GABAB antagonist, improves learning and memory performance in the Morris water maze. In order to better characterize the effects of the GABAB receptor on complex behavior, we compared the effect of two distinct doses of these GABAB ligands on the performance of male Sprague-Dawley rats in the Morris water maze. Additionally, we examined the effect of co-administration of baclofen and phaclofen on performance. While the deficits due to baclofen followed an ascending concentration pattern, the effects of phaclofen were more interesting. The low dose of phaclofen improved learning and memory performance, but the higher dose did not impact learning. We then examined the effect of these ligands when co-administered utilizing the doses of the ligands that did not, by themselves, alter learning and memory. Interestingly, the co-administration of the GABAB receptor agonist and antagonist impaired performance in the Morris water maze. We also analyzed hippocampal tissue for alterations to numerous protein markers in order to link any changes to these neural targets with performance in the behavioral task. Our data indicate specific concentrations associated with both the agonist and antagonist that are capable of altering learning and memory. The data further indicate a limited range of effect of the GABAB receptor antagonist, which may contribute to some of the differences present in the literature. Finally, our data also indicate that co-administration of these two ligands impairs behavior.

These data suggest a potential interaction of the two ligands that may be possibly tied to pre- versus postsynaptic GABAB receptor mechanisms.

Disclosures: C.F. Heaney: None. **M.M. Bolton:** None. **A.S. Murtishaw:** None. **M.A. Langhardt:** None. **J.W. Kinney:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.08/B59

Topic: B.03. G-Protein Linked Receptors

Support: Immunostar Corp

Title: 5HT6 receptor distribution in rat brain using agonist-induced FOS and antireceptor antibody immunocytochemistry

Authors: S. STEWART[1], L. DAILEY[1], A. HEFFERNAN[2], A. MITZEY[1], *M. S. BROWNFIELD[1];
[1]Dept Comp Biosci., Univ. Wisconsin, Madison, WI; [2]Vet. Clin. Sci., Univ. of Minnesota, Minneapolis, MN

Abstract: The 5-HT6 receptor is one of the most recently discovered serotonin receptors. This receptor appears to play a big role in cognition and memory, as evidenced by the paradoxical ability of both agonists and antagonists to have anti amnesic effects. Many brain illnesses that involve cognitive dysfunction, including schizophrenia and Alzheimer's disease, involve the 5-HT6 receptor. The 5-HT6 receptor has been found throughout the brain, primarily in the striatum, the dentate gyrus of the hippocampus, and the olfactory tubercle. Previous studies investigating the positioning of the 5-HT6 receptor, however, have been limited by the techniques they used, typically in-situ hybridization and Northern blotting. These techniques, which probe for mRNA associated with the receptor, can only tell us if the gene for the receptor is present; they cannot give any information on functionality. We have probed the entire rat brain for the 5-HT6 receptor using immunocytochemistry for the receptor protein. To do this, we generated an antibody to the 5-HT6 receptor in our laboratory (using the sequence LERPPGTPRHPPGPPLW-amide), and we used this antigen to affinity purify the antibody. We also looked at agonist-induced fos expression. Fos is the product of an intermediate early gene (IED) that is expressed when a neuron is activated; we gave rats a 5-HT6 agonist, EMD 386088, and observed where fos was expressed. We found the receptor in all the areas it had previously been suggested to be, as well as in several areas it has never been reported to be; these places

include, but are not limited to, neurons and fibers in the paraventricular nucleus of the thalamus, the lateral and medial, as well as central, nuclei of the amygdala, the habenula, and the subfornical organ. We found high concentrations of the 5-HT6 receptor throughout the hippocampus (CA1, CA2, CA3, dentate gyrus, and subiculum); the presence of the receptor in the hippocampus may mediate the 5-HT6 receptor's effect on memory.

Disclosures: S. Stewart: None. **L. Dailey:** None. **A. Heffernan:** None. **A. Mitzey:** None. **M.S. Brownfield:** Other; Immunostar.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.09/B60

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant MH072672

NIH Grant MH100652

Title: Interleukin 6 attenuates serotonin 2A receptor signaling through the JAK-STAT pathway

Authors: *J. J. DONEGAN[1,2], T. A. CHAVERA[1,2], M. S. PATTON[1,2], K. A. BERG[1,2], D. A. MORILAK[1,2], M. GIROTTI[1,2];
[1]Pharmacol., [2]Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: Cognitive flexibility, the adaptive ability to modify behavior in response to environmental change, is impaired in psychiatric diseases, such as depression. Reversal learning is a type of cognitive flexibility mediated by the orbitofrontal cortex (OFC). We have shown that reversal learning is facilitated by both the serotonin 2A (5-HT2A) receptor and the cytokine, interleukin-6 (IL-6), in the OFC. In the current studies, we tested the hypothesis that 5-HT2A receptor- and IL-6-mediated signaling interact. We first determined if IL-6 signaling occurs in OFC neurons that also express 5-HT2A receptors. Rats were injected with an adeno-associated virus to over-express IL-6 and activate the downstream JAK-STAT pathway in the OFC. Dual-fluorescence immunohistochemistry was used to label 5-HT2A receptors and phosphorylated STAT3, a marker of pathway activation. Nearly 100% of 5-HT2A receptor-expressing cells show JAK-STAT activation in response to IL-6 over-expression, suggesting that there is the

potential for cross-regulation between the IL-6 and 5-HT2A receptor systems *in vivo*. To determine if IL-6 influences 5-HT2A receptor signaling in an *in vitro* cell model, we measured inositol phosphate (IP) accumulation in response to the 5-HT2 receptor agonist, DOI in the neuronal cell line, A1A1. In the presence of IL-6 (50 ng/ml), the maximal response to DOI was reduced from 45.96 ± 3.37% to 24.92 ± 3.58% above basal, suggesting that IL-6 regulates 5-HT2A receptor signaling. To identify the signaling pathway that mediates this effect, cells were pretreated with the JAK-STAT inhibitor, JSI-124 (50 μM), or the ERK inhibitor, PD-98059 (50 μM) and DOI-mediated IP accumulation was measured in the presence or absence of IL-6. The IL-6 effect on DOI-induced IP accumulation was abolished following treatment with JSI-124, but not PD-98059, suggesting that IL-6 acts through the JAK-STAT pathway to influence 5-HT2A receptor signaling. To determine if the effect of IL-6 generalizes to other Gq-coupled receptors, bradykinin (BK) B2 receptor-mediated IP accumulation in response to maximal concentrations of BK (100 nM) was measured in the presence or absence of IL-6. IL-6 had no effect on BK-mediated IP accumulation, suggesting that IL-6 may specifically attenuate 5-HT2A receptor signaling. Together, these findings suggest that the IL-6 and 5-HT2A receptor systems can interact, resulting in a functionally distinct outcome. Both IL-6 and the 5-HT2A receptor have been implicated in psychiatric disease. Therefore, further understanding of this interaction, and its functional effect on cognitive flexibility, may provide new insights into the treatment of psychiatric disease.

Disclosures: J.J. Donegan: None. **T.A. Chavera:** None. **M.S. Patton:** None. **K.A. Berg:** None. **D.A. Morilak:** None. **M. Girotti:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.10/C1

Topic: B.03. G-Protein Linked Receptors

Title: Co-expression of 5-HT7 and 5-HT2 receptors in molluscan muscle

Authors: *D. R. MCPHERSON, J. A. LOVETT;
SUNY-Geneseo, GENESEO, NY

Abstract: Serotonin is a potent modulator of contraction in foot and buccal muscle of the marine gastropod *Aplysia californica*. Serotonin enhances the force of contraction and also increases the rate of relaxation from contraction. And in bivalve molluscs, serotonin is

responsible for release from the catch state of contraction in slow adductor muscles of the valves and in the anterior byssal retractor muscle (ABRM) of mussels such as *Mytilus edulis*. We have previously isolated and cloned a 5-HT_7 receptor that is expressed in *Aplysia* foot muscle (McPherson and Lovett, 2008, Soc. Neurosci. Abstr.). We have subsequently found that this receptor is expressed in the I-5 muscle of the *Aplysia* buccal mass, and that homologous receptors are expressed in foot tissue of the terrestrial gastropod *Helix aspersa* and in the ABRM of *Mytilus edulis*. More recently we have explored whether other serotonin receptors are expressed in the foot of *Aplysia,* and have discovered expression of a 5-HT_2 receptor. We are presently exploring whether co-expression of 5-HT_7 and 5-HT_2 receptors is a widespread feature of molluscan muscle organization.

Disclosures: D.R. McPherson: None. **J.A. Lovett:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.11/C2

Topic: B.03. G-Protein Linked Receptors

Support: NIDA 62-6037

NIDA T32-DA00007278

Title: Localization and function of 5-HT6 receptor mutants to neuronal primary cilia in striatum

Authors: *M. BRODSKY, A. LESIAK, K. GARCIA NAKATA, J. SULLIVAN, J. NEUMAIER;
Univ. of Washington, Seattle, WA

Abstract: The 5-HT_6 serotonin receptor is an excitatory G_S-coupled metabotropic receptor which is found almost exclusively in the brain, is abundant in striatum, and is localizes to the primary cilia of neurons. These receptors are implicated in a variety of cognitive and emotional phenotypes including anxiety, depression, habitual behaviors and reward learning. The primary cilium is a sensory organelle stemming from the cell body of most mammalian neurons. The primary cilium is an antenna-like, microtubule-supported structure that receives both chemical and mechanical signals from other cells and the surrounding environment. Recently, neuronal primary cilia have become a major target of research as they play crucial roles in a variety of

disorders known as “ciliopathies”; they have also been implicated in Huntington’s and Alzheimer’s diseases. However, the role of primary cilia in normal cognitive functions is not understood, but there is evidence that impairments of ciliary signaling produce cognitive deficits. GPCRs that localize to cilia generally have a cilia targeting sequence (CTS) of conserved amino acids in the iC3 loop that has been hypothesized to be needed for intraflagellar transport, thereby permitting selective trafficking into primary cilia. Previous work has shown that the absence of a satisfactory CTS leads to default trafficking to the somatodendritic compartment of the neuron. We investigated whether manipulating 5-HT_6 receptor structure and expression levels alter their trafficking to neuronal primary cilia as well as their effects on cilia morphology and signaling. We introduced a set of discrete mutations to the iC3 loop of the 5-HT_6 receptor. Our results thus far suggest that some deletions and mutations to the CTS disrupt trafficking of the mutant receptor to cilia. Trafficking was, to some extent, altered by the extent of overexpression. Further, some of these mutations can interfere with the mutant receptor’s ability to activate adenylate cyclase (whether localized in the cilia or cell body). We are currently investigating whether an additional CTS in the C-terminal is necessary or sufficient for cilia trafficking. Our results suggest that regulation of cilia trafficking is strongly affected by the iC3 loop CTS, but additional features are likely to also be involved in 5-HT_6 receptor trafficking to neuronal primary cilia.

Disclosures: **M. Brodsky:** None. **A. Lesiak:** None. **J. Sullivan:** None. **J. Neumaier:** None. **K. Garcia Nakata:** None.

Poster

784. Serotonin and GABAB Gpcrs

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 784.12/C3

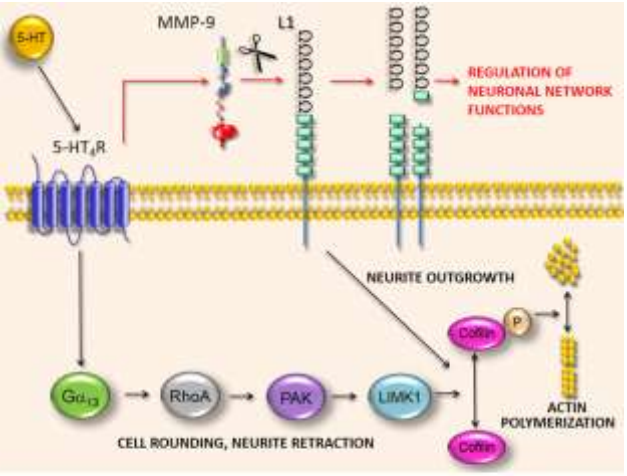
Topic: B.03. G-Protein Linked Receptors

Title: New serotonergic pathway regulating neuronal morphology and functions via adhesion molecule L1

Authors: *D. GUSEVA[1], Y. SCHILL[1], M. BIJATA[2], J. WLODARCZYK[2], E. PONIMASKIN[1]; [1]Cell. Neurophysiol., Hannover Med. Sch., Hannover, Germany; [2]Nenski Inst., Warsaw, Poland

Abstract: Serotonin (5-HT) is an important neurotransmitter regulating a wide range of physiological and pathological functions including many aspects of neural development. Serotonin operates via the activation of multiple 5-HT receptors, whereby in the present study

we focused on the 5-HT4 receptor (5-HT4R). In the mammalian brain this receptor contributes to the regulation of learning and long term memory and is involved in various central and peripheral disorders, including neurodegenerative disease and depression. The 5-HT4R stimulation results in the activation of the small GTPase RhoA, leading to cell rounding and neurite retraction. Here we have demonstrated that this effect can be mediated by phosphorylation of cofilin, important mediator of the temporal and spatial control of actin filament organization. The neuronal adhesion molecule L1 is critically involved in axonal development and can contribute to stress-related disorders in humans. Noteworthy, L1-mediated neurite outgrowth is regulated by cofilin phosphorylation. Thus, cofilin may represent a common downstream effector for both 5-HT4R and L1. In present study we have shown that neuronal L1 can be proteolytically cleaved by the matrix metalloproteinase-9 (MMP-9). Interestingly that stimulation of 5-HT4R induces the release of enzymatically active MMP-9 from hippocampal neurons. Moreover, 5-HT4R and L1 are tightly co-localized at the synapses. Taken together, our results demonstrate that 5-HT4R might regulate L1 shedding in an MMP-9-dependent manner, and can thus represent a novel molecular mechanism by which serotonin can regulate the formation and plasticity of neuronal networks. From these investigations we hope to identify novel targets for pharmacological intervention into stress-related disorders such as depression.



Hypothetical model of the functional cross-talk between 5-HT$_4$R, MMP-9 and L1 signaling.

Disclosures: **D. Guseva:** None. **Y. Schill:** None. **M. Bijata:** None. **J. Wlodarczyk:** None. **E. Ponimaskin:** None.

Poster

785. Spine Dynamics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 785.01/C4

Topic: B.07. Synaptic Transmission

Support: DFG grant Exc 257

DFG grant JO 1079/1-1

DFG grant SFB 665

DFG grant RU1660

Title: Ryanodine receptor activation induces long-term plasticity of spine calcium dynamics

Authors: *F. W. JOHENNING[1], A.-K. THEIS[1], U. PANNASCH[1], M. RÜCKL[2], S. RÜDIGER[2], D. SCHMITZ[1];
[1]Charité Univ. Med. Berlin, Berlin, Germany; [2]Humboldt Univ., Berlin, Germany

Abstract: Dendritic spines compartmentalize signalling at excitatory synapses. Upon action potential firing, the majority of spines are subject to global backpropagating action potential (bAP) mediated Ca2+ transients (CaTsbAP). Here, we demonstrate that bAPs are electrochemically coupled to Ca2+ release from intracellular stores. We describe a new function of ryanodine receptor (RyRs) mediated spine intracellular Ca2+ release, the activity-dependent long-term enhancement of the CaTbAP. This form of plasticity is highly compartmentalized and independent of dendritic Ca2+ regulation. Further, this functional state change depends exclusively on bAPs travelling antidromically into dendrites and spines, without requirement for concomitant synaptic transmission. Induction, but not expression, of CaTbAP enhancement is a spine-specific function of the RyR Ca2+-nanodomain. We describe a new form of spine Ca2+ transient plasticity that constitutes a storage mechanism of neuronal suprathreshold activity patterns

Disclosures: F.W. Johenning: None. **A. Theis:** None. **U. Pannasch:** None. **M. Rückl:** None. **S. Rüdiger:** None. **D. Schmitz:** None.

Poster

785. Spine Dynamics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 785.02/C5

Topic: B.08. Synaptic Plasticity

Support: NIH RO1N5062736

Title: Heterosynaptic structural plasticity on local dendritic segments of hippocampal CA1 neurons

Authors: *L. K. PARAJULI, W. OH, K. ZITO;
Univ. of California, Davis, Davis, CA

Abstract: Competition between neighboring synapses contributes to activity-dependent refinement of the peripheral nervous system during development. However, it remains yet unknown if the local competition between synapses play a similar role during experience-dependent plasticity in the cerebral cortex. Here, we examined the role of activity-mediated competitive interactions in regulating dendritic spine structure and function on hippocampal CA1 neurons. We found that high-frequency glutamatergic stimulation at individual spines, which leads to input-specific synaptic potentiation, induces shrinkage and weakening of nearby unstimulated spines in the same dendrite. Heterosynaptic plasticity requires potentiation of multiple neighboring spines, suggesting that a local threshold of neural activity exists beyond which inactive synapses are punished. Notably, inhibition of calcineurin or IP3Rs blocked heterosynaptic shrinkage without blocking structural potentiation, and inhibition of CaMKII blocked structural potentiation without blocking heterosynaptic spine shrinkage, supporting a model in which activity-induced shrinkage signal, and not competition for limited synaptic resources, drives heterosynaptic structural and functional depression during neural circuit refinement.

Disclosures: L.K. Parajuli: None. **W. Oh:** None. **K. Zito:** None.

Poster

785. Spine Dynamics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 785.03/C6

Topic: B.08. Synaptic Plasticity

Support: CONICYT-PFB 12/2007

Predoctoral fellowship from CONICYT

Title: Wnt5a modulates the postsynaptic density of dendritic spines and the distribution of synaptic mitochondria in hippocampal neurons

Authors: *M. S. ARRÁZOLA, D. ORDENES, N. C. INESTROSA;
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Abstract: Wnt signaling plays a key role in the development of the nervous system and participates in synapse formation. Wnt5a regulates the synaptic structure and function in hippocampal neurons and has shown postsynaptic effects stimulating dendrite spine morphogenesis and increasing glutamatergic neurotransmission through the up-regulation of the NMDA receptor-mediated currents, facilitating the induction of long-term potentiation (LTP). In agreement with the postsynaptic effects described, it has been shown that Wnt5a induces the clustering of PSD-95 in dendritic spines of cultured hippocampal neurons, which is associated with an increase in the density of dendritic protrusions. On the other hand, it has been reported that increasing dendritic mitochondrial content enhances the number and plasticity of spines, suggesting that dendritic distribution of mitochondria is essential for the support of synapses. In agreement with this idea, overexpression of the mitochondrial fission protein Drp1 increases the density of PSD-95 puncta, spines and synapses. By electron microscopy analysis from mouse hippocampal slices, we studied the effects of Wnt5a on the ultrastructure of the postsynaptic terminal. We observed a time-dependent increase in the length of the postsynaptic density (PSD) in dendritic spines in response to Wnt5a and a latter increase in the number of synaptic contacts. We also observed an increase in the percentage of dendritic spines that present segmented PSD, which has been associated with the dendritic spine changes in response to LTP induction and synaptic potentiation. Therefore, we evaluated whether these morphological changes were associated with the distribution of mitochondria at the synapse. We observed that Wnt5a induced an increase in the number of dendritic protrusions with mitochondria and in the number of mitochondria in dendritic spines. This redistribution of mitochondria at the postsynaptic terminal was correlated with a decrease in mitochondrial area and with the activation of the Drp1, which suggest that Wnt5a induces mitochondrial fission probably to support the morphological changes that occurs on the dendritic spines to produce new synapses.

Disclosures: M.S. Arrázola: None. **D. Ordenes:** None. **N.C. Inestrosa:** None.

Poster

785. Spine Dynamics

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Program#/Poster#: 785.04/C7

Topic: B.08. Synaptic Plasticity

Support: NIH RO1NS062736

Title: The role of metabotropic and ionotropic glutamate receptor signaling in activity-dependent spine shrinkage

Authors: *I. S. STEIN[1], W. OH[2], K. ZITO[1];
[1]Ctr. for Neurosci., UC Davis, Davis, CA; [2]Max Planck Florida Inst., Jupiter, FL

Abstract: Structural refinement of neuronal circuits is thought to underlie experience-dependent modification of brain function. Pruning of superfluous synaptic contacts during development as well as input-specific elimination of synapses in the adult have been correlated with behavioral performance. We are especially interested in the signaling mechanisms leading to shrinkage and elimination of dendritic spines, the principal sites of excitatory synaptic input in the mammalian cerebral cortex. Morphological changes in dendritic spines are closely linked to changes in synaptic strength and function; increases in synaptic strength through the induction of long-term potentiation (LTP) are associated with spine enlargement, while decreases in synaptic strength via the induction of long-term depression (LTD) are associated with spine shrinkage or loss. Work from our lab recently demonstrated long-lasting synaptic weakening and spine shrinkage following low-frequency glutamatergic activity at individual spines. This input-specific spine shrinkage required NMDAR activation and, intriguingly, was differentially regulated in small and large spines. In addition to NMDAR activity, the shrinkage of large spines also was contingent on group I mGluR activation and the downstream release of Ca2+ through IP3Rs. We are currently investigating more closely the role of metabotropic and ionotropic glutamate receptor signaling in this activity-induced spine shrinkage using subtype- and binding site-specific antagonists.

Disclosures: I.S. Stein: None. **W. Oh:** None. **K. Zito:** None.

Poster

785. Spine Dynamics

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Program#/Poster#: 785.05/C8

Topic: B.08. Synaptic Plasticity

Title: Modeling spine dynamics in recurrently connected spiking networks

Authors: *J. HUMBLE[1], H. KASAI[2], T. TOYOIZUMI[1];
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Abstract: Cortical circuits rewire in an activity-dependent way. A major biological mechanism underlying this rewiring process is Hebbian plasticity. Hebbian plasticity is often regarded unstable in nature because of its positive feedback, e.g., as synapses become stronger, they are more efficient at driving their postsynaptic neurons and consequently get potentiated further. This kind of instability can manifest in a recurrently connected circuit with multiple loops of connections, which may cause non-physiological growth of neuronal activity and/or merge multiple patterns of stored memories unless a plasticity rule is finely tuned to provide stabilization and competition of synapses under a wide range of conditions. The biological mechanisms that achieve synaptic stabilization and competition under ongoing Hebbian plasticity are unknown. However, it was previously reported that intrinsic fluctuation of spine volume is roughly proportional to spine-size, and this fluctuation can gradually set the distribution of spine-sizes to a stereotypical shape in the absence of neural activity (Yasumatsu et al. 2008). In this study, we investigate the interaction of spike-timing-dependent plasticity (STDP) and fluctuations of synaptic strengths. Specifically, we demonstrate with a model of recurrently connected spiking neuron networks, that Hebbian (STDP) plasticity can be stabilized by intrinsic synaptic fluctuation, which increases linearly with synaptic strength. The model reproduces the previously reported distribution of synaptic strengths, with a fat tail, in the presence of ongoing Hebbian plasticity. Moreover, we show that persistent activation of arbitrary subsets of neurons forms independent cellular assemblies, which specifically and persistently elevate their firing rates and synaptic connections even after termination of the stimulus without causing synaptic instability or fusion of assemblies. Thus, memories of past stimuli are retained in our recurrent networks as cellular assemblies under noticeable fluctuations of synaptic strength.

Disclosures: J. Humble: None. **T. Toyoizumi:** None. **H. Kasai:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: KAKENHI 19200029

Title: Myosin II ATPase activity mediates the biphasic movement of stable F-actin bound by drebrin A between dendritic spines and the parent dendrite in long-term potentiation

Authors: T. SHIRAO[1], T. MIZUI[1], N. KOGANEZAWA[1], H. SHIMIZU[1], *H. YASUDA[1], Y. SEKINO[1,2];
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Abstract: The neuronal actin-binding protein drebrin A forms a stable structure with F-actin in dendritic spines. NMDA receptor activation causes an exodus of F-actin bound by drebrin A (DA-actin) from dendritic spines, suggesting a pivotal role for DA-actin exodus in synaptic plasticity. In the present study, we first used stochastic optical reconstruction microscopy (STORM) to reveal localization of drebrin in nanoscale. We prepared primary hippocampal neuronal culture from embryonic day-18 rat brain using Banker's method. For drebrin imaging, we fixed cultured neurons at 21 days *in vitro* (DIV) and immunolabeled drebrin with Alexa 568. The neurons were transfected with GFP to detect the neuron shape and it is also immunolabeled by anti-GFP antibody conjugated with Alexa 647 to do STORM analysis. Drebrin A is localized in the central region of dendritic spine heads at rest. We then quantitatively assessed the extent of DA-actin localization to spines using the spine-dendrite ratio of drebrin A in cultured hippocampal neurons, and found that (1) chemical long-term potentiation (LTP) stimulation induces rapid DA-actin exodus and subsequent DA-actin re-entry in dendritic spines, (2) Ca^{2+} influx through NMDA receptors regulates the exodus and the basal accumulation of DA-actin, and (3) the DA-actin exodus is blocked by myosin II ATPase inhibitor, but is not blocked by myosin light chain kinase (MLCK) or Rho-associated kinase (ROCK) inhibitors. These results indicate that myosin II mediates the interaction between NMDA receptor activation and DA-actin exodus in LTP induction. Furthermore, myosin II seems to be activated by a rapid actin-linked mechanism rather than slow MLC phosphorylation. Thus the myosin-II mediated DA-actin exodus might be an initial event in LTP induction, triggering actin polymerization and spine enlargement.

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Poster

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Topic: B.02. Ligand-Gated Ion Channels

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Title: The dynamics of interneuronal spines are regulated by N-methyl-D-aspartate receptors

Authors: *M. PEREZ-RANDO[1], E. CASTILLO-GOMEZ[1], R. GUIRADO[2], H. CARCELLER[1], J. NACHER[1];
[1]Cell Biol., Univ. of Valencia, Burjasot, Spain; [2]Neurosci. Ctr., Univ. of Helsinki, Helsinki, Finland

Abstract: Dendritic spines are membranous protrusions specialized in receiving neuronal input. Although they have been typically studied on excitatory pyramidal neurons, they are also present in some populations of inhibitory interneurons, playing the same role. These neurons express plenty of neurotransmitter receptors, but glutamate N-methyl-D-aspartate receptors (NMDAR) are especially important. Despite extensively studied in order to understand learning and memory, little is known about their role on the structural plasticity of interneurons. In this study we try to unravel how NMDAR influence the spine dynamics of a subpopulation of interneuronal dendritic spines of hippocampus. In order to do so, we use the highly selective NMDAR antagonist MK-801 on organotypical entorhino-hippocampal cultures, performed on transgenic mice that express the enhanced green fluorescent protein (eGFP) under the promoter of the glutamic acid decarboxylase (GAD67) gene. This technique allows us to study either longitudinally or tangentially spine dynamics and the relative spine density. In the present study, we describe how the apparition turnover rate of interneuronal spines is rapidly decreased 4 hours after the antagonist treatment, remaining low 24 hours later. In addition, we report a decreased relative spine density 24 hours after the treatment. Since NMDAR hypofunction on inhibitory networks is the most accepted hypothesis explaining the molecular basis of schizophrenia, this study can also help to shed light on this important subject.

Disclosures: M. Perez-Rando: None. **E. Castillo-Gomez:** None. **R. Guirado:** None. **J. Nacher:** None. **H. Carceller:** None.

Poster

785. Spine Dynamics

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Topic: B.07. Synaptic Transmission

Title: A novel RhoGAP, ARHGAP12, interacting with F-BAR protein CIP4 regulates spine morphology and AMPA receptor function

Authors: *W. BA[1], J. VAN DER RAADT[2], M. M. SELTEN[1], L.-L. LI[3], M. BENEVENTO[1], A. R. OUDAKKER[2], H. VAN BOKHOVEN[2], M. J. COURTNEY[3], N. NADIF KASRI[1];
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Abstract: Activity-dependent changes in the strength of excitatory synapses are thought to be key cellular mechanisms that contribute to the plasticity of neuronal networks underlying learning and memory. Key mechanisms for the regulation of synaptic strength are the dynamic change in size and number of dendritic spines and the synaptic incorporation and removal of AMPA-type glutamate receptors (AMPAr). As key regulators of the actin cytoskeleton the Rho subfamily of GTP-binding proteins play a critical role in synaptic development and plasticity. They shuttle between the active GTP- bound form and the inactive GDP- bound form under the regulation of dedicated guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). More than 80 human GEFs and GAPs have been identified, most of which are expressed in the brain with a specific spatial and temporal expression pattern. Surprisingly, the cellular function of most GEFs and GAPs in the brain has not yet been elucidated. Here, we report the functional characterization of ARHGAP12, a RhoGAP that negatively regulates Rac1 signaling. ARHGAP12 is specifically expressed in the CA1 and dentate gyrus region of the hippocampus and its expression decreases after the first postnatal week, coinciding with the maturation process of excitatory synapses. At the cellular level ARHGAP12 localizes to the postsynaptic site of mature excitatory synapses. Via a direct interaction with the F-BAR-containing protein CIP4, ARHGAP12 bidirectionally regulates spine volume and the ratio of mature and immature spines of CA1 pyramidal neurons. Overexpression of ARHGAP12 in hippocampal neurons reduces the number of excitatory synapses and dramatically decreases the frequency and amplitude of AMPAr-mediated miniature excitatory postsynaptic currents. Conversely downregulation of Arhgap12 rapidly converted silent synapses to active synapses,

therefore enhancing AMPAr-mediated excitatory synaptic transmission. Together our data suggest that endogenous ARHGAP12 functions as a synaptic break during development and can bidirectionally regulate excitatory synaptic strength. Interestingly, Arhgap12 mRNA was recently identified as a target of Fragile X mental retardation protein, which regulates local protein translation during metabotropic glutamate receptor LTD. In accordance we found that ARHGAP12 is rapidly translated in response to mGluR activation in and is required for mGluR-LTD expression in hippocampal brain slices. Together our data show that ARHGAP12 is a novel synaptic RhoGAP that plays a critical role in regulating excitatory synaptic structure and function.

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Poster

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Title: NOS1AP regulates synapse formation and synaptic strength in rat cortical neurons

Authors: *K. HERNANDEZ[1], P. SWIATKOWSKI[1], N. R. DUDZINSKI[1], L. M. BRZUSTOWICZ[2], B. L. FIRESTEIN[1];
[1]Cell Biol. and Neurosci., [2]Genet., Rutgers Univ., Piscataway, NJ

Abstract: Proper neuronal circuitry is dependent upon the appropriate dendritic patterning of neurons as well as spine formation and maintenance. Schizophrenia is one of several neurodevelopmental disorders that are characterized by alterations in dendrite branching and spine density, resulting in the manifestation of the disease phenotype. NOS1AP (nitric oxide

synthase 1 [neuronal] adaptor protein) is a protein encoded by a schizophrenia susceptibility gene, and studies from the Firestein and Brzustowicz laboratories have shown that its expression is upregulated in the dorsolateral prefrontal cortex of patients with schizophrenia. In addition, our laboratory reported that NOS1AP negatively regulates dendrite branching in cultured rat hippocampal cells. The regulation of actin polymerization and depolymerization is important to the formation of new branch points and spines, which are small actin-rich protrusions, and thus, we studied the association of NOS1AP with actin. Using a co-immunoprecipitation assay, we found that the short isoform of NOS1AP (NOS1AP-S) associates with F-actin in adult rat brain, further strengthening a possible role for NOS1AP in actin dynamics. To elucidate NOS1AP's role in synapse formation and synaptic strength, we cultured rat cortical neurons exogenously overexpressing NOS1AP during the developmental time points of synapse formation and maturation. Both the short (NOS1AP-S) and long (NOS1AP-L) isoforms of NOS1AP increase spine density when overexpressed in cortical neurons from day *in vitro* (DIV) 14 to DIV 17. Using mutants of NOS1AP-L, we show that the PDZ-binding motif, which is present in both NOS1AP-S and NOS1AP-L, is necessary for mediating the effects of NOS1AP on spine density. The ability of NOS1AP to compete with PSD-95 for binding to NOS1 via its PDZ binding motif suggests that NOS1AP may sequester NOS1 away from NMDA receptors. The decoupling of NOS1 and NMDA receptors would prevent the activation of NOS1 and its downstream signaling pathways. To study the synaptic strength of cortical cells overexpressing NOS1AP, whole-cell patch clamping was performed. We find that NOS1AP alters the amplitude and frequency of excitatory postsynaptic currents (EPSCs). Our findings show that NOS1AP alters neuronal morphology as well as synaptic function.

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Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: NIH [NCRR-RR003037]

PSC CUNY[66663-0044]

Title: Juvenile rats exposed to environmental enrichment show increased densities of long-thin and filopodia spines, clustering of GluA2 and PSD-95 in the hippocampus and enhanced spatial learning

Authors: *A. AUBRY[1,2], A. ALLIGER[2], D. MUSHEYEV[2], J. AVILA[1,2], P. SERRANO[1,2]; [1]CUNY Grad. Ctr., New York, NY; [2]Hunter Col., New York, NY

Abstract: Environmental enrichment (EE) has been shown to increase synaptic plasticity and improve learning and memory. As a result of EE, many studies have identified increases in overall density of dendritic spines across various brain regions using traditional Golgi staining. Recent advances in both Golgi and imunnohistochemistry techniques now allow for the identification of specific spine types and the expression of synaptic proteins within these spines (Sebastian et al 2013, PLoS One, vol8, e79077). We focused on four distinct spine types (filopodia, stubby, long-thin, and mushroom) and two synaptic markers (GluA2 and PSD-95), which are found to cluster with protein kinase M zeta (PKMζ) during episodes of learning and synaptic plasticity. We therefore investigated the effects of 6 weeks of EE on spatial learning, spine morphology and the clustering of these synaptic markers in juvenile rats. Following 6 weeks of EE or standard housing conditions brains were prepared for Golgi-IHC. Spine morphology and the expression of GluA2 and PSD-95 were quantified with IMARIS software. Our results show that in the stratum radiatum of area CA1, EE increased the density of filopodia and the co-localization of GluA2 and PSD-95 within this spine type. In CA3, EE increased the density of long-thin spines, the expression of PSD-95 and the co-localization with GluA2. Filopodia and stubby spines in CA3 also reflected EE-induced increases in the expression PSD-95 and the co-localization with GluA2. Spatial learning was assessed during the 6th week of EE or standard housing treatment. For spatial learning assessment, animals were given 3 days of radial-8 arm maze training (10 trials per day/3d) where 4 of 8 arms were baited with sweetened oatmeal mash, which served as a food reward. We quantified daily acquisition performance as percent correct (number of correct arm entries divided by the total number of arm entries per trial). EE animals exhibited significantly higher percent correct scores compared to standard housed animals. Our results suggest that EE can increase immature spine types that are associated with improved learning. Together with the increase in the co-localization of GluA2 and PSD-95, EE may be acting to lower the threshold to induce morphological changes in the immature spine types resulting in larger, more stable spines (mushroom and long-thin) following learning. These morphological and neurochemical changes may reflect a mechanistic explanation for the increased acquisition of a spatial memory observed in rats reared in an enriched environment.

Disclosures: **A. Aubry:** None. **A. Alliger:** None. **D. Musheyev:** None. **J. Avila:** None. **P. Serrano:** None.

Poster

785. Spine Dynamics

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Topic: B.07. Synaptic Transmission

Support: NIH Grant NS39444

Title: Comparison of axospinous synapses onto glutamatergic and GABAergic forebrain neurons

Authors: *R. J. WEINBERG[1], E. KIM[3], A. C. BURETTE[2];
[1]Cell & Developmental Biol., [2]Cell Biol. & Physiol., Univ. North Carolina, CHAPEL HILL, NC;
[3]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: The dendrites of principal cells in the mammalian forebrain are covered with dendritic spines, which provide the main target for excitatory afferent input. These axospinous synapses exhibit a characteristic "asymmetric" morphology at the electron microscope, displaying a pronounced post-synaptic density. Accumulating evidence shows an important role for synaptic size: synaptic contacts with large surface area (typically between large presynaptic terminals and large postsynaptic spines) are stabler and express more AMPA receptor than small contacts, but it remains unclear whether there are other differences between the axo-spinous synapses on different types of principal cells in forebrain. The pyramidal cells of hippocampus and cortex are glutamatergic, whereas the medium spiny neurons (MSNs) of neostriatum are GABAergic, leading us to investigate possible differences in the structure and molecular organization of synapses onto these two basic cell types. The two classes of axospinous synapses have PSDs of similar length and thickness, but they appear to display different curvature: the postsynaptic membrane of spines on pyramidal cells typically exhibits a flat or convex curvature, whereas the postsynaptic membrane of spines on MSNs is often concave. Perhaps related to this, we find that the I-BAR protein IRSp53 lies preferentially at the center of the synapse in pyramidal cells, but is uniformly distributed along the synapse in MSNs. In contrast, our current evidence suggests only subtle differences in the organization of glutamate receptor subunits in these two types of synapses.

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Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH094268

Title: Aberrant spine pruning in adolescence in a model of schizophrenia and related disorders

Authors: *S. K. BARODIA, J. R. MOORE, S.-H. KIM, A. SAWA, H. JAARO-PELED; Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Disturbances in glutamatergic synaptic connectivity underlie schizophrenia (SZ) and associated disorders. One major question is whether reduction of spine density frequently observed in autopsied brains from SZ patients occurs by excess synaptic pruning in adolescence or malformation of synaptic connection from early development. It is very difficult to address this question in human brains. Thus, animal models that represent SZ-relevant endophenotypes in behaviors, neurochemistry, and histology may provide an opportunity to address this question. Among multiple choices that satisfy these criteria of animal models, we chose a transgenic model expressing a putative dominant-negative DISC1 (DN-DISC1 Tg) (Hikida, Jaaro-Peled et al, PNAS 2007; Johnson, Jaaro-Peled et al PNAS 2013; Jaaro-Peled et al, Hum Mol Genet 2013). Involvement of DISC1 in synaptic dynamics has also been reported (Takagi-Hayashi et al, Nat Neurosci 2010; Brandon et al, Nat Rev Neurosci 2011). In the present study, we examined the spine change in the two regions of the prefrontal cortex in the time course from early adolescence to adulthood. Heterozygous line from DN-DISC1 mouse was crossbred with homozygous line of Thy-1YFP mouse to genetically label pyramidal neurons selectively in layer 5/6. Dendritic spine density was assessed at early adolescence (P28), late adolescence (P49) and adulthood (P90) in the medial prefrontal cortex and ventrolateral orbitofrontal cortex. We did not observe robust difference in the spine density at P28, whereas major spine loss existed at P90, between DN-DISC1 and control mice. Interestingly, dramatic difference between these two groups occurred between P28 and P49, but not between P49 and P90. This suggests the existence of pathological pruning in adolescence in DN-DISC1 model. It is under investigation how administration of PAK inhibitors in late adolescence can ameliorate the spine change and adult behaviors.

Disclosures: S.K. Barodia: None. **J.R. Moore:** None. **S. Kim:** None. **A. Sawa:** None. **H. Jaaro-Peled:** None.

Poster

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Topic: B.07. Synaptic Transmission

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Title: Dendritic spine neck resistance measurements using voltage sensitive dyes

Authors: M. POPOVIC[1], N. CARNEVALE[1], B. ROZSA[2], *D. ZECEVIC[3];
[1]Yale Univ., New Haven, CT; [2]Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary; [3]Dept Cell & Mol. Physiol, Yale Univ. Sch. Med., NEW HAVEN, CT

Abstract: Whether dendritic spines play an electrical role in signal processing based on hypothetical electrical isolation of the spine head from the parent dendrite by elongated spine necks is an open question. There is no direct information on the electrical resistance of the spine neck, the critical functional variable in this context. We investigated the transfer of unitary excitatory postsynaptic potentials (EPSPs) from synapses on spine heads across the spine neck to the parent dendrite using high resolution voltage sensitive dye imaging in the wide-field epi-fluorescence microscopy mode. L5 pyramidal neurons in mouse cortical slices were labeled by intracellular application of a membrane impermeable voltage-sensitive dye. To improve the signal-to-noise ratio in monitoring electrical events from individual spines we advanced the sensitivity of recording by: (a) using monochromatic excitation light from a laser at a near-optimal wavelength; (b) increasing the excitation light intensity to a level close to chromophore saturation; (c) by restricting the illuminated area to a small square region (18 x 18 μm) around the recorded spine and (d) briefly reducing the oxygen concentration in the extracellular medium during recording to minimize photodynamic damage. Unitary EPSPs were evoked by localized high-speed iontophoretic glutamate ejections from extracellular sharp electrodes onto individual spines. Alternatively, synapses were activated using focal 2-photon photolysis of caged glutamate with diffraction limited spot of 720 nm laser light. Somatic patch-electrode

measurements verified that the size and the kinetics of evoked excitatory postsynaptic currents (EPSCs) and potentials (EPSPs) were comparable to the spontaneously occurring events. Optical recordings of evoked subthreshold signals from dendritic spines showed that a temporal average of 9-25 trials was sufficient to record EPSPs that had somatic amplitude in the range of 0.3-0.8 mV with the S/N of >5. The ratio of recorded peak EPSP amplitudes in the spine heads/parent dendrites was close to 1. An attenuation ratio (EPSPspine/EPSPdendrite) of ~1 is in agreement with diffusion equilibration studies as well as with multicompartmental modeling (NEURON 7.2), indicating that the axial resistance of the spine neck is much smaller than the dendritic input resistance in most areas of the dendritic arbor. The time course of the signal from the spine head and from the parent dendrite was practically identical. This result indicates that the small membrane surface area of both the spine head and the dendritic compartment immediately below the spine provided negligible local capacitance.

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Poster

785. Spine Dynamics

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Topic: B.07. Synaptic Transmission

Title: Morphological and Functional analysis of glutamatergic synapses in the dopamine neurons of the Substantia nigra pars compacta

Authors: ***M. JANG**, M. PARK;
Physiol., Sch. of Medicine, Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Midbrain dopamine neurons play critical roles in motivation and reward-based learning through glutamatergic signaling. Dopamine neurons of the substantia nigra pars compacta (SNc) are known to receive approximately 8,000 synaptic inputs and among them at least 30% are glutamatergic. However, little is known about the morphological and functional properties of glutamatergic synapses in the dopamine neuron dendrites. Here, using two-photon confocal microscopy we demonstrate morphological features of dendrites and typical structures of glutamatergic synapses in the dopamine neurons of the mouse SNc. The SNc dopmaine neurons in brain slices showed simple and low-branching dendritic arborizations, when

compared to those of CA1 pyramidal neurons. Interestingly, we found that there were three types of common dendritic spines in the SNc dopamine neurons; the thin, mushroom, and stubby spines, whose dimensions were similar to those of CA1 pyramidal neurons. However, the SNc dopamine neurons had a very low number of spines than CA1 pyramidal neurons. By analysis of PSD-95, GluR1 and GluN1 expressions with immunostaining and glutamate uncaging techniques, we identified functional dendritic spines for glutamatergic synapses in dopamine neurons which had a different AMPAR/NMDAR ratio to those in dendritic shafts. Therefore, we first provide a new piece of evidence showing the morphological and functional substrate for glutamatergic synapses in the SNc dopamine neurons.

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Poster

785. Spine Dynamics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 785.15/C18

Topic: B.08. Synaptic Plasticity

Support: Whitehall Foundation Research Grant

Title: Repeated ketamine exposure during early development impairs learning-dependent dendritic spine plasticity in adulthood

Authors: L. HUANG, *G. YANG;
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Abstract: General anesthetics are commonly used for surgical operations in infants and young children. Recent studies in rodents and monkeys suggest that repeated and prolonged anesthetic exposure, during early postnatal development, can lead to cognitive and behavioral impairments later in life. However, the mechanisms underlying anesthesia-related learning impairment remain unclear. Here, we have investigated motor learning and learning-dependent synaptic plasticity in adult mice that received repeated anesthetic exposure during development. We found that multiple ketamine (20 mg/kg)-xylazine (3 mg/kg) (KX) injections at early (postnatal days 7-18), but not late stage (postnatal days 21-25) of brain development, impair the animals' motor skill learning in a rotarod running task in adulthood. Using *in vivo* transcranial two-photon microscopy to track the formation and elimination of postsynaptic dendritic spines in the primary motor cortex, we observed no significant difference in either dendritic spine density or baseline

spine turnover rates between KX- and saline-treated mice. However, motor learning-induced formation of new dendritic spines is significantly reduced in adult mice with early KX treatments. Notably, enriched motor experience for ten days following anesthetic exposure ameliorates anesthesia-induced behavioral and synaptic deficits. Taken together, our study demonstrates that repeated exposure to ketamine-xylazine during early development impairs learning and learning-dependent dendritic spine plasticity in adulthood. Furthermore, these deficits can be reduced by environmental enrichment following anesthetic exposure.

Disclosures: L. Huang: None. **G. Yang:** None.

Poster

785. Spine Dynamics

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NIH RO1NS027177-26 to RYT

NIH Grant GM103412 to MHE

NIA fellowship F32AG039127 to JNS

Title: Very long-term memories may be stored in the pattern of holes in the perineuronal net

Authors: *V. LEV-RAM[1], E. A. BUSHONG[2], J. N. SAVAS[4], D. C. PRASHER[3], S. F. PALIDA[3], J. R. YATES, 3rd[4], M. H. ELLISMAN[2], R. Y. TSIEN[5];
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Abstract: We are carrying out several experiments to test the hypothesis that life-long memories are stored as the pattern of holes in the perineuronal net (PNN) (R.Y.Tsien PNAS 2013). The PNN is a specialized form of extracellular matrix deposited around selected neurons during critical periods of development in specific parts of the brain, interrupted by holes where synapses occur. We postulate that the PNN is a long-lived structure and that new memories are created by cutting new holes in the PNN or expanding existing holes to enable formation of new synapses or to strengthen existing ones. There is much circumstantial evidence implicating the PNN in synaptic plasticity. If correct, the PNN would be something like an enormously convoluted punched card, with information continuously being stored in the location and size of

the interruptions in its coverage of the neuron - what we are calling holes. A basic premise of this hypothesis is that the card stock, i.e. the bulk of the PNN, should not undergo metabolic renewal from the first age at which memories are retained until senescence, whereas the active constituents of synapses i.e. membranes, receptors, channels, kinases, phosphatases, intracellular scaffolds, etc. turn over much more frequently and would therefore be poorer substrates for permanent information storage, unless they are equipped with incredibly accurate copying mechanisms. We are now testing this hypothesis using several experimental approaches: 1. The intertwining of PNN and synapses is being imaged by Serial Block Face Scanning EM to more fully reconstruct their 3D relationship. 2. Lifetimes of PNN proteins vs. intrasynaptic components are compared using mass spectroscopy and Stable Isotope Labeling in Mammals (SILAM). We grow mice on a ^{15}N spirulina diet until postnatal day 45, just beyond the final brain critical period and maturation of the PNN. Thereafter, the mice are fed a ^{14}N normal diet. At progressive times from the end of the ^{15}N pulse, different brain areas are prepared for Multidimensional Protein Identification Technology (MudPIT) to identify interesting proteins and those with the lowest turnover, i.e. $^{14}N/^{15}N$ ratio. 3. The role in very long-term plasticity of matrix metalloproteinase-9 (MMP-9), one candidate for cutting new holes or enlarging existing holes in the PNN, is tested in genetic knockouts and with pharmacological inhibitors during cued and contextual fear conditioning. We will present preliminary data from these three types of experiment to investigate the hypothesis that the PNN plays a role in life-long memory.

Disclosures: V. Lev-Ram: None. **E.A. Bushong:** None. **S.F. Palida:** None. **M.H. Ellisman:** None. **R.Y. Tsien:** None. **D.C. Prasher:** None. **J.N. Savas:** None. **J.R. Yates:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: Hungarian Scientific Research Fund OTKA#K83830

Bólyai János Research Fellowship of the Hungarian Academy of Sciences

Research Faculty Grant 2014 of Szent István University, Faculty of Veterinary Science

Title: Ultrastructural changes in hippocampal synaptic architecture caused by reduced food intake

Authors: *B. L. RACZ, R. B. BABITS, T. MAGYAR, D. NOVÁK-HAZAI, P. SÓTONYI; Szent Istvan Univ., Budapest, Hungary

Abstract: Neuronal mechanisms regulating food intake are a topic of current interest. Behavioral patterns of food intake involve learning and memory functions. The hippocampus plays a key role in encoding relationships between internal states (e.g. hunger) and action, providing a mechanism by which motivation and memory are coordinated to guide behavior. Learning paradigms can induce synaptic plasticity and physiological changes in the hippocampus, but it is unclear whether metabolic state can induce synaptic plasticity. Recent evidence shows that intestinal hormones (e.g. ghrelin) may initiate synaptogenesis in hippocampus. As activity-dependent 'morphing' of spines is linked to changes in synaptic efficacy, we studied spine-rich areas (CA1 and dentate gyrus) pivotal in long-term synaptic plasticity. Using quantitative electron microscopy we analyzed the effect of food restriction (FR) on the biophysical characteristic of hippocampal spines. We found that short-term FR induces significant ultrastructural changes in the architecture of hippocampal synapses. Interestingly, these changes were similar to those observed during long-term potentiation.

Disclosures: B.L. Racz: None. **R.B. Babits:** None. **P. Sótonyi:** None. **D. Novák-Hazai:** None. **T. Magyar:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: NH grant MH092809

NSF graduate research fellowship

Title: Impermanence of dendritic spines in live adult CA1 hippocampus

Authors: *A. ATTARDO[1,2], J. E. FITZGERALD[1,3], M. J. SCHNITZER[1,2]; [1]Stanford Univ., Stanford, CA; [2]Howard Hughes Med. institute, Chevy Chase, MD; [3]Harvard Univ., Cambridge, MA

Abstract: The mammalian hippocampus is crucial for episodic memory formation and transiently retains information for ~3-4 weeks in adult mice and longer in humans. Although

neuroscientists widely believe neural synapses act as elemental sites of information storage, there has been no direct evidence hippocampal synapses persist for time intervals commensurate with the duration of hippocampal-dependent memory. Here we experimentally tested the prediction that the lifetimes of hippocampal synapses match the measured longevity of hippocampal memory. For this test, we monitored synapses by imaging dendritic spines, where most excitatory synapses have their post-synaptic structures and whose turnover dynamics are thought to reflect those of synaptic connections. By using time-lapse, two-photon microendoscopy we tracked CA1 pyramidal neurons' basal dendritic spines to investigate their dynamics over multiple weeks in live mice. Mathematical modeling revealed that, among a wide class of kinetic models, the data best matched models in which there is a single population of dendritic spines with a mean lifetime of ~1-2 weeks. This implies ~100% turnover in ~2-3 times this interval, a near full erasure of the synaptic connectivity pattern. Environmental enrichment did not alter these dynamics, whereas NMDA receptor blockade caused a temporary increase in spine loss. Together, these results quantitatively support the idea that the transience of hippocampal memory may directly reflect the turnover dynamics of the synapses that store these memories. Moreover, the turnover dynamics of CA1 dendritic spines appear distinct from those in neocortex, with a far greater fraction of spines undergoing turnover in hippocampus but with a slower time constant. These differences may reflect the different functional roles for hippocampus and neocortex in memory formation and storage. Our work also paves the way to visualization of synaptic dynamics in other deep brain areas and the cell biological mechanisms by which the brain encodes aspects of the external world and life experiences.

Disclosures: **A. Attardo:** None. **J.E. Fitzgerald:** None. **M.J. Schnitzer:** None.

Poster

785. Spine Dynamics

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Program#/Poster#: 785.19/C22

Topic: B.08. Synaptic Plasticity

Title: Picomolar beta-amyloid modulates hippocampal synaptic plasticity via inhibitor of apoptosis protein regulation of caspase-3 activity and rho gdp dissociation inhibitor

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Abstract: Picomolar concentrations of beta-amyloid (Abeta) positively modulates hippocampal synaptic plasticity and memory, whereas nanomolar and higher concentrations lead to inhibition of long-term potentiation (LTP), loss of spines and eventually neuronal death. We have previously shown that the toxic effects of Abeta are mediated by activation of caspase-2, despite concurrent activation of caspase-3 (caspase-3 is neither necessary nor sufficient for toxicity). In the current work we examine the function of caspase-3 in the effects of pM Abeta. Treatment of primary hippocampal neurons with 300 pM Abeta leads to a rapid increase in spine density that is accompanied by a rapid increase in caspase-3 activity in purified synaptosomes. Effects are seen within 30 minutes of treatment of the cultures; injection of 300 pM Abeta *in vivo* also causes an increase in spine density. As this effect is rapid, we posited that the synapses contain cleaved (activated) caspase-3 that is inhibited by endogenous inhibitor of apoptosis proteins, IAPs. We found that the synaptosomal fraction contains cIAP1 and XIAP, and co-immunoprecipitation (co-IP) shows that there are complexes of cIAP1-cleaved caspase-3 and XIAP-cleaved caspase-3. Treatment of cultures with 300 pM Abeta induces a decrease in the cIAP1-cleaved caspase-3 interaction, but there is no change in the XIAP-cleaved caspase-3 interaction, suggesting that the increase in caspase-3 activity is modulated by cIAP1. Actin, the major component of spines, is a substrate of caspase-3, and we find that Abeta leads to an increase in cleaved actin (f-actin). siRNA knockdown of caspase-3 prevents the effects of Abeta and siRNA knockdown of cIAP1 potentiates the effects of Abeta on spine density. Surprisingly we found that siRNA knockdown of XIAP prevented the effects of Abeta. A recent study has shown that XIAP can bind to RhoGDI (Rho GDP dissociation inhibitor), leading to an increase in f-actin. Co-IP carried out in our lab shows that 300 pM Abeta increases the XIAP-RhoGDI interaction. Our data show that spine dynamics can be regulated by Abeta through both induction of caspase-3 activity by a decrease in cIAP1 levels and sequestration of RhoGDI by XIAP.

Disclosures: **M. Pero:** None. **E.M. Ribe:** None. **Y.Y. Jean:** None. **C.M. Troy:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: DFG KO1674/8-1

Title: Profilin isoforms as important modulators of structural spine plasticity and memory formation

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Abstract: Actin dynamics in neurons are equally important for structural stability, allowing proper signal transduction, and for plastic changes of neuronal structure. This tremendous task is fulfilled with the help of an ever increasing number of factors discovered to tightly regulate actin polymerization and F-actin structure in space and time. In our work, we concentrated on the neuronal profilin isoforms PFN1 and PFN2a regulating actin polymerization and known to be recruited to dendritic spines in an activity-dependent manner. Here, we were interested in the role of PFN1 and PFN2 for neuronal function and structure in murine hippocampal neurons. Fluorescence recovery after photobleaching (FRAP) using GFP-actin revealed alterations of spine actin dynamics in loss of function experiments in organotypic slices cultures using biolistic gene transfer of shRNAs against PFN1/ PFN2a. The loss of PFN1 led to an increase of the stable F-actin fraction in dendritic spines whereas shPFN2a had the opposite effect. As a next step, long-term potentiation (LTP) was induced via a chemical protocol in primary hippocampal cultures as well as in hippocampal slice cultures using Glycine. Calcium imaging experiments showed that synapses of PFN1/ PFN2a deficient cells were equally potentiated compared to control neurons. In contrast to this, PFN2a was found to be crucially involved in activity-dependent structural plasticity as spine head growth following LTP induction was prevented specifically in PFN2a deficient CA1 neurons. Finally, we investigated expression patterns of profilins following spatial memory formation in the Morris Water Maze (MWM) in the mouse model of the fragile X syndrome (FXS, Fmr1 KO mice). It was shown previously that the mRNA of the *Drosophila* homolog of profilin (chickadee) is bound by the fragile x mental retardation protein (FMRP). Interestingly, we found an impairment in spatial memory formation in Fmr1 KO animals together with a significant elevation of PFN1 expression levels in Fmr1 KO mice but not in WT littermates after spatial training. Most notably, PFN2a levels were not altered compared to WT littermates. This finding is in line with our results from co-immunoprecipitation studies where we could identify the mRNA of PFN1 but not PFN2a as a target of FMRP. Taken together our results indicate that both profilin isoform can perform even opposing roles - stabilizing versus destabilizing - on spine actin dynamics and that only the neuron specific isoform PFN2a is involved in activity-dependent structural plasticity. In addition, we could identify PFN1 but not PFN2a as part of the molecular mechanisms underlying FXS.

Disclosures: K. Michaelsen-Preusse: None. **S. Zessin:** None. **M.H. Korte:** None. **F. Scharkowski:** None. **J. Feuge:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: Founded by the DFG (ZA 554/3-1)

Title: Mechanism of Nogo-A action in regulating functional and anatomical synaptic plasticity

Authors: *M. ZAGREBELSKY[1], Y. KELLNER[1], S. KRAMER[2], M. E. SCHWAB[2], M. KORTE[1];
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Abstract: The functions of neural circuits in the adult brain are determined by the architecture of axons and dendrites and of the synaptic structures connecting them. Changes in synaptic connectivity - structural plasticity - have been correlated to functional changes at synapses - functional plasticity - and are thought to underlie learning and memory processes. Yet, long-term *in vivo* imaging in the adult brain reveals that the overall architecture of the neuronal network is remarkably stable. These observations suggest the need for a set of molecules regulating the balance between stability and plasticity of mature neuronal networks, ensuring the spatial and temporal specificity of plastic changes and preventing interference between different memory events. The myelin-associated neurite growth inhibitor Nogo-A and its receptors, Nogo-66 receptor 1 (NgR1) and sphingosine 1-phosphate receptor 2 (S1PR2) are expressed pre- and post-synaptically and have been shown to negatively modulate neuronal architecture and to control synaptic plasticity, by restricting long-term potentiation (LTP). However, whether Nogo-A regulates structural plasticity at a fast time scale and what the molecular mechanisms mediating this action are, is so far unknown. Here we addressed this question by combining time-lapse confocal imaging, Fluorescence recovery after photobleaching (FRAP) and calcium imaging with a series of loss- or gain-of-function approaches for Nogo-A and its receptors in mature hippocampal cultures. Our results show that Nogo-A signalling acutely modulates the spine actin cytoskeleton dynamics to control structural plasticity at dendritic spines at a fast time scale. Indeed, Nogo-A signalling loss-of-function experiments transiently increase F-actin stability within minutes and results in an increase in dendritic spine density and length. In addition, Nogo-A acutely restricts AMPA receptor (AMPAR) insertion and formation of new AMPAR clusters. Finally, calcium imaging reveals a role for Nogo-A in negatively modulating synaptic strength and number of active synapses at spines. Our data provide a cellular and molecular mechanism

mediating the role of Nogo-A signalling in controlling activity-dependent synaptic plasticity thereby maintaining the balance between the plasticity and stability of the neuronal circuitry in the mature central nervous system.

Disclosures: M. Zagrebelsky: None. **S. Kramer:** None. **M.E. Schwab:** None. **M. Korte:** None. **Y. Kellner:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: NSF-GRFP

Alfred P. Sloan Foundation

Title: The role of alpha2-chimaerin in dendrite morphogenesis and synaptic plasticity

Authors: *C. VALDEZ, A. A. BEG;
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Abstract: Neurons maintain a dynamic cellular morphology that can be modulated in response to synaptic activity. Dendritic spines are fine neuronal processes that can balance neuronal structure and function. These fine structural components localize biochemical signals and spatially restrict the recruitment of membrane receptors to activated synapses. The superfamily of Rho-GTPases have been shown to modulate cytoskeletal proteins that regulate dendritic arborization and spine shape. Importantly, these proteins can form activity-dependent complexes with signaling molecules that mediate surface levels and post-translational modifications of synaptic receptors. Our laboratory studies a Rac1 GTPase activating protein named alpha2-chimaerin. We have detected that alpha2-chimaerin is predominantly expressed during timepoints that coincide with developmental stages of synaptic connectivity. Our goal is to determine if the loss of alpha2-chimaerin results in long-term changes to dendritic morphology and synaptic transmission. We hypothesize that alpha2-chimaerin is important for neuronal development, and the subsequent loss results in long-term changes to dendritic morphology, synaptic function and behavior.

Disclosures: C. Valdez: None. **A.A. Beg:** None.

Poster

785. Spine Dynamics

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Topic: A.06. Synaptogenesis and Activity-Dependent Development

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Alzheimer's Association Grant NIRG

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Title: Regulation of spine architecture by δ-catenin

Authors: *L. YUAN[1,2], E. SEONG[1], J. L. BUESCHER[1], J. ARIKKATH[1];
[1]Developmental neuroscience, [2]Pharmacol. and experimental neuroscience, Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: The cadherin-catenin complex regulates multiple aspects of neural and synaptic development and plasticity. δ-catenin is a component of the cadherin-catenin complex and is predominantly expressed in the central nervous system. δ-catenin associates with cadherin at the juxtamembrane domain. We have previously demonstrated that loss or knockdown of δ-catenin leads to an increase in spine density and synaptic function. We now present evidence for a critical role for δ-catenin in regulating synaptic architecture. Loss or knockdown of δ-catenin leads to a decrease in spine headwidth and length in primary cultured rat neurons. The ability of δ-catenin to induce alterations in synaptic architecture is dependent on its ability to associate with cadherin. We are currently examining the molecular and cellular basis of how the cooperation between cadherin and δ-catenin contribute to the control of synaptic architecture and synaptic structural plasticity.

Disclosures: L. Yuan: None. **E. Seong:** None. **J.L. Buescher:** None. **J. Arikkath:** None.

Poster

785. Spine Dynamics

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R37-AG06647

Title: Morphometric analysis of human pyramidal neurons of Brodmann Areas 39 and 40

Authors: J. C. ZINN[1], W. G. M. JANSSEN[2], J. EVANS[5], A. D. SHARAN[5], M. R. SPERLING[6], K. SIMONYAN[1], *P. R. HOF[3], J. H. MORRISON[2], F. HAMZEI-SICHANI[4,5]; [1]Neurol., [2]Neurosci., [4]Neurosurg., [3]Icahn Sch. of Med. At Mount Sinai, NEW YORK, NY; [5]Neurosurg., [6]Neurol., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Although previous studies have described geometric properties of human neurons, the quality of tissue has prevented reliable characterization of structure on the sub-micrometer scales that characterize crucial structures of neurons such as dendritic spines. Here, we present detailed, high-resolution analyses of layer II/III and layer V pyramidal neurons of two adult patients with epilepsy. Regions were identified as functionally normal via intracranial EEG and cortical mapping. High quality Lucifer Yellow filled neurons were selected and imaged at high magnification (63X, 1.4 N.A. 100 nm cubic voxel) using confocal laser scanning microscopy. Neuronal morphology was extracted from stacks of scanned images using a custom-designed algorithm (NeuronStudio). Morphometric analysis provided unbiased measurements of both local and global structure of the pyramidal neurons. In an effort to explore parameters of interest to electrotonic structure of neurons, spine volume and density were described as a function of spine type (mushroom or thin) and the parent dendrite (basal vs. apical). Gamma but not normal distributions were found to be more consistent with observed distribution of volumes of both types of spines in pyramidal cells of Brodmann areas 39 and 40. Nonparametric post-hoc analysis was therefore performed on spine volume measurements. Taken together, cells indicated significantly positive differences in mean volumes of basal and apical mushroom spines ($p < 0.001$, corrected). Basal mushroom spines were found to have average densities greater than both those found on apical dendrites ($p < 0.001$, corrected) and basal & apical spines ($p < 0.001$, corrected), using bootstrapped spine counts. In general, basal mushroom spines populated the neurons more densely than did thin spines ($p < 0.001$, corrected). The distribution of spine densities across type showed increases in density out to 100 micrometers away from the soma. Our preliminary findings offer insights into the functioning of healthy cells in Brodmann areas 39 and 40, which will prove useful for future modeling of human neocortical areas. In particular, differences in mushroom morphology as a function of position on basal or apical dendrites suggest specialization of basal/apical mushroom spines in neuronal signaling. Further study will

address other factors such as spine clustering and dendritic width that also affect eletrotonic structure of human neocortical pyramidal neurons.

Disclosures: **J.C. Zinn:** None. **W.G.M. Janssen:** None. **J. Evans:** None. **A.D. Sharan:** None. **M.R. Sperling:** None. **K. Simonyan:** None. **P.R. Hof:** None. **F. Hamzei-Sichani:** None. **J.H. Morrison:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH-IRP

NIH HD25938

Title: Involvement of Cadherin 13 in cocaine-related behaviors in mice

Authors: *J. DRGONOVA[1], D. WALTHER[1], O. RIVERO MARTIN[2], B. RANSCHT[3], K.-P. LESCH[2], G. R. UHL[1];
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Abstract: Proper function of brain circuitry requires accurate expression and regulation of cell adhesion molecules (CAMs), including those of the calcium-dependent cadherin family. Cadherin-13 (CHD13; T-cadherin) is an atypical cadherin in that it lacks its intracellular domain and is localized to the plasma membraine via a glycosylphosphatidylinositol anchor. In mice, CDH13 is expressed in cerebral cortex, hippocampus, amygdala, striatum, ventral tegmental area, and other brain regions interesting in the context of drug addiction. Molecular genetic studies in humans associate variants in CDH13 with vulnerability to substance dependence and ability to quit smoking. In human postmortem frontal cerebral cortical samples, we found that levels of CDH13 mRNA expression correlated with CDH13 genotype. CDH13 knockout mice (CDH13-KO) provide a good model for possible behavioral and anatomical influences of variation at this gene locus. We now describe data from constitutive and conditional CDH13-KO mice which suggest that altered levels of CDH13 expression can influence cocaine reward as assessed by conditioned place preference test. These results are complemented by the data from

wild-type mice that show that levels of CDH13 mRNA variants are regulated by cocaine and strongly correlate with cocaine conditioned place preference and locomotor sensitization. These behavioral observations and the anatomical distribution of CDH13 are consistent with contributions of variants in this gene to human phenotypes in addiction. **Support:** NIH IRP (NIDA); NIH HD25938 (BR).

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Poster

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Topic: B.08. Synaptic Plasticity

Support: Fritz Thyssen Stiftung Grant

Title: Role of Cadherin-13 in interneuron function and hippocampal-related behavior

Authors: ***O. RIVERO**[1], S. SICH[1], S. POPP[1], L. BACMEISTER[1], D. NAGEL[1], C. GROSS[2], E. AMENDOLA[2], K.-P. LESCH[1];
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Abstract: Cadherin-13 (Cdh13) is an atypical cell adhesion molecule that has been recently associated with several neuropsychiatric conditions, namely attention deficit/hyperactivity disorder, autism, drug abuse and depression. Cdh13 has role as a regulator of neurite outgrowth and axon guidance during brain development and it also takes part in the formation of excitatory and inhibitory synapses in the hippocampus. In this study, we aimed to better understand the regional and cellular pattern of Cdh13 in the mouse brain. Our investigations showed that Cdh13 is found in regions that are important for cognitive control and attention, such as the prefrontal cortex (PFC), the reticular nucleus of the thalamus (TRN) and the hippocampus, among others. Especially enticing is the presence of Cdh13 in the TRN, a GABAergic nucleus known to regulate the activity of thalamocortical pathways. Indeed, double fluorescence studies proved that Cdh13 mRNA and protein are found in GABAergic interneurons of the TRN and the hippocampal stratum oriens. Coexpression studies of Cdh13 with different interneuron markers also showed that Cdh13 is located in distinct subpopulations of hippocampal interneurons, with

the higher coexpression levels found with Parvalbumin (PV) and Somatostatin (SOM). The presence of Cdh13 in GABAergic interneurons encouraged us to study the effects of Cdh13 deficiency in GABAergic and glutamatergic signaling. For this purpose, we compared gene expression levels via quantitative PCR in the hippocampus of knockout (Cdh13-/-), heterozygote (Cdh13+/-) and wildtype (Cdh13+/+) adult mice. Although the genes involved in glutamatergic signaling were unaffected, the expression of some GABAergic genes, such as Vgat, Gphn or some GABA-A receptor subunits, was significantly different in Cdh13-/- mice. Currently, we are also evaluating whether the number of PV- and SOM-positive interneurons are different in the stratum oriens of Cdh13-/- mice. Subsequently, an evaluation of different behavioral domains revealed that Cdh13-/- mice present increased locomotor activity as well as impaired spatial learning and contextual fear memory, two cognitive domains which are directly related to hippocampal function. Presently, we are also studying the effect of Cdh13 deficiency in attentional behavior. In summary, our study shows that Cadherin-13 has a role in the function of particular subpopulations of hippocampal GABAergic interneurons, reflected by changes in the expression of genes involved in the inhibitory synaptic machinery in Cdh13-/- mice. These molecular changes can be correlated with behavioral alterations in domains that involve hippocampal function.

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Poster

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NIH Grant P30NS045892

Charles and Johanna Busch Biomedical Award

Title: NrCAM regulates semaphorin 3f-induced spine morphogenesis in developing neocortex

Authors: V. MOHAN[1], G. P. DEMYANENKO[1], X. ZHANG[1], L. BRENNAMAN[1], K. E. S. DHARBAL[3], T. S. TRAN[4], P. B. MANIS[2], *P. F. MANESS[1];
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Abstract: NrCAM (Neuron-Glial Related Cell Adhesion Molecule) is an Ig superfamily molecule implicated as a risk factor in autism spectrum disorders (ASD). NrCAM null mice display ASD-related impairment in sociability, reversal learning, and visual cortical responses. We found that NrCAM deletion in mice increases spine densities on dendrites of pyramidal neurons in visual cortex (V1) and other cortical areas.Spine density was specifically elevated on apical but not basal dendrites of star pyramidal cells, the principal target of thalamic inputs in layer 4 of V1. Whole cell recordings under voltage clamp in V1 slices showed that loss of NrCAM increased miniature EPSC frequencies in star pyramidal cells with no change in amplitude, consistent with an excess of functional excitatory synapses, which was confirmed by electron microscopy. NrCAM localized to postsynaptic sites on dendritic spines, and was co-expressed with Sema3F in layers 4, 6 of V1. In cortical neuron cultures, Sema3F-Fc fusion protein induced spine retraction on apical, but not basal dendrites of WT but not NrCAM null neurons. NrCAM co-immunoprecipitated with the Sema3F receptor complex, Neuropilin2 (Npn-2) and PlexinA3 from brain, as well as with synapse associated protein 102 (SAP102), a postsynaptic density scaffold protein. The binding site for Npn-2 was identified within the Ig1 domain of NrCAM (TARNER).Sema3F-induced spine retraction was rescued in NrCAM-minus neurons by WT NrCAM but not by mutants deleted in Npn2 or PDZ binding motifs. An *in vivo* genetic interaction test with heterozygotes demonstrated that Sema3F and NrCAM pathways interact to regulate cortical spine density. These findings reveal that NrCAM regulates postnatal spine remodeling in cortical pyramidal neurons, as an integral component of the Sema3F receptor complex, thus playing a vital role in establishing excitatory/inhibitory balance in cortical circuits. Altered expression of NrCAM may contribute to increased spine density and cortical hyperexcitability in ASD.

Disclosures: V. Mohan: None. **G.P. Demyanenko:** None. **X. Zhang:** None. **L. Brennaman:** None. **K.E.S. Dharbal:** None. **T.S. Tran:** None. **P.B. Manis:** None. **P.F. Maness:** None.

Poster

785. Spine Dynamics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 785.28/C31

Topic: B.08. Synaptic Plasticity

Support: NIDA-IRP

Title: Cadherin 13 (cdh13) gene knockout affects ethanol consumption

Authors: ***F. S. HALL**[1], S. O. GOLUB[1], A. M. MORROW[1], J. DRGONOVA[1], B. RANSCHT[2], G. R. UHL[1];
[1]Mol. Neurobio. Br., Natl. Inst. On Drug Abuse, IRP/NIH/DHHS, BALTIMORE, MD; [2]Sanford Burnham Med. Res. Inst., La Jolla, CA

Abstract: The gene for the cell adhesion molecule Cadherin 13 (CDH13) has been repeatedly associated with drug addiction, alcoholism, smoking and smoking cessation in genome wide association studies (GWAS). Furthermore, gene variants associated with addiction in the CDH13 gene are also associated with differences in the level of expression of CDH13 mRNA in human brain tissue samples post mortem. To model these differences in level of expression of the CDH13 gene on ethanol consumption we have used littermate CDH13 knockout mice (+/+, +/- and -/-). Mice were initially assessed for ethanol consumption using a home-cage two-bottle test (ethanol versus water) in which ethanol concentrations were varied over a period of 10 days. The initial concentration was 2% ethanol (vol/vol), and the ethanol concentration was raised every third day in the following progression: 2%, 4%, 8%, 16% and 32%. Subsequently, after 4 days without ethanol, 8% ethanol (and water) was made available on a limited access basis, 2 times per week for 24 hours. This "escalation" procedure was continued for 6 weeks. This procedure has been previously shown produce escalation of ethanol consumption over a period of 3 weeks. In the first procedure, CDH13 -/- mice were found to have elevated consumption of the highest ethanol concentration (32%). This effect was sex-dependent; consumption of this concentration was significantly increased in female but not male CDH13 KO mice, although male mice showed a similar trend. Escalation of ethanol consumption was observed in mice of all genotypes, but no differences between genotypes were observed. These experiments support a role for the CDH13 gene in ethanol consumption, and in particular consumption of high ethanol concentrations that may be more typical of alcoholics. Further experiments are needed to see if differences in ethanol consumption between these genotypes can be observed under other conditions, including escalation of intake for higher ethanol concentrations. Further experiments are also being conducted to determine if similar changes are observed for other drugs in CDH13 KO mice, including nicotine and methamphetamine. (**Support:** NIDA-IRP)

Disclosures: **F.S. Hall:** None. **S.O. Golub:** None. **A.M. Morrow:** None. **J. Drgonova:** None. **G.R. Uhl:** None. **B. Ranscht:** None.

Poster

786. Synaptic Plasticity: Other

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 786.01/C32

Topic: B.08. Synaptic Plasticity

Support: Grant-in-Aid for JSPS Fellows

Title: cAMP signaling is involved in activity-dependent reduction of calcium responses in *Drosophila* mushroom body

Authors: *S. SATO[1], K. UENO[2], T. SAKAI[1];
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Abstract: In the fruitfly, *Drosophila* melanogaster, behavioral and genetic studies have been extensively used to elucidate the molecular and neural mechanisms in learning and memory. In *Drosophila*, the mushroom body (MB), which is considered to be the memory center, receive olfactory information via the dendritic calyx mainly from the antennal lobe (AL). In cultured *Drosophila* brain, the simultaneous stimulation of the AL and ascending fibers of the ventral nerve cord (AFV) establishes long-term enhancement (LTE) in AL-induced Ca2+ responses. LTE at the AL-MB synapse is thought to be a cellular model for olfactory memory. In this current study, in an isolated cultured *Drosophila* brain, we report activity-dependent reduction of Ca2+ responses in the MB using electrical stimulation of AL (AL-stimulation). In wild-type, Ca2+ responses in the MB were reduced after repetitive-stimulation of AL (more than 40 bursts repeated with a 1 sec inter-burst interval, and 30 pulses at 100 Hz per burst). The reduction of Ca2+ responses induced by 40 repeated bursts was maintained for at least 30min. However, 30 repeated bursts were not sufficient to induce the reduction of Ca2+ responses. Therefore, to form the reduction of Ca2+ responses in the MB is required for intense and/or continuous neuronal excitation. One of the Ca2+/calmodulin-dependent adenylyl cyclase, Rutabaga (Rut), is expressed in the MBs and Rut-dependent cAMP production is required for short-term memory induced by olfactory conditioning. Thus, we next examined whether rut mutations affect activity-dependent reduction of Ca2+ responses. The reduction of Ca2+ responses induced by repetitive-stimulation of AL completely disappeared in rut mutant flies. In addition, targeted expression of wild-type rut in the MB in rut mutant flies restored rut mutant phenotype. These results suggest that activity-dependent reduction of Ca2+ responses is formed by postsynaptic Rut-dependent cAMP production in *Drosophila* brain.

Disclosures: S. Sato: None. **K. Ueno:** None. **T. Sakai:** None.

Poster

786. Synaptic Plasticity: Other

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 786.02/C33

Topic: B.08. Synaptic Plasticity

Support: NSF Grant IOS-1121054

Title: *Drosophila* WDR40A is critical for synaptic growth and neurotransmitter release at the neuromuscular junction

Authors: *L. A. PATRON, M. IMAD, K. NAGATOMO, K. ZINSMAIER;
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Abstract: We conducted a forward genetic screen to identify novel genes regulating neurotransmitter release. Two of the isolated lethal alleles, B332 and B417, mutate the *Drosophila* gene CG3313, which encodes an ortholog of the mammalian WD repeat-containing protein 40A (WDR40A). WDR40A belongs to a class of WD40-repeat proteins that likely serve as substrate adaptor proteins for the DDB1-CUL4 E3 ubiquitin ligase complex. *Drosophila* WDR40A (dWDR40A) is expressed in the cytoplasm and nucleus of neurons and muscles. In neurons, dWDR40A is especially enriched in axons and axon terminals, where it is found in close apposition to the plasma membrane. In neurons, dWDR40A levels in the nucleus are low. However, in mitotically active muscles, dWDR40A is strongly expressed in nuclei and enriched in foci that depend on the presence of CUL4 and DDB1, suggesting that dWDR40A can interact with the CUL4 ubiquitin ligase complex. dWDR40A mutant neuromuscular junctions (NMJs) show a reduction in the number of glutamatergic type I boutons and display abnormal octopaminergic type II axonal innervation. In addition, dWDR40A mutants exhibit a severe impairment of evoked neurotransmitter release to 30% of control flies. The defects in evoked release are partially rescued by presynaptic expression of normal dWDR40A protein. At higher stimulation frequencies (10 Hz), dWDR40A mutants show an initial facilitation of release that is sustained for ~1 min. Thereafter, evoked release gradually depresses to less than 40% of the original value. These data suggest that dWDR40A is critically required for presynaptic function and likely an important component of the machinery that facilitates the probability of evoked release, as well as SV trafficking and/or recycling. The structural synaptic defects cannot explain the more severe functional defects. Therefore, we propose that dWDR40A has 2 mechanistically independent synaptic roles. Notably, postsynaptic dWDR40A is part of an unknown trans-synaptic signaling mechanism that controls presynaptic dWDR40A protein levels at the fly NMJ. RNAi-mediated KD of dWDR40A in the muscle reduces dWDR40A protein levels in both the

muscle and the presynaptic motor neuron. Similarly, RNAi-mediated KD of DDB1 or CUL4 in the muscle reduces presynaptic dWDR40A levels. Ongoing experiments aim to identify the biological significance of dWDR40A-mediated trans-synaptic signaling and how it may regulate neurotransmitter release and/or synaptic growth.

Disclosures: **L.A. Patron:** None. **M. Imad:** None. **K. Nagatomo:** None. **K. Zinsmaier:** None.

Poster

786. Synaptic Plasticity: Other

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Topic: B.08. Synaptic Plasticity

Support: F31 MH095401

SFARI 206919

Title: Protocadherin 17 (Pcdh17), a common target of MEF2 and FMRP, is required for MEF2-dependent synapse pruning

Authors: ***C. F. HALE**[1,2], C.-W. CHANG[2], K. Y. HUNG[3], J. C. DARNELL[3], K. M. HUBER[2], C. W. COWAN[1];
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Abstract: Synapses are formed during early brain development, but neuronal activity also promotes the elimination of synapses to either prune excess synapses or to homeostatically maintain a steady-state number of synaptic connections. The myocyte enhancer factor 2 (MEF2) transcription factors were recently identified as important activity-dependent regulators of synapse elimination in the developing and adult brain, findings that have been correlated with a role for MEF2 in mediating various behaviors, including learning and memory. Recently, we found that Fragile X Mental Retardation Protein (FMRP) is required for MEF2-dependent excitatory synapse elimination, suggesting that MEF2 and FMRP function together to regulate common transcripts to induce elimination of excitatory synapses. To examine this hypothesis, we utilized high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) of FMRP to identify MEF2-regulated gene targets whose transcripts associate with FMRP in neurons. We identified a large overlap in MEF2 target genes and RNAs associated with

FMRP, consistent with their shared roles in synapse elimination. More specifically, we observed that one FMRP-associated mRNA coding for Protocadherin 17 (Pcdh17) is upregulated upon activation of MEF2, and it exhibits differential binding to FMRP following MEF2 activation. Pcdh17 is partially enriched at excitatory synapses in postnatal hippocampus, and we find that it is required for MEF2-induced dendritic spine elimination of hippocampal CA1 pyramidal neurons. However, reducing Pcdh17 levels in the absence of MEF2 activation did not alter structural or functional glutamatergic synapses, suggesting that regulation of Pcdh17 is necessary, but not sufficient, for regulating MEF2-induced synapse elimination. Ongoing studies are examining the molecular and cellular mechanisms by which Pcdh17 mediates MEF2-dependent synapse elimination.

Disclosures: C.F. Hale: None. **C. Chang:** None. **J.C. Darnell:** None. **K.M. Huber:** None. **C.W. Cowan:** None. **K.Y. Hung:** None.

Poster

786. Synaptic Plasticity: Other

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Program#/Poster#: 786.04/C35

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS085176

Title: EZH2 regulates learning and memory via controlling the synaptogenesis in mouse brain

Authors: *M. ZHANG[1], C. LIU[2], S. XXX[2], Z. JIAO[2], F. ZHOU[2];
[1]Orthopeadic surgury, Johns Hopkins Univ., Baltimore, MD; [2]Orthopaedic surgery, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Polycomb group protein Ezh2 is an essential epigenetic regulator of embryonic and adult neurogenesis in mouse brain, but its role in the neuronal morphogenesis during development is unknown. We generated a novel conditional knockout mouse line (NEX-EZH2) in which Cre, under the control of the NEX promoter, drives the deletion of EZH2 specifically in early postmitotic, excitatory neurons of the developing brain. Here we show that loss of EZH2 impairs the spatial learning and memory ability of mice. In addition, in a small portion of postnatal EZH2 mutant mice, they show abnormal cortical layer morphology, as well as abnormal dendritic morphology. We found a substantial change in the density of dendritic spines, which play critical roles in synaptic transmission and plasticity. Taken together, our

results suggest that EZH2 functions in modulating synaptic plasticity and long-lasting changes of neural circuits, which in turn regulates learning and memory.

Disclosures: **M. Zhang:** None. **C. Liu:** None. **S. Xxx:** None. **Z. Jiao:** None. **F. Zhou:** None.

Poster

786. Synaptic Plasticity: Other

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Topic: B.08. Synaptic Plasticity

Support: NIH grant EY12782

DOD grant W81XWH-08-2-0136

Title: The effect of sustained vs. transient epochs of irregular conditioning on inhibitory synaptic plasticity in visual cortex

Authors: ***G. DAVIS**[1,2], D. KALIKULOV[2], Q. FISCHER, Ph.D.[2], M. FRIEDLANDER[2,1]; [1]Virginia Tech. Carilion Sch. of Med., Roanoke, VA; [2]Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: A better understanding of the role of excitatory and inhibitory synaptic plasticity can contribute to improved design of strategies for deep brain stimulation. Many studies of inhibitory synaptic plasticity in visual cortex have used conditioning with high-frequency stimulation (~50 Hz) with equal distributions of inter-stimulus intervals. We sought to examine more natural stimulation patterns by using a lower frequency (10 Hz), applied in sustained or transient epochs, and with a highly irregular interstimulus interval distribution with a coefficient of variation (CV) of 1.0. Glutamatergic signaling was blocked with bath-applied (in uM) 50 D-AP5, 25 CNQX, 1 LY341495, 10 MPEP hydrochloride, and 30 LY367385. Inhibitory field potentials (FPs) were evoked by stimulation in layer 4 and recording in layer 2/3 in visual cortical slices of 10-12 week old LE rats. Slices under pharmacologic glutamatergic blockade that received a conditioning epoch at 10 Hz, CV=1, with 9 sub-epochs of 100 stimuli each separated by 8 equal rest intervals (transient, n=8) had a mean post-conditioning/pre-conditioning FP peak amplitude ratio of 0.92 (±0.06), with no significant change in the majority of experiments (n=6) and significant long term depression (LTD) in the minority of experiments (n=2). Slices under glutamatergic blockade stimulated at 10 Hz, CV=1, for 900 pulses over 90 seconds (sustained, n=8) had a mean

post/pre FP peak amplitude ratio of 1.05 (±0.06), with variable plasticity outcomes ranging from significant LTD (n=3) to no significant change (n=2) to significant long term potentiation (LTP) (n=3). Response latency was measured as the time between the stimulus and the FP peak amplitude. The sustained stimulation group experienced no change, but the transient stimulation group underwent an increase in average response latency from 1.90±0.10 msec to 2.02±0.17 msec (post/pre ratio 1.05, p=0.32). In conclusion, synaptic inhibition between layers 4 and 2/3 in rat visual cortex responds with weak LTD to periods of spaced, transient synaptic conditioning with medium frequency and irregular interstimulus intervals. Sustained epochs of otherwise similar conditioning lead to greater and more variable long term synaptic plasticity. Such specific effects on long term plasticity may be useful in better predicting how downstream neural circuits respond to sustained deep brain stimulation directed at cortical pathways.

Disclosures: **G. Davis:** None. **D. Kalikulov:** None. **Q. Fischer:** None. **M. Friedlander:** None.

Poster

786. Synaptic Plasticity: Other

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 786.06/C37

Topic: B.08. Synaptic Plasticity

Support: 5T32DA031111-03

Title: *In vivo* morphine exposure generates silent synapses

Authors: ***N. M. GRAZIANE**[1], Y. HUANG[2], Y. WANG[3], O. SCHLUETER[4], Y. DONG[1]; [1]Neurosci., [2]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [3]The Univ. of British Columbia, Vancouver, BC, Canada; [4]Göttingen Grad. Sch. for Neurosciences, Biophysics, and Mol. Biosci., Gottingen, Germany

Abstract: Morphine addiction is a debilitating problem, likely initiated as neuroadaptations in the brain's reward circuit. Cascades of cellular and circuit alterations ultimately leading to addictive phenotypes in vulnerable populations may be triggered by initial neuroadaptations that occur during drug exposure. Here, we demonstrate a prominent initial neuroadaptation following exposure to morphine. We show that AMPA receptor-silent glutamatergic synapses are generated on medium spiny neurons in the nucleus accumbens ventromedial shell, a brain region involved in the acute reinforcing effects of abused drugs. Using electrophysiological approaches in brain slices we found that *in vivo* morphine exposure gradually generated silent synapses on

nucleus accumbens medium spiny neurons reaching significance after five days. Since a synaptic pruning-like phenomenon is observed one month following morphine administration (Robinson et al., 2002), we investigated whether morphine-generated silent synapses were initiated by AMPAR internalization and subsequent synapse degeneration. To do this we administered GluR2-3Y peptide in order to block activity-dependent AMPA receptor endocytosis (Brebner et al., 2005). Systemic GluR2-3Y administration blocked morphine-generated silent synapses, while the scrambled control peptide had no effect. Our results suggest that silent synapses are generated by AMPAR internalization following morphine administration. This morphine-generated silent synapse mechanism may lead to downstream neuroadaptations such as synaptic pruning and re-arrangement of excitatory circuits within the nucleus accumbens.

Disclosures: **N.M. Graziane:** None. **Y. Huang:** None. **Y. Dong:** None. **Y. Wang:** None. **O. Schlueter:** None.

Poster

786. Synaptic Plasticity: Other

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Program#/Poster#: 786.07/C38

Topic: B.08. Synaptic Plasticity

Title: Chronic administration of the antidepressant fluoxetine predisposes hippocampal network to elevated plasticity

Authors: *D. POPOVA[1], T. TAIRA[2], E. CASTREN[3];
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Abstract: Recent studies demonstrate that chronic administration of the antidepressant fluoxetine (FLX) promotes neurogenesis, synaptogenesis and synaptic plasticity in the hippocampus, cortex and amygdala. In the present study we have further investigated the cellular and molecular changes associated with the FLX administration in the mouse hippocampus. After 21 days of FLX exposure in 8-11 week old mice recordings of field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of Schaeffer collaterals in the area CA1were done in acutely prepared hippocampal slices. We found that the basic synaptic transmission was upregulated after the FLX treatment as indicated by increased input-output curve of the fEPSPs. Moreover, FLX treatment increase Pair-pulse ratio of the fEPSPs and resulted in elevated level of long-term potentiation (LTP) thus indicating changes in short- and long-term synaptic

plasticity. Western blot analysis done in hippocampal slices after induction and maintenance of LTP revealed that the levels of expression of key synaptic proteins (synaptophysin, synaptotagmin, PSD 93/95, CaMKII, ERK and CREB) were altered after the FLX treatment. Thus, chronic FLX administration accentuates activity-dependent plasticity in the mice hippocampus paralleled by changes in the key marker proteins of synaptic plasticity.

Disclosures: D. Popova: None. **T. Taira:** None. **E. Castren:** None.

Poster

786. Synaptic Plasticity: Other

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 786.08/C39

Topic: B.08. Synaptic Plasticity

Support: INAIL 2/2009 to AP.

MIUR GR 2009 to AP

Title: Exposure to low dose pesticides precipitates synaptic plasticity alterations in mice heterozygous for the Parkinson's gene PINK1

Authors: *G. MARTELLA[1,3], G. MADEO[2], M. MALTESE[5,3], V. VANNI[3], E. FERRARO[4], E. M. VALENTE[6], T. SCHRINZI[2], L. BONANNI[7], J. SHEN[8], G. MANDOLESI[3], P. BONSI[3], A. PISANI[9];
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Abstract: Loss-of-function mutations in the PTEN-induced kinase 1 (PINK1) gene have been linked to early onset Parkinson Disease (PD). Compound PINK1 heterozygous mutations have been proposed as a susceptibility factor contributing to the risk of developing PD. Accordingly, homozygous PINK1 null mice (-/-) exhibit bidirectional striatal synaptic plasticity impairment, whereas heterozygous mice show only subtle plasticity changes, associated to a significant

decrease of evoked dopamine (DA) release (Kitada et al, 2007; Madeo et al 2014). In this work we investigated if the exposure of mice carrying the heterozygous mutation of PINK1 gene to low-dose pesticides precipitates bidirectional striatal synaptic plasticity alterations, mimicking the homozygous condition, by a multidisciplinary approach. Chronic treatment with low doses of pesticides, rotenone and paraquat (i.p. for 7 days respectively), did not cause any nigral or striatal neurodegeneration. Moreover, basal levels of both striatal and nigral ATP, measured by ATP bioluminescence assay were normal after chronic pesticides treatment. Electrophysiological recordings, by means of conventional sharp and patch clamp techniques, of striatal medium spiny neurons (MSNs) from chronically treated (+/-) mice revealed the complete loss of bidirectional corticostriatal plasticity, whereas electrophysiological properties of nigral dopaminergic neurons were unaffected. A pre-treatment with amphetamine, a compound able to increase the DA availability in the synaptic cleft, restored the plasticity deficits in chronic treated (+/-) mice, similarly to the plasticity rescue previously showed in (-/-) mice (Kitada et al, 2007). Interestingly, a preventive treatment with Trolox, an antioxidant agent able to counteract the mitochondrial oxidative stress, determined a fully rescue of corticostriatal plasticity abnormalities in (+/-) mice chronically treated, as well as in (-/-) mice. Our experimental data suggest that synaptic plasticity disruption might represent an early hallmark in familial parkinsonism determined by the interaction between genetic predisposition and exposure to environmental factors.

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Poster

786. Synaptic Plasticity: Other

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 786.09/C40

Topic: B.08. Synaptic Plasticity

Title: Effects of FK506 on kainic acid-induced neural injury in hippocampal slice culture

Authors: *M. TANIOKA[1,2], K. LEE[3], U. KIM[1], B. LEE[1,2];
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Korea, Republic of; [3]Dept. of Dent. Hygiene, Div. of Hlth. Sci., Dongseo Univ., Busan, Korea, Republic of

Abstract: FK506, classified as an immunosuppressant, has shown the neurotrophic function *in vitro* and also neuroprotective effect on some neurological injuries. We observed long-term changes in synaptic efficacy following electrical and/or pharmacological manipulation of synaptic function by using electrophysiological and histological assays. Through optical imaging, we investigated the effects of FK506 on synaptic plasticity in organotypic hippocampal slice culture (OHSC). Hippocampal slices of 6-7 day-old rats were obtained using a tissue chopper which were placed on a membrane insert. Significant delayed neuronal death (18 hr after kainic acid (KA) treatment) was quantified by propidium iodide, NeuN, and TUNEL staining. The neuronal death was prevented significantly at 24 hr after 0.1 μM FK506 treatment. In our electrophysiology study, optical signals were observed by long-term potentiation (LTP) followed by the stimulation of the Schaffer collateral pathway. The improvement in amount of LTP was found in the FK506-treated group. These results suggest that FK506 may have a beneficial role in the recovery of synaptic efficacy following KA-induced neuronal injury. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2013R1A1A4A01009332).

Disclosures: M. Tanioka: None. **K. Lee:** None. **U. Kim:** None. **B. Lee:** None.

Poster

786. Synaptic Plasticity: Other

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Topic: B.08. Synaptic Plasticity

Support: CDMRP grant #W81XWH-08-2-0136 to MJF

Title: Effect of interstimulus interval regularity and mild traumatic brain injury (mTBI) on synaptic plasticity outcomes in adult rat visual cortex

Authors: *Q. S. FISCHER, D. KALIKULOV, M. J. FRIEDLANDER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Typical protocols for inducing synaptic plasticity use stimulus patterns with constant interstimulus intervals (ISIs), while neurons *in vivo* receive synaptic input with irregular ISIs. A study of more physiologically salient stimulation patterns is important to understand the role that irregularity may play in determining synaptic plasticity outcome. Here we evaluate how stimulus regularity influences the induction of synaptic plasticity in visual cortex of normal and mTBI rats. We applied a defined pattern of stimulation to layer 4 in acute slices and made whole cell recordings of evoked postsynaptic potentials (PSPs) from 99 layer 2/3 pyramidal cells in 10-12 week-old rats (2-3 weeks after mTBI). Conditioning stimulation consisted of 900 pulses at 1Hz with 1 of 3 different patterns of ISI regularity defined by the coefficient of variation (CV): regular (CV=0), slightly irregular (CV=0.2), or highly irregular (CV=1). Averaged PSP amplitudes evoked by 0.1Hz stimulation were measured before and after conditioning stimulation, and their ratio used to assess plasticity outcome. In controls, LTD was observed in 68% of cells for regular stimulation (n=25) and 74% of cells for slightly irregular stimulation (n=19), but in just 44% of cells for highly irregular stimulation (n=23). Plasticity (LTP or LTD) did not occur in 16% of cells for regular or slightly irregular stimulation, increasing to 39% of cells for highly irregular stimulation (remaining cells showed LTP). Notably, the distribution of plasticity outcomes was significantly different for slightly vs. highly irregular stimulation (KS test, $P<0.05$). In mTBI rats, LTD occurred in 100% of cells for slightly irregular stimulation (n=8), 64% of cells for highly irregular stimulation (n=11), and 39% of cells for regular stimulation (n=13). Plasticity did not occur in 39% of cells for regular stimulation, and 27% of cells for highly irregular stimulation (remaining cells showed LTP). Importantly, the distribution of plasticity outcomes was significantly different for slightly irregular vs. regular stimulation (t-test, $P<0.05$). We also compared the half width, rise time, tau decay rate, and latency of PSPs before and after conditioning stimulation. In controls, slightly irregular stimulation induced a significant increase in rise time and latency (t-test, $p<0.05$), while highly irregular stimulation induced a significant decrease in half width and tau decay rate (t-test, $p<0.05$). In contrast, mTBI rats showed no change in these parameters for any stimulus pattern. Our results suggest that stimulus regularity modifies the efficacy of synaptic plasticity induction and that mTBI can alter this interaction.

Disclosures: Q.S. Fischer: None. **D. Kalikulov:** None. **M.J. Friedlander:** None.

Poster

786. Synaptic Plasticity: Other

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Topic: B.08. Synaptic Plasticity

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Title: Novel form of depolarization-induced depression of GABAergic IPSCs in the Purkinje cells

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Abstract: It is well known that the elevation of intracellular Ca^{2+} caused by depolarization can induce various forms of synaptic plasticity of inhibitory transmission at the cerebellar Purkinje cell (PC). These are rebound potentiation, depolarization-induced suppression of inhibition and depolarization-induced potentiation of inhibition. However, all these studies employed high Cl^- pipette solution which can record $GABA_A$ receptor-mediated current as an inward current. In this study, we attempted to observe depolarization-induced synaptic plasticity of inhibitory transmission under more physiological condition. We performed the whole cell voltage-clamp recording with low Cl^- pipette solution using young rat cerebellar slices. The direction of evoked IPSC (eIPSC) changed from outward to inward immediately after PC depolarization. Thereafter, the amplitude of eIPSC was depressed (Depolarization-induced Depression of Inhibition: DDI) for more than 20 min in spite of pharmacological blocking the well established depolarization-induced synaptic plasticity of inhibitory transmission. Exploring the biophysical properties revealed that the reversal potential of GABA-mediated current was positively shifted. Thus, $[Cl^-]_i$ of PC was increased by depolarization pulses. Furthermore, this phenomenon was abolished by BAPTA, CaMKII inhibitor, calcium-activated chloride channel (CaCC) blockers and cation-chloride cotransporter (CCC) inhibitors. Hence, CaCC and CCC were responsible to the elevation of $[Cl^-]_i$ via CaMKII activation. Finally, we examined the effects of DDI action on the spontaneous spikes of PCs. After the repetitive stimulation of climbing fiber to depolarize PC, 50% of observations showed weakened exogenous GABA-mediated inhibitory action on the spike generation. Altogether, our finding is distinct postsynaptic mechanism from well established depolarization-induced plasticity of the GABAergic transmission at the cerebellar PCs.

Disclosures: **H. Satoh:** None. **F. Saitow:** None. **H. Suzuki:** None.

Poster

786. Synaptic Plasticity: Other

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant AG10435

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Title: Homogenous thalamic input across layer 5 corticospinal subpopulations becomes biased toward task-relevant neurons following motor learning

Authors: *J. BIANE[1], J. M. CONNER[2], M. SCANZIANI[2], M. H. TUSZYNSKI[2,3];
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Abstract: Layer 5 neurons of the neocortex receive direct and relatively strong input from the thalamus. However, the intralaminar distribution of these inputs and their capacity for plasticity in adult animals are largely unknown. Here, we examine the allocation of thalamic input to distinct subpopulations of layer 5 corticospinal neurons, and whether such input is modulated following acquisition of a skilled motor behavior. In young adult rats, channelrhodopsin (ChR2) was expressed in pyramidal neurons of the motor thalamus via focal injection of AAV2-ChR2 into VA/VL. Approximately three weeks later, we simultaneously recorded from pairs of corticospinal neurons associated with control of distinct motor outputs - distal forelimb versus proximal forelimb - in slices containing primary motor cortex (M1). Despite known differences in dendritic morphology and baseline spine density between recorded corticospinal subpopulations, activation of thalamic afferents in M1 produced equivalent responses in monosynaptic excitation, suggesting thalamic resources are evenly dispersed across layer 5 corticospinal neurons under baseline conditions. Following 10 days of skilled forelimb grasp training, however, thalamocortical input was biased toward the subpopulation of task-relevant corticospinal neurons associated with control of the distal forelimb, inducing a greater excitatory response in these cells compared to corticospinal neurons controlling the proximal forelimb. The

underlying synaptic mechanisms of this alteration are currently under investigation. These findings elucidate modifications in synaptic signaling between thalamus and layer 5 cortex that support learning during adulthood.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

Mentoring Environment Grant BYU

Title: Expression of endocannabinoid biosynthesizing enzyme mrna and protein in hippocampal stratum oriens neurons

Authors: *L. N. FRIEND, C. B. MERRILL, R. C. WILLIAMSON, S. T. NEWTON, Z. H. HOPKINS, J. G. EDWARDS;
Brigham Young Univ., Provo, UT

Abstract: The hippocampus is thought to mediate learning and memory by altering the strength of synapses within its circuitry. In many cases, this synaptic plasticity can be induced by signaling molecules. Lipid-based signaling molecules called endocannabinoids, have been shown to modulate synaptic plasticity among hippocampal pyramidal cells and stratum radiatum interneurons; however, the role of endocannabinoids in mediating synaptic plasticity among interneurons in the stratum oriens is still unclear and indeed what mediates some forms of plasticity, including one novel form of LTP in these interneurons, has yet to be characterized. Our goal was to determine whether stratum oriens interneurons have the machinery necessary for endocannabinoid production and, if so, whether this machinery is expressed in a cell sub-type specific manner and can potentially modulate oriens interneuron activity. Using patch-clamp electrodes to extract single cells we analyzed the expression of endocannabinoid biosynthetic enzyme mRNA using RT-PCR. In this analysis, we examined cellular expression of two interneuron markers, GAD65 and GAD67, as well as several calcium-binding proteins and

neuropeptides to determine interneuron subtype. We analyzed cellular expression of several endocannabinoid biosynthetic enzymes, including N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), diacylglycerol lipase alpha, and 12-lipoxygenase, as well as type I mGluRs. In addition, our data suggests that stratum oriens interneurons coexpress mRNA necessary for endocannabinoid biosynthetic enzymes with type I mGluRs. We identified interneurons that coexpress mRNA for somatostatin and diacylglycerol lipase as well as parvalbumin positive basket cells coexpressing NAPE-PLD, suggesting that both basket cells and O-LM cells, or another somatostatin-positive interneuron subtype possess the enzymes necessary to produce various endocannabinoids. In addition, using immunohistochemistry, interneurons were identified to express NAPE-PLD, DAGLα, and mGluR5 to show that target mRNA is converted to protein and that the targets are co-localized to GAD producing interneurons. Currently we are performing whole-cell electrophysiological experiments to examine the potential involvement of endocannabinoids in modulating stratum oriens interneuron activity.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS098645

Mentoring Environment Grant BYU

Title: Ventral tegmental area dopamine and GABA neurons: Physiological properties and expression of mRNA for endocannabinoid biosynthetic enzymes and type I mGluRs

Authors: *C. B. MERRILL, L. N. FRIEND, S. T. NEWTON, Z. H. HOPKINS, J. G. EDWARDS;
PDBio, Brigham Young Univ., Provo, UT

Abstract: The ventral tegmental area (VTA) is known to control the processing of rewarding and addictive behaviors. The VTA contains dopamine (DA) cells, which release DA to

downstream targets in response to rewarding stimuli, and GABA cells, which modulate DA cell activity. Therefore, both cell types are involved in associative reward learning. Synaptic plasticity plays an important role in adaptive reward signaling within the VTA. Endocannabinoids can mediate or modulate synaptic plasticity at several synapses within the reward circuit. However, the source of endocannabinoids within the VTA is not well understood. Therefore, our goal was to describe the distribution of endocannabinoid biosynthetic enzyme mRNA within VTA neurons. We extracted single VTA neurons via whole cell patch clamp and used single-cell real-time quantitative PCR to identify DA and GABA neurons based on mRNA expression of cell-type specific targets. DA neurons were identified by the presence of tyrosine hydroxylase and DA transporter mRNA, while GABA neurons expressed GAD65 and GAD67 mRNA. Additionally, electrophysiological properties such as action potential frequency and sag potential amplitude were examined between the two cell types. Concurrent with established observations, slower firing frequencies and larger Ih potentials were observed in DAergic neurons, however overlap was identified between these two cell types. VTA neurons were then probed for endocannabinoid/eicosanoid biosynthetic enzyme mRNA, such as N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD), diacylglycerol lipase α (DAGLα), and 12-lipoxygenase. We also probed for type I metabotropic glutamate receptor (mGluR) mRNA, as endocannabinoid synthesis requires mGluR activation in many cases. Our data demonstrate that endocannabinoid biosynthetic enzyme mRNA is expressed in both DAergic and GABAergic cells with concurrent expression of type I mGluRs. Next, to ensure mRNA expression was representative of protein content, slices were stained using immunohistochemistry for GAD67, DAGLα, NAPE-PLD and type I mGluRs. Positive labeling for these targets was observed in VTA neurons, supporting our RT-PCR results. Collectively, these data suggest DAergic and GABAergic cells of the VTA have the capability to produce endocannabinoids and potentially alter synaptic plasticity involved in reward and addiction.

Disclosures: C.B. Merrill: None. **L.N. Friend:** None. **S.T. Newton:** None. **Z.H. Hopkins:** None. **J.G. Edwards:** None.

Poster

786. Synaptic Plasticity: Other

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Topic: B.08. Synaptic Plasticity

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GRK1123

Title: Endocannabinoids mediate autocrine inhibition of hippocampal principal cells

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Abstract: Endocannabinoids (eCBs) exert major control over neuronal activity by activating G Protein-coupled cannabinoid receptors (CBRs). The functionality of the eCB system is primarily ascribed to the well-documented retrograde activation of presynaptically located CBRs. We find that action potential-driven release of the eCB 2-arachidonoylglycerol leads to a long-lasting membrane potential hyperpolarisation in hippocampal CA3 pyramidal cells. The hyperpolarisation can be mimicked and occluded by CBR agonists. In contrast to depolarisation-induced suppression of inhibition, this mechanism occurred in a purely autocrine manner, as suggested by dual recordings from neighbouring cells. To conclude, we describe an auto-regulatory plasticity in the hippocampus that emphasises the importance of CBR function in the central nervous system.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: CNMPB, Cluster of Excellence 171

Title: STED microscopy of filamentous actin in the visual cortex of adult mice

Authors: *K. I. WILLIG[1,2], H. STEFFENS[1], W. WEGNER[2], C. GREGOR[1], S. W. HELL[1];
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Abstract: Two-photon microscopy and less extensive also confocal microscopy are widespread techniques to visualize fluorescently labeled cells in tissue. However, the limit of this technique is the diffraction limited resolution of about half of the wavelength of light (~200-350nm). This barrier had been overcome by a whole family of superresolution microscopy or nanoscopy concepts such as STED, RESOLFT, PALM, STORM etc. They all rely on modulating the fluorescence emission so that adjacent features fluoresce sequentially in time. From all the superresolution microscopy techniques, STED microscopy stands out for its imaging capabilities in tissue: It is live-cell compatible, especially when using standard fluorescent proteins such as EGFP or EYFP, it is able to record 3D images from inside transparent tissue, and the imaging speed is fast compared to other superresolution methods. Recently, we have developed an upright scanning STED microscope to image the dynamics of dendritic spines in the molecular layer of the visual cortex in a living mouse. We implemented virus infection methods (Semliki Forest Virus and Adeno-associated virus) to label filamentous actin in the living mouse with Lifeact, an actin binding peptide, and the yellow fluorescent protein (EYFP). We recorded actin in dendritic arborisation and spines with a resolution of 50 - 70 nm at a depth down to 40 µm. Here we present the use of red fluorescent proteins for *in vivo* STED microscopy which has several advantages: Due to the red-shifted laser light compared to the previously used EYFP settings we were able to observe morphological changes of filamentous actin over a time scale of hours without any sign of photo toxicity and only limited by photo bleaching. The higher scattering length of the red light enables further a better penetration depth. With red fluorescent proteins we recorded morphological changes of actin over hours in the living mouse. These results show that STED microscopy becomes a valuable tool to study morphological changes in the living mouse brain.

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Poster

786. Synaptic Plasticity: Other

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Title: Disrupting PSD-95-mediated protein-protein interaction in neonatal rats impairs hippocampal neuronal function, learning, and memory

Authors: *O. FURMANSKI[1], C. LI[3], Y. YANG[2], Y. SATO[4], Q. CHEN[5], P. TANG[5], F. TAO[1], R. A. JOHNS[1];
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Abstract: Introduction: Evidence from human and animal studies suggests that early postnatal anesthesia, using agents such as isoflurane (ISO), causes long-term impairment of learning and memory. These cognitive deficits in animals correlate with mitochondrial dysfunction, reduced neuronal complexity, and hippocampal cell death. However, the mechanisms of ISO toxicity remain poorly understood. Previously, we have shown via biochemical techniques that ISO inhibits N-methyl-D-aspartate (NMDA) receptor interaction with PSD-95 in a dose-dependent manner. We hypothesize that disrupting interactions between NMDA receptors and the PDZ2 domain of PSD-95 during development may impair cognition later in life. Methods: Male Sprague-Dawley rats were exposed to vehicle air (VA) control or 3.4% ISO for 4 hours on either postnatal day (PND) 7 or 60. Animals were tested for spatial and auditory memory in a fear conditioning paradigm. Animals were sacrificed 7 days later, and brains were processed for either electron microscopy or immunofluorescence. Acute hippocampal slices were prepared from animals exposed to VA or ISO and sacrificed 7 days later. Hippocampal long-term potentiation (LTP) was recorded at Schaffer collateral synapses on CA1 pyramidal neurons using whole-cell patch clamping. Finally, cultured hippocampal neurons were exposed to VA or ISO for 4 hours after either 7 or 14 days *in vitro* (DIV). Cultures were stopped on DIV15 for staining and microscopy. Results: ISO impaired hippocampus-dependent spatial fear memory and reduced cell proliferation in the hippocampi of rats treated on PND7, but not on PND60. Electron microscopy revealed that ISO reduced synaptic contacts and disrupted mitochondrial membranes on PND7 but not PND60. Western blotting showed that the actin-associated protein drebrin was reduced in the hippocampus after PND7 ISO, suggesting impaired postsynaptic spine development. To further examine the effects of disrupting NMDAR-PSD-95 interactions, recombinant PSD-95 PDZ domain 2 was conjugated to the cell-permeable Tat peptide (Tat-PDZ2). Tat-PDZ2 inhibited LTP in juvenile mouse hippocampal slices, and ISO in rats inhibited LTP after PND7 treatment but not after PND60. Recent data suggest that ISO and/or Tat-PDZ2 treatment in cultured hippocampal neurons rapidly and persistently reduced immunofluorescent staining for drebrin. Conclusions: These data show that PSD-95-mediated protein-protein interactions are important to the proper development and function of hippocampal neurons.

Disrupting PSD-95 binding to NMDA receptors is one possible mechanism by which ISO could impair synaptic function, synaptogenesis, and neurogenesis.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01MH087631

Title: Modulation of heterosynaptic plasticity by adenosine receptors in the rat neocortex *in vitro*

Authors: ***N. M. BANNON**, M. CHISTYAKOVA, M. VOLGUSHEV;
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Abstract: Heterosynaptic plasticity can be induced in neocortical neurons by intracellular tetanization: bursts of spikes in the postsynaptic cell induced by depolarizing current pulses in the absence of presynaptic stimulation. Intracellular tetanization can result in potentiation, depression, or no change, depending on the initial paired-pulse ratio (PPR). Generally, synapses with a high release probability (low initial PPR) depress, while synapses with a low release probability (high PPR) potentiate. The dependence of the plastic change on the initial state of the synapse implicates a retrograde messenger which confers information about postsynaptic spiking to the presynaptic terminal. Adenosine is released from neurons and glial cells in an activity-dependent manner, modulating synaptic transmission by acting on pre and postsynaptic receptors. Because induction of heterosynaptic plasticity depends on release probability, we asked whether manipulation of adenosine receptors can modulate susceptibility of synapses for plastic changes. To test this, we made *in vitro* whole-cell recordings from layer 2/3 pyramidal neurons in slices of rat visual cortex and studied synaptic responses evoked with two pairs of stimulating electrodes placed in layer 4. After recording control EPSPs for 9-13 min, synaptic stimulation was stopped and intracellular tetanization applied to the cell, consisting of three trains (1/min) of ten bursts (1 Hz) of five pulses (5 ms, 100 Hz, 0.4-1.1 nA). The current intensity evoked 4-5 spikes per burst. Following tetanization, synaptic stimulation was resumed, and evoked EPSPs were recorded for 40-50 min. We compared the induction of heterosynaptic

plasticity under conditions of normal artificial cerebral spinal fluid, and with 20uM adenosine or 30nM of the selective A1R antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) added to the recording medium. Adenosine increased the median PPR during the control period, suggesting a reduction in release probability. The dependence of plastic changes on initial release probability, and a negative correlation between changes of EPSP amplitude and changes of the PPR were maintained on the background of adenosine. Under A1R blockade, inputs still showed potentiation, depression, or no change, but the dependence of changes on the initial PPR was abolished. This suggests that biochemical cascades activated by adenosine are not directly interacting with the mechanism by which long term changes are expressed and maintained, but rather adenosine receptor signaling serves a modulatory role, linking the initial state of the presynaptic input to the outcome of intracellular tetanization.

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Poster

787. Oscillations: Connectivity

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Topic: B.09. Network Interactions

Support: NIH Grant 5T90DA032484

Title: Mechanisms underlying thalamocortical phase-amplitude switches due to the anaesthetic propofol

Authors: ***A. SOPLATA**[1], S. LEE[3], S. CHING[4], E. BROWN[5,6,7], P. PURDON[7,6], N. KOPELL[2]; [1]Grad. Program in Neurosci., [2]Mathematics, Boston Univ., Boston, MA; [3]Dept. of Neurosci., Brown Univ., Providence, RI; [4]Electrical and Systems Engin., Washington Univ. in St. Louis, Saint Louis, MO; [5]Dept. of Brain and Cognitive Sciences, MIT-Harvard Div. of Hlth. Sci. and Technol., MIT, Cambridge, MA; [6]Harvard Med. Sch., Boston, MA; [7]Dept. of Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp., Boston, MA

Abstract: Thalamocortical dynamics display highly detectable signals associated with state changes of consciousness. Many different anesthetics can induce unconsciousness, and their spectral characteristics are often even more differentiated than those between the multiple stages of non-REM sleep. Propofol, a common anesthetic that acts as a GABA-A agonist and hyperpolarization-activated current (H-current) suppressor, has been shown to produce a

dynamic phase-amplitude coupling between alpha (8-14 Hz) and slow wave (0.1-1 Hz) rhythms (Purdon et al., 2013) in human EEG recordings. At approximately the time that the subject loses consciousness, "trough-max" coupling occurs, in which alpha power peaks at the trough of the thalamocortical slow wave (i.e., the cortical OFF state), while during full unconsciousness, there is "peak-max" coupling in which alpha power is highest at the peak of the slow wave (i.e., the cortical ON state). Building on previous models for alpha generation under propofol (Ching et al., 2010) that inherit from thalamic models (Destexhe et al., 1996), we have used a computational model to explore how propofol-induced changes in baseline voltage, the decay time of the GABA-A induced inhibition, and the maximal conductance of the H-current (gH) change the phase of the alpha rhythm in a cortically-generated slow-wave rhythm. We found that the predominant reason for the switch from trough-max to peak-max is the propofol-induced reduction of gH, as decreasing this current changes the interaction between the latter and the T-type Calcium bursting current, which is key for many thalamic oscillations (Destexhe et al., 1996). When the thalamus is hyperpolarized enough to burst, modeling an increase in the maximal conductance and time scale of decay of inhibition can increase the frequency of spontaneous thalamic bursting, but these, as well as direct hyperpolarization and modulation of potassium leakage strength, are all insufficient for generating the full range of the phase-amplitude coupling. The results of this model illustrate the level of complexity in the mechanisms by which anesthetics modulate brain interactions. Ching, S., et al. Thalamocortical model for a propofol-induced alpha-rhythm associated with loss of consciousness. PNAS December 13, 2010, doi:10.1073/pnas.1017069108 Destexhe, A., et al. (1996). Ionic mechanisms underlying synchronized oscillations and propagating waves in a model of ferret thalamic slices. Journal of Neurophysiology, 76(3), 2049-2070. Purdon, P. L., et al. Electroencephalogram signatures of loss and recovery of consciousness from propofol. PNAS March 4, 2013, doi:10.1073/pnas.1221180110

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Poster

787. Oscillations: Connectivity

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Support: Federal Ministry of Education and Research (BMBF) Germany, Grant Number 01GQ1005B

EU-FP7 MSCA IEF 330792 (DynViB)

Title: Layered architecture shapes context-dependent response and input integration of a cortical circuit

Authors: *M. HELMER[1,2], X.-J. CHEN[3], W. WEI[4], F. WOLF[1,2], D. BATTAGLIA[5,1]; [1]BCCN Goettingen, Goettingen, Germany; [2]Dept. of Nonlinear Dynamics, Max Planck Inst. for Dynamics and Self-Organization, Göttingen, Germany; [3]Psychology Dept., Brandeis Univ., Waltham, MA; [4]Ctr. for Neural Sci., New York Univ., New York, NY; [5]Inst. de Neurosciences des Systèmes, Aix-Marseille Univ., Marseille, France

Abstract: Lamination is a landmark feature of cortical architecture. But even if functional specializations of individual layers have been suggested (Raizada & Grossberg 2003; Hirsch & Martinez 2006), the role played by interlayer connections in shaping the dynamical responses of a cortical column has not yet been fully elucidated. Here, we analyze a mean-field model of a cortical column, embedding realistic interlayer connections (the one-column "connectome" of Binzegger et al. 2004). Systematically varying efficacy of excitation and inhibition in the model we find phase diagrams showing a great diversity of possible dynamical regimes. In particular, due to the presence of delayed inhibition and the interaction of layers, oscillations can be generated. Oscillations in different layers may be phase-locked or phase-precessing and have different frequencies. In some regions of the phase diagrams high frequencies (gamma-like) predominate in L23 while low beta-like frequencies predominate in L5. Remarkably, while this experimentally observed tendency is usually attributed to the different cortical sources to different layers, here it arises spontaneously in an isolated model column, resulting from the multilayer connectivity. The column is thus intrinsically predisposed to communication-through-coherence processes over multiple frequency bands. Furthermore, we find that horizontal or top-down currents, mediating perceptual context information, are non-linearly amplified and that vertical inter-layer interactions alone already contribute to contextual modulations of the column response, besides other interactions here not explicitly modeled. Our model predicts inter-layer competition behaviors which could be probed experimentally by selective optogenetic (in)activation techniques. Finally, the robustness of our findings is discussed by comparison with alternative wiring diagrams.

Disclosures: **M. Helmer:** None. **X. Chen:** None. **W. Wei:** None. **F. Wolf:** None. **D. Battaglia:** None.

Poster

787. Oscillations: Connectivity

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Topic: B.09. Network Interactions

Support: EU-FP7 MSCA IEF 330792 (DynViB)

Federal Ministry of Education and Research (BMBF) Germany, Grant Number 01GQ1005B

Title: Dynamics of cortical circuits lead to switching resting state functional connectivity

Authors: *V. K. JIRSA[1], E. HANSEN[1], A. SPIEGLER[1], G. DECO[2], D. BATTAGLIA[1,3]; [1]Faculté de Médecine, Inst. De Neurosciences Des Systemes, Marseille, France; [2]Univ. Pompeu Fabra, Barcelona, Spain; [3]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany

Abstract: Functional connectivity opens a window on the interactions between different brain regions. Besides basic research, it is clinically relevant for applications in Alzheimer's disease, schizophrenia, presurgical planning, epilepsy, and traumatic brain injury. Simulations of whole-brain mean-field computational models with realistic connectivity determined by tractography studies enable us to reproduce average functional connectivity in the resting state with remarkable accuracy. Previous computational studies, however, did not address the prominent non-stationarity in resting state functional connectivity, which may result in large intra- and inter subject variability and thus preclude an accurate individual predictability. As we show here, this non-stationarity reveals a rich structure, characterized by rapid transitions switching between a few discrete functional connectivity states. We show that state-of-the-art computational models fail to reproduce these spontaneous state transitions and, thus, are not qualitatively superior to simplified linear stochastic models, which account for the effects of structure alone. We then demonstrate that a slight enhancement of the non-linearity of the network nodes is sufficient to vastly broaden the repertoire of possible network behaviors, leading to modes of fluctuations which are strongly reminiscent of some of the most frequently observed Resting State Networks. Because of the noise-driven exploration of this dynamical as well as functional repertoire, the dynamics of functional connectivity changes now qualitatively and displays non-stationary switching as in empirical resting state recordings. It thus bears promise to serve as a better biomarker of resting state dynamics.

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Poster

787. Oscillations: Connectivity

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Topic: B.09. Network Interactions

Support: National Science Foundation Graduate Research Fellowship under grant number DGE-1313667

Title: Spatio-temporal dynamics of low frequency oscillations in resting-state fMRI

Authors: *D. MASTROVITO, S. HANSON, C. HANSON;
Rutgers Univ., Newark, NJ

Abstract: Resting-state fMRI has been shown to capture intrinsic fluctuations in brain activity. These fluctuations are thought to mediate long distance neuronal synchronization and have a characteristic power spectrum dominated by low frequency oscillations (<.1 Hz). Using techniques such as correlation-based functional connectivity analysis, studies have identified structure in resting-state time series related to functional networks. However, functional connectivity analysis assumes temporal stationarity of the MRI time series and provides no indication of asymptotic convergence. Evidence suggests that functional connectivity changes over time, however few studies have attempted to characterize the spatio-temproal dynamics of resting-state functional connectivity. Those that have, employed techniques such as PCA or ICA that force constraints such as orthogonality or statistical independence on the data that may not be true of the underlying signals. The current study explores the dynamics of resting-state functional connectivity using state-of-the-art high temporal resolution multiband resting-state functional magnetic resonance imaging (rsFMRI) (TR = 645 msec). 63 lateralized regions of interest (ROIs) from the Montreal Neurological Institute (MNI) 2mm atlas were selected based on their high power in the low frequency band between .02 and .1 Hz as indicated by Fourier analysis. Temporal dynamics of the relationships between each of the 63 ROIs were explored using continuous wavelet transform. Wavelet analysis reveals changes in spectral power for each ROI over time, which were then mapped into an audible frequency range. Additionally, using these identified ROIs, sliding window correlations were used as an input similarity measure to map the ROIs into a 3 dimensional non-parametric multidimensional scaling (MDS-Kruskal-Shepard) space. A solution recovering 3 dimensions was able to account for 90 percent of the variation in the time series and provided a spatial visualization of the resting state network as it evolved over time. The resultant visualization of the ROI dynamics showed a highly structured

interplay between ROIs with periodic local collapse of ROIs towards the center of the space and subsequent expansion.

Disclosures: **D. Mastrovito:** None. **S. Hanson:** None. **C. Hanson:** None.

Poster

787. Oscillations: Connectivity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 787.05/C54

Topic: B.09. Network Interactions

Title: Structural architecture supports local and global functional interactions in healthy aging

Authors: ***J. ZIMMERMANN**[1], R. MCINTOSH[1], P. RITTER[2], S. ROTHMEIER[2], M. SCHIRNER[2];
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Abstract: Neuroscience research suggests that local and global functional interactions throughout the human brain are modulated by the underlying anatomical architecture. Age-related changes of anatomical tracts suggests a potential for concommitant changes in the structure-function relationship. We compared functional connectivity inferred from spontaneous BOLD fMRI with structural connectivity inferred from DTI among 49 participants between 18 and 82 years of age. Using multivariate Partial Least Squares analysis, we noted that the density of individual axonal tracts (r = .82, p <.01, 95% CI [0.77, .90]), and strength of functional interactions between regions (r = 0.54, p < .01, 95% CI [0.53, 0.82]) changed in the aging process. Although the direction of change was region-dependent, the majority of structural and functional connections decreased with age. Interestingly, we observed that structure-function correlations *(*individual participants: r = 0.19 to 0.36, and r = -0.40 to 0.66, for whole network and regionwise respectively), vary across the age spectrum*(r* = 0.69, p < .01, 95% CI [0.68, 0.83]), and that these differences varied by region. We also examined how the structural architecture upholds the functional modular organization, and whether this changes with age. First, the relationship between structure and function across modules was assessed with the participation coefficient function in the Brain Connectivity Toolbox (https://sites.google.com/site/bctnet/Home). The degree to which a region participates outside its functionally-defined module was significantly correlated with how structurally connected it was outside of its structural module (r = .32, p < .05). In a second analysis, we examined how

structure supports within-module function. We partitioned the structural network according to the modular organization of the functional network, and calculated the density (proportion of existing connections to all possible connections), and subsequently the Modularity Index (MI was quantified as the difference between density in the functionally defined structural partitions and the density across the entire structural network) of each region. MI's were significantly > 0 (t = 2.69, p < .01), such that regions were more densely connected within their functionally defined module, and MI's varied with age (r = 0.73, p < .01, CI [0.71, 0.85]) depending on region. For the first time, we provide a comprehensive view of how anatomical connectivity throughout the brain modulates regionwise functional interactions, intermodular integration, and community segregation changes in healthy aging.

Disclosures: J. Zimmermann: None. **R. McIntosh:** None. **P. Ritter:** None. **S. Rothmeier:** None. **M. Schirner:** None.

Poster

787. Oscillations: Connectivity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 787.06/C55

Topic: B.09. Network Interactions

Support: EU-FP7 MSCA IEF 330792 (DynViB)

Federal Ministry of Education and Research (BMBF) Germany, Grant Number 01GQ1005B

Title: Brain state-dependent liquid information processing

Authors: *D. BATTAGLIA[1,2], P. QUILICHINI[1], C. BERNARD[1];
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Abstract: How does information flow through cortical networks: via fixed and discrete internal pathways or via routes that are continuously dynamically reconfigured? Here we show, through information theoretical analysis of LFP and single unit recordings, that functional connectivity maps between entorhinal cortex neurons display dynamic reconfiguration between theta and slow oscillations *in vivo*. At the level of field potentials, the flow information follows fixed paths within and between cortical layers in a brain state-dependent manner. In contrast, at the single

cell level, the information flows in continuously reconfiguring networks, without changing the global amount of transferred information. We conclude that information processing operates differently at different scales: it is "liquid" at the neuronal scale, whilst it follows stable pathways at the network scale. Both modus operandi are brain-state specific, with theta oscillations being associated generally to enhanced information transmission between cortical layers.

Disclosures: D. Battaglia: None. **P. Quilichini:** None. **C. Bernard:** None.

Poster

787. Oscillations: Connectivity

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 787.07/C56

Topic: B.09. Network Interactions

Support: NHMRC Early Career Fellowship (SC)

NHMRC Project Grant 1068140 (DR)

Title: Functional connectivity of the claustrum during resting wakefulness

Authors: *D. H. RESER[1], S. C. KOLBE[2];
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Abstract: Despite widespread connectivity with sensory and motor control areas of the cerebral cortex, the role of the claustrum in normal and pathological brain function remains unclear. We have recently proposed that the claustrum may be involved in regulation or transition among functional networks of the cerebral cortex. Here we examined the resting state functional connectivity (FC) of the left and right claustrum using voxelwise correlation analysis seeded from regions of interest (ROIs) in 13 right handed healthy subjects (6m/7f, mean ± SD age = 32 ± 4.7yrs). Subjects were scanned at 3T (Skyra, Siemens, Erlangen) while viewing a blank (grey) screen with eyes open. Regions of interest were manually drawn on high-resolution T1-weighted scans of each subject and linearly registered to each subject's functional MRI scans using FLIRT (FMRIB, Oxford). Seed-based connectivity maps of the whole brain were calculated for each subject and non-linearly transformed to the MNI atlas using ANTs (PICSL, UPenn) to create a group average connectivity map. Analysis of the average resting state connectivity of the claustrum yielded a pattern of bilaterally symmetrical connectivity with areas of peak correlation

in sensory cortices (visual, auditory and somatosensory), spatial attention areas (frontal eye fields, parietal cortex), supplementary motor areas, dorso-lateral prefrontal cortex, insula, and medial thalamus. Overall, connectivity was stronger in the hemisphere ipsilateral to the seed. However, the patterns of connectivity from left and right claustrum were highly consistent between hemispheres. The resulting connectivity maps coincide well with recent descriptions of the FC between visual cortex and the salience network as measured during the eyes open condition (Riedl et al., J. Neurosci. 2014. 34(18):6260-6266). Our data indicate that the claustrum may be a ripe target for analysis of FC in resting state networks, and indicate the need to differentiate activity in the claustrum from that of surrounding structures in future imaging studies.

Disclosures: D.H. Reser: None. **S.C. Kolbe:** None.

Poster

787. Oscillations: Connectivity

Location: Halls A-C

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Program#/Poster#: 787.08/C57

Topic: B.09. Network Interactions

Support: NIH P50-MH094263

NIH F32-NS083340

Title: Targeted enhancement of hippocampal brain networks and associative memory with transcranial magnetic stimulation

Authors: *J. X. WANG[1,2], K. L. BRANDSTATT[1,2], E. Z. GROSS[1,2,4], A. J. RYALS[1,2], L. M. ROGERS[5], M. DOKUCU[3], M. S. HERMILLER[2], J. L. VOSS[1,2];
[1]Med. Social Sci., [2]Interdepartmental Neurosci. Program, [3]Psychiatry and Behavioral Sci., Northwestern Univ., Chicago, IL; [4]Psychology, Wayne State Univ., Detroit, MI; [5]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Few findings substantiate the influential notion that the hippocampus supports associative memory by binding together elements of experience that are individually processed by distributed brain regions. We explored the causal role of hippocampus by using noninvasive electromagnetic stimulation of human hippocampal brain networks to identify changes in associative memory caused by enhancing the interactivity of hippocampus with distributed

regions. Individualized measures of intrinsic functional connectivity of hippocampus were used to locate subject-specific stimulation targets. A total of 16 subjects participated in our experimental design that counterbalanced one week of treatment stimulation with one week of sham control stimulation. Target sites were stimulated with repetitive transcranial magnetic stimulation (rTMS) for a total of five daily sessions. Stimulation increased functional connectivity within hippocampal networks versus the sham condition, and this was associated with selective improvements in associative memory performance that outlasted the period of stimulation by ~24 hours. Furthermore, treatment-induced changes in seed-based connectivity with respect to hippocampal targets were spatially selective to individual target locations. No improvements were identified for tests of attention, language, and perceptual functions. These findings demonstrate that hippocampal brain networks and associative memory can be enhanced in humans using noninvasive stimulation, providing direct evidence that hippocampal interactivity with distributed regions plays a causal role in associative memory.

Disclosures: **J.X. Wang:** None. **K.L. Brandstatt:** None. **E.Z. Gross:** None. **A.J. Ryals:** None. **L.M. Rogers:** None. **M. Dokucu:** None. **M.S. Hermiller:** None. **J.L. Voss:** None.

Poster

787. Oscillations: Connectivity

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Program#/Poster#: 787.09/C58

Topic: B.09. Network Interactions

Support: ONR grant N000141310672

NIH grant R01 MH099645

NIH grant R01 EB009282

Title: Origin of resting state slow spontaneous neuronal oscillations in brain networks

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Abstract: Resting or baseline brain activity occurring at very low frequencies (0.01-0.1 Hz) has been observed in fMRI and EEG recordings. These oscillations were found to be correlated across brain regions and are thought to reflect functional connectivity between interacting areas of the brain. Here, we used a biophysically realistic, conductance based cortical network model

to show that spontaneous very slow oscillations (less than 0.1 Hz) may rise from the ion concentration dynamics. The model included ion concentration dynamics for intra- and extracellular K+ and Na+ and intracellular Cl-, Na+/K+ exchange pump, and KCC2 pump. A random Poisson input was applied to each neuron to mimic *in vivo* conditions. Our study revealed that network oscillation around 0.01-0.05 Hz arises from progressive slow accumulation of extracellular [K+] and its removal. The network oscillations were more prominent in larger network as compared to the smaller networks, and the amplitude of oscillations depended on the random input to the network. While K+ dynamics and diffusion contributed to the local synchrony, long-range synaptic connectivity provided correlated activity between distinct populations of neurons. This finding may explain observation that long-range synaptic connectivity influences the correlation between locally generated oscillations in remote cortical regions. We conclude that the ion concentration dynamics and long-range connections may originate the very slow large-scale spontaneous fluctuations of brain activity found with fMRI recordings.

Disclosures: G.P. Krishnan: None. **M. Bazhenov:** None. **O. Gonzalez:** None.

Poster

787. Oscillations: Connectivity

Location: Halls A-C

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Program#/Poster#: 787.10/C59

Topic: B.09. Network Interactions

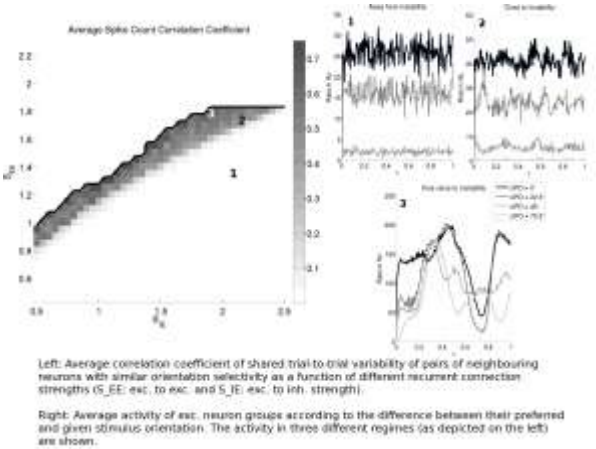
Support: DFG Grant GRK1589/1

Title: Correlated variability in a network model of primary visual cortex

Authors: *R. MEYER[1,2], K. OBERMAYER[1,2];
[1]Fakultät IV, Technische Univ. Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany

Abstract: Cortical neurons are organized into functional columns, where individual cells share many common inputs. Correlated trial-to-trial variability among the activity of cortical neurons is believed to be influenced by the functional and anatomical connectivity of a cortical circuit. There is experimental evidence that correlated variability varies between different layers of primary visual cortex [1]. Here we investigate the hypothesis that correlations of trial-to-trial variability depend on the topology of a neural network as proposed by [1]. We simulate a

network of inhibitory and excitatory Hodgkin-Huxley neurons receiving tuned afferent stimulus input as in [2]. We measure correlated trial-to-trial variability and systematically vary the recurrent connectivity spread as well as recurrent synaptic strengths. Our findings suggest that besides the connectivity profile, correlated trial-to-trial variability is most strongly modulated by the recurrent connectivity strength of a network. As previously shown, balance of excitatory and inhibitory recurrent activity can actively decorrelate neural responses [3]. Accordingly, we observe correlated activity in network regimes where the balance between excitation and inhibition is broken and excitatory recurrent activity is slightly stronger than the inhibitory one. Moreover, networks operating in regimes with correlated responses undergo a substantial change in behaviour where individual neurons show fast spiking variability and slow changes in rate dynamics where average activity oscillates, comparable to findings in [4]. [1] B. J. J. Hansen, et al., Neuron 76, 590 (2012). [2] M. Stimberg, et al., Cerebral Cortex 19, 2166 (2009). [3] A. Renart, et al., Science 327, 587 (2010). [4] A. Litwin-Kumar & B. Doiron, Nat. Neurosci. 15, 1498 (2012)



Left: Average correlation coefficient of shared trial-to-trial variability of pairs of neighbouring neurons with similar orientation selectivity as a function of different recurrent connection strengths (S_EE: exc. to exc. and S_IE: exc. to inh. strength).

Right: Average activity of exc. neuron groups according to the difference between their preferred and given stimulus orientation. The activity in three different regimes (as depicted on the left) are shown.

Disclosures: **R. Meyer:** None. **K. Obermayer:** None.

Poster

787. Oscillations: Connectivity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 787.11/C60

Topic: B.09. Network Interactions

Title: Are spontaneous oscillatory brain dynamics impacted by the gastric slow-wave? An investigation of cerebro-gastric coupling

Authors: *C. G. RICHTER[1], M. BABO-REBELO[1], A. DUCORPS[2], C. TALLON-BAUDRY[1]; [1]Lab. de Neurosciences Cognitives, L'Ecole Normale Superieure, Paris, France; [2]CENIR - Ctr. de Neuroimagerie de Recherche, Paris, France

Abstract: Spontaneous brain activity is highly structured in space, time and frequency, and impacts both neural processing and behavior. It is usually considered that the structure of spontaneous brain dynamics derives solely from constraints intrinsic to the brain. Here, we examine an alternative view: spontaneous brain dynamics could also be influenced by an extra-cerebral pacemaker. The stomach wall contains cells (interstitial cells of Cajal, or ICCs) that have an intrinsic pacemaker activity giving rise to a stable ~ 0.05 Hz electrical oscillation (the gastric slow-wave) in healthy adults. Because the ICC matrix and brain are anatomically densely interconnected via ascending and descending neural pathways, we hypothesized that the low frequency activity originating in the stomach may be functionally coupled with spontaneous brain dynamics. Indeed, it is known that the amplitude of brain rhythms, in particular in the alpha (8-12 Hz) and beta (14-30 Hz) ranges, spontaneously waxes and wanes very slowly. We test here whether the infra-slow modulation (0.01-1Hz) of brain rhythms is coupled to the stomach pacemaker. To assess this coupling we simultaneously recorded the electrogastrogram (EGG) from electrodes placed over the abdominal surface and magnetoencephalogram (MEG) in 18 healthy human subjects (9 females) during 12 minutes of resting fixation. The gastric-slow wave was identified from the EGG of each subject (mean frequency, 0.046 Hz ± 0.001 s.e.m.). We then assessed phase amplitude coupling (PAC) between the amplitude envelopes of MEG activity in the 1 – 15 Hz range, and the EGG phase, using the coefficient of variation as a modulation index over each MEG sensor, envelope frequency, and subject. Using a cluster-based randomization procedure, we tested for a group effect by comparing these values to the median values obtained from a surrogate distribution produced via rotating the relationship between the EGG and MEG data by a value greater than +/-3 cycles of each subject's gastric slow-wave peak frequency. This resulted in a significant cluster (cluster statistic = 86.21, p = 0.004) of EGG-MEG modulation of 10 - 11 Hz oscillatory activity, lateralized to the left-hemisphere. These results indicate that cerebro-gastric coupling is a significant contributor to alpha rhythm amplitude variability.

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Poster

787. Oscillations: Connectivity

Location: Halls A-C

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Topic: B.09. Network Interactions

Support: NIMH Grant MH080838

Davimos Family Endowment for Excellence in Science

Title: Extracellular field contributions to a globally conscious brain

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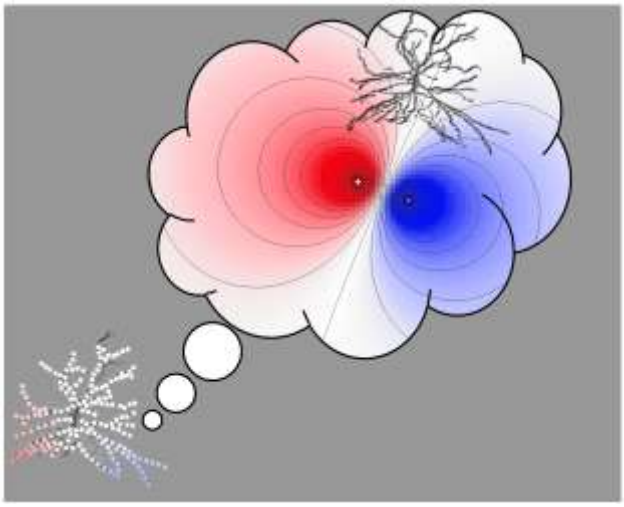
Abstract: Brain function depends on the rapid exchange of information across spatially-dispersed neural ensembles. It is well established that information is communicated proximally by virtue of synaptic connections: one neural structure -the dendrite- receives the information that another- the axon- emits. In this scenario, information exchange between distant neural groups suffers delays of tens of milliseconds. Here, we raise the theoretical possibility that some neurons attune themselves to extracellular brain fields, such that local neural ensembles are near instantaneously informed of the global pattern of brain activity. On the extracellular side, dendrites are exposed to a local ionic environment that changes over time under the influence of proximal synaptic release and global activity-dependent electromagnetic fluctuations. Fluctuations arising from local synapses have the largest magnitude and as such have received most experimental attention. Nevertheless, fluctuations of extracellular fields driven by the activity of distant neural populations have the potential to convey information. We explore the possibility that neurons are directionally tuned to remote neural populations, by sampling extracellular field gradients with their tridimensional -spatially extended- dendritic arborescence. Recent empirical evidence (ephaptic coupling, dendritic computation, extracellular volume/consciousness connection) renders this hypothesis plausible, with implications for both empirical and theoretical neuroscience. In particular, it is argued that the Extracellular Field hypothesis has potentially profound consequences for artificial neural networks and for brain in silico: for both implementations, the spatial arrangement of neural populations, distance and orientation, have yet to be included as key variables. This hypothesis may also shed new light on spatial transformations occurring in the evolving and developing brain: a directionally favorable arrangement of neural ensembles may help explain idiosyncrasies in human performance.



Disclosures: **E. Tognoli:** None. **J. Kelso:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.01/C62

Topic: C.02. Alzheimer's Disease and Other Dementias

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HKU Alzheimer's Disease Research Network under Strategic Research Theme on Aging

Generous donation from Ms Kit Wan Chow

Title: Investigating the effects of anesthetic dexmedetomidine on tau phosphorylation

Authors: *R. C. CHANG[1,2,4], C. HUANG[3], Y. S. HO[1,5], O. T. W. NG[1], M. G. IRWIN[3], G. T. C. WONG[3];
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[5]Macau Inst. Applied Res. in Med. and Hlth., Macau Univ. of Sci. and Technol., Macau SAR, China

Abstract: Effects of anesthetics on tau phosphorylation have received increasingly attention in these years. Most of the studies have shown that anesthetics-induced hypothermia is a key factor for inducing hyperphosphorylation of tau. Among different anesthetics, dexmedetomidine is widely used in different types of surgery and as sedative in Intensive Care Unit. It is not a conventional anesthetic agent in which its action is independent of GABA receptor. Its action mechanism is via the alpha-2 adrenergic receptor. However, it is unclear of whether dexmedetomidine can also trigger phosphorylation of tau. The major purpose of this study is to investigate its effects on tau phosphorylation in neurons. We hypothesize that dexmedetomidine can promote tau phosphorylation. We had established both *in vitro* and *in vivo* experiments for this study. Primary culture of cortical neurons established from Sprague-Dawley (SD) rat embryos were exposed to dexmedetomidine for 1 or 6 h; and the levels of tau phosphorylation at the AT8, AT180 and S396 sites were assessed by Western-blot analysis. To assess and compare their relative *in vivo* effects, the same agent was administered intravenously to 8 - 10 week-old male SD rats and titrated to the loss of the righting reflex for 2 h. After 1 h of recovery, the rats were euthanized and tissues from the cortex and hippocampus were harvested for Western-blot and immunohistochemical analysis. The *in vitro* studies revealed significant phosphorylation only at the S396 site; but such changes returned to normal level at 6 h. With temperature control, dexmedetomidine significantly induced increase in phosphorylation at AT8 site in the cortex and hippocampus and at AT180 in the hippocampus. The direct effect of anesthetic agents on differentiated cortical neurons was epitope specific and short lived. Its effects in rats were more complicated and depended not only on the phosphorylation site but the regions of the brain. While hypothermia has been considered to play important roles in inducing phosphorylation of tau, our findings suggest that dexmedetomidine can induce tau phosphorylation under normothermic conditions. Further studies are warranted to determine the long-term impact of this anesthetic on tau pathology and even cognitive functions.

Disclosures: **R.C. Chang:** A. Employment/Salary (full or part-time):; Laboratory of Neurodegenerative Diseases, Department of Anatomy, LKS Faculty of Medicine, The University of Hong Kong, Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong, State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong. **C. Huang:** None. **Y.S. Ho:** None. **O.T.W. Ng:** None. **M.G. Irwin:** None. **G.T.C. Wong:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.02/C63

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association

BrightFocus Foundation

CurePSP

NIEHS ES020395

Title: Tau accumulation is regulated by RNA translation: A role for stress granules in tauopathies

Authors: *D. APICCO, T. VANDERWEYDE, P. ASH, B. WOLOZIN;
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Abstract: Pathological accumulation of microtubule associated protein tau is a key feature of various neurodegenerative disorders, including Alzheimer's disease (AD) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), which are collectively referred to as tauopathies. However, the cellular processes that regulate tau accumulation in disease remain unclear. Here, we demonstrate a role for RNA binding proteins (RBPs) associated with stress granules (SGs) in promoting tau pathology. SGs are cytoplasmic complexes of RNA and protein that form in response to stress-induced inhibition of RNA translation. Immunohistochemistry showed that SGs are highly prevalent in AD, FTDP-17, and rTg4510 transgenic mouse cortical tissues compared to control tissues, and that RBPs associated with SGs, particularly T cell intracellular antigen 1 (TIA-1), strongly co-localize with phosphorylation and conformational tau epitopes associated with disease (recognized by PHF1 and MC1 antibodies, respectively). In immortalized hippocampal neurons, overexpression of TIA-1 led to co-localization of TIA-1 with MC1-positive tau and increased the number and size of MC1-positive tau inclusions. The effect of TIA-1 was attenuated by expression of a tau construct with 14 serine/threonine residues mutated to alanine, indicating that phosphorylation of tau is required for the interaction with TIA-1. A kinase inhibitor screen of tau kinases associated with AD identified p38 MAPK as a critical regulator of the association of tau with SGs. Since SG formation requires translational inhibition, we investigated the effect of modulating RNA translation on tau using two translation inhibitors with distinct mechanisms of action: puromycin, which causes premature chain termination leading to SG formation, and cycloheximide, which blocks chain elongation but does not induce SGs. Interestingly, puromycin treatment increased the level of total and MC1-positive tau while cycloheximide had no effect on tau levels. In contrast, stimulation of RNA translation by GADD34 overexpression reduced tau levels. These results show that tau accumulation is regulated by the translational machinery and suggest that

the disease-associated phosphorylation of tau contributes to a biochemical pathway that regulates the response of RBPs to stress, highlighting a central role for SG signaling pathways in the progression of tauopathies.

Disclosures: D. Apicco: None. **T. Vanderweyde:** None. **P. Ash:** None. **B. Wolozin:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.03/C64

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG026249

Fellowship from Japan Society for the Promotion of Science (ST)

Title: Neuron-to-neuron propagation of high-molecular-weight tau species derived from tau-transgenic mouse and human Alzheimer's disease brain

Authors: *S. TAKEDA[1], S. WEGMANN[1], H. CHO[2], A. D. ROE[1], C. COMMINS[1], D. IRIMIA[2], B. T. HYMAN[1];
[1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]BioMEMS Resource Ctr., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Tau pathology is known to spread in a predictable pattern in Alzheimer's disease (AD) brain and accumulating evidence suggests a trans-synaptic mechanism of tau transfer between neurons. However, it remains controversial which tau species are involved in neuron-to-neuron propagation. To identify specific tau species responsible for propagation, we compared uptake and propagation properties of different tau species derived from tau-tg mice and human AD brains. First, we prepared two different brain extracts from rTg4510 mice (PBS-soluble, 3k g or 150k g ext.), incubated them with mouse primary neurons, and assessed tau uptake immunohistochemically. After 2 days incubation, significant uptake of human tau occurred from the 3k g brain extract, which contains high-molecular-weight (HMW) tau species. The involvement of HMW tau in neuronal uptake was confirmed by separating the same brain extract by SEC and incubating each fraction with primary neurons. The most extensive uptake was observed in HMW fractions, suggesting that HMW tau species were responsible for uptake. Brain extracts from rTg21221 mice, which overexpress WT human tau, did not contain these

HMW tau species, and no tau uptake was observed into neurons. Importantly, brain ISF, collected from rTg4510 using a large pore microdialysis probe (1,000kDa MWCO), contained extracellular HMW tau, which was taken up by neurons, demonstrating that transmissible tau species are present in the extracellular space. We studied the transfer of tau between neurons inside a new microfluidic neuron culture platform. The design of the platform included three distinct chambers connected through arrays of channels such that the growth of axons and the formation of synaptic connections are precisely controlled between neurons in different chambers. rTg4510 brain-derived tau was taken up and propagated between neurons in the device within a week. Notably, human tau taken up by the 1st chamber neuron was propagated to the next chamber neurons even after washing out the human tau from the medium. HMW tau species extracted from AD brain were highly phosphorylated and also taken up by primary neurons. On the other hand, HMW tau species extracted from control brain or prepared from recombinant tau were not taken up by neurons. Further biochemical analysis showed that the HMW tau species appear to be complexes containing both human mutant and mouse endogenous tau, suggesting an interaction between these tau species in the brain. These findings suggest that PBS-soluble HMW p-tau species, present in the extracellular space, are involved in propagation and could be a target for therapeutic intervention and biomarker development.

Disclosures: S. Takeda: None. **S. Wegmann:** None. **A.D. Roe:** None. **C. Commins:** None. **B.T. Hyman:** None. **H. Cho:** None. **D. Irimia:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.04/C65

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT. Grant No. 142293

CONACyT. Grant No.127357

Title: Site-specific phosphorylations of tau are associated with cell division in SH-SY5Y cells. A mechanism of DNA protection?

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Abstract: In Alzheimer's disease, tau protein is the major constituent of paired helical filaments, and undergoes several post-translational modifications (hyperphosphorylation and truncation). It has been reported that the main function of tau is to stabilize microtubules and promote the movement of organelles through the axon of neurons. However, little is known regarding other functions in which tau protein might be involved. We performed double and triple immunolabelling for a variety of tau markers in non-differentiated SHSY-5Y cells and analyzed them using confocal microscopy. We detected phosphorylated tau protein in the cell nucleus in small dense dots, which colocalize with intranuclear structures known as speckles. We observed that tau phosphorylated at Thr231 is closely associated with cell division. It appears that different phosphorylated forms of tau protein are associated with specific functions during mitosis. Our results suggest that the presence of tau might be involved in the separation of sister chromatid during anaphase, that it maintains the integrity of DNA throughout the prophase and protects chromosomes during cell division.

Disclosures: P.R. Flores: None. **V. Ibarra-Bracamontes:** None. **N. Zarco:** None. **A. Navarrete:** None. **A. Alonso:** None. **R. Mena:** None. **J. Luna-Muñoz:** None. **B. Floran-Garduño:** None. **J. Segovia-Vila:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.05/C66

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Conacyt-Mexico Grant 152535

Title: Overexpression of tau protein induces aberrant plasma membrane blebbing in glial cells through actin cytoskeleton remodeling

Authors: *F. GARCIA-SIERRA[1], F. M. TORRES-CRUZ[1], F. RODRIGUEZ-CRUZ[1], J. AVILA[2], G. BASURTO-ISLAS[3];
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Abstract: Abnormal intracellular aggregation of tau protein is a pathological condition leading to neuronal death in Alzheimer´s disease (AD). Fibrillar and nonfibrillar aggregates of tau protein alter the normal functioning of neurons by disturbing the organization of distinct membranous organelles including the mitochondria and nucleus. However, the mechanism by which tau produces these alterations is still under investigation. In this study we evaluated whether full-length tau protein and its acid aspartic^{421}-truncated variant (D^{421}-truncated tau) also produce alterations in the normal organization of the cytoskeleton and plasma membrane (PM) when expressed in cultured glial cells. We transiently transfected the glial C6 cell line with plasmids (pcDNA3.1Zeo(-)) containing the sequence for either full-length tau protein (hTau40) or D^{421}-truncated tau. After transfection, cell viability and alteration in the normal organization of the cytoskeleton and PM were evaluated by multilabeling-immunofluorescence and confocal microscopy. After 48 hours of transfection, the radial microtubule lattice emerging from microtubule-organization centers in glial C6 cells was changed to more pericentric and cortical arrays of thick microtubule bundles. This effect was produced by both hTau40 and Asp^{421}-truncated tau molecules. Moreover, both tau variants induced severe alteration in the glial morphology by producing extensive blebbing along the PM. The formation of these PM blebs was closely associated with remodeling of the actin cytoskeleton. When tau-transfected cells were incubated with drugs that depolymerize the actin cytoskeleton the PM blebbing was reverted. Moreover when glial cells showing tau-induced PM blebbing were incubated with inhibitors of the Rho-GTPase-Rho-associated kinase (ROCK) signaling pathway, the formation of these abnormal membrane blebs was avoided. Interestingly, under this condition the actin cytoskeleton underwent a new organization in close association with the rings of abnormally formed microtubules bundles. These results are new evidence about alterations of the actin cytoskeleton, indirectly produced by the expression of tau protein. This effect may represent a new mechanism of tau toxicity, in which activation of the Rho-GTPase-ROCK pathway mediating the remodeling of cortical actin and MP blebbing is produced by tau-induced microtubule bundling. In the disease, this tau-induced blebbling of the PM may occur and contribute to the impairment of glial activity

Disclosures: F. Garcia-Sierra: None. **F.M. Torres-Cruz:** None. **F. Rodriguez-Cruz:** None. **J. Avila:** None. **G. Basurto-Islas:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.06/C67

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Model of tau pathology in induced pluripotent stem cell-derived human neurons

Authors: *M. USENOVIC, S. NIROOMAND, M. COSDEN, B. VOLETI, J. J. RENGER, S. PARMENTIER-BATTEUR;
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Abstract: The pathology of neurodegenerative disorders, like Alzheimer's disease (AD), may spread throughout the brain via transcellular propagation of misfolded aggregated proteins. Abnormal accumulation of tau protein into intracellular hyperphosphorylated aggregates is a pathological hallmark of AD. The principle of tau transmission in AD has emerged due to spatial and temporal progression of tau pathology observed in AD patients' brains, as well as evidence showing that tau misfolded proteins gradually propagate from one brain region to another in animal models. However, the molecular and cellular mechanisms of tau transmission still remain unknown. We developed a novel clinically relevant cellular model to study tau aggregation and transmission using induced pluripotent stem (iPS) cell-derived human neurons. We seeded these neurons with full length human tau monomers and oligomers. The effects of tau seeding were examined for 4 weeks. Using high content imaging and a microfluidic device that enables the separation of neuronal soma and axon in two compartments, we showed that tau oligomers were internalized and transported along the axons. Also, oligomer-treated neurons exhibited an increase in aggregated and phosphorylated pathological tau. Interestingly, these effects were associated with progressive changes in neuronal morphology with neurite retraction and loss of synapses in time dependent manner. We did not observe any changes in neurons treated with tau monomers. Taken together, our data provide support for the pathological tau propagation hypothesis in a new human neuronal model for studying mechanisms involved in tau pathology to identify novel therapeutic targets.

Disclosures: **M. Usenovic:** A. Employment/Salary (full or part-time):; Merck & Co. **S. Niroomand:** A. Employment/Salary (full or part-time):; Merck & Co. **M. Cosden:** A. Employment/Salary (full or part-time):; Merck & Co. **B. Voleti:** A. Employment/Salary (full or part-time):; Merck & Co. **J.J. Renger:** A. Employment/Salary (full or part-time):; Merck & Co. **S. Parmentier-Batteur:** A. Employment/Salary (full or part-time):; Merck & Co..

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Ellison Foundation Senior Scholar in Aging Award

Cure Alzheimers Fund

Leopoldina Postdoctoral Fellowship

NYSCF Druckenmiller Postdoctoral Fellowship

Title: Elucidating the role of pathogenic Tau in neurodegeneration using neurons generated from patient-derived stem cells

Authors: *D. PAQUET[1], A. CHEN[1], A. SPROUL[2], S. JACOB[2], A. GREGG[1], D. KWART[1], K. OLSEN[1], O. OLSEN[1], S. NOGGLE[2], M. TESSIER-LAVIGNE[1];
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Abstract: Our aging society is confronted with a dramatic increase in patients suffering from Tauopathies, such as Frontotemporal dementia (FTD) and Alzheimer's disease (AD). These disorders are characterized by neuropathological lesions including Tau hyperphosphorylation and aggregation and massive cortical cell death. No mechanism-based cures are currently available. Animal models, although useful for understanding aspects of Tau pathology, do not capture key aspects of the disease process and have been of limited use in developing treatments. Furthermore, certain aspects of Tau biology, such as isoform expression, differ between rodents and human. A human Tauopathy model that reproducibly develops disease-relevant molecular pathology in cortical neurons would enable functional studies and serve as an improved screening platform for drug development. Recent advances in stem cell research have allowed reprogramming of mutant patient-derived cells to induced pluripotent stem cells (iPSCs) and subsequent differentiation into cortical neurons. Although the first studies using this approach have shown promising results for AD and atypical Tauopathies, no human model of FTD has been described. Moreover, early studies lacked appropriate isogenic controls to confirm phenotype specificity. Newer studies have used isogenic controls generated by zinc finger nuclease-mediated genome editing, but these are very difficult to assemble in non-expert labs. We have generated iPSC lines from patients carrying different FTD-causing mutations affecting several aspects of Tau biology, such as splicing, microtubule binding or aggregation. Using TALENs and CRISPR genome-editing tools, we (1) have generated multiple isogenic control lines, (2) knocked out genes to study aspects of normal and disease-associated Tau biology, and

(3) are inserting transgenes in safe-harbor genomic loci to label cells, characterize phenotypes and introduce disease-modifiers. The splice mutant neurons show altered isoform expression. Other disease-relevant phenotypes, such as Tau phosphorylation, aggregation and cell death, are under investigation. An update on these phenotypes will be presented at the meeting.

Disclosures: **D. Paquet:** None. **A. Chen:** None. **A. Sproul:** None. **S. Jacob:** None. **A. Gregg:** None. **D. Kwart:** None. **K. Olsen:** None. **O. Olsen:** None. **S. Noggle:** None. **M. Tessier-Lavigne:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ohio University Baker Fund

Title: Optimization and utilization of hybrid culture between rat and fly primary neurons

Authors: K. L. KRAUS, *R. A. COLVIN, C. QIAN, D. LEE;
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Abstract: Various *Drosophila* lines provide strong genetic models of human neurological disorders that can be efficiently produced at low cost; however, fly cultures less closely model the development, synaptic organization and neurotransmitter utilization of the human brain with respect to rodent primary neuronal cultures. A multidisciplinary approach to culturing neurons *in vitro* can be utilized to create a hybrid culture model consisting of both rodent and *Drosophila* primary neurons that amplifies the selective benefits of culturing each type of neuron. With clearly distinguishable pre- and post-synaptic targets, a hybrid model will be useful in studying various aspects of synaptic physiology, such as pre- and post-synaptic mechanisms and the cell-to-cell propagation of proteins. Preliminary data have demonstrated that fly and rat neurons can co-exist in primary culture and have the potential to establish cross-species contacts. Fly embryonic neuroblast cells were added to established rat (Sprague-Dawley) cortical neurons prepared from E18 embryos and the resulting hybrid culture was incubated at 24°C. Fly and rat neurons in mass culture were distinguished through their distinct morphological differences and the use of a transgenic fly line that selectively expresses GFP in cholinergic neurons. The fully established, self-organized hybrid neural network was then investigated for functional cross-

species contacts using a vertebrate nicotinic acetylcholine receptor (nAChR) antibody (mAb270 from DSHB). In pure primary rat cortical culture, nAChR expression is negligible. However, the rat neurons in close proximity to the fly neurons in the hybrid culture expressed significant levels of nAChR, indicating the possibility of functional cross-species interactions. The next step will be discovery of optimal long-term survival culture conditions (media composition, temperature, etc.). Using innate species differences in major excitatory neurotransmitter utilization and whole-cell patch clamp techniques to measure excitatory post-synaptic currents, it will be possible to definitively demonstrate cross-species functional contacts, and thus the potential applications of the hybrid model. Many neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, are believed to involve the cell-to-cell propagation of characteristic misfolded proteins. The novel development of an *in vitro* hybrid model of neurodegenerative disease would permit the study of trans-synaptic protein propagation under controlled conditions with clear pre- and post-synaptic targets.

Disclosures: K.L. Kraus: None. **R.A. Colvin:** None. **C. Qian:** None. **D. Lee:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

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Program#/Poster#: 788.09/C70

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Owens Family Foundation

NIH Training Grant T32 GM008136

Title: Disruption of intraneuronal tau by extracellular tau oligomers containing individual or mixed tau isoforms

Authors: E. M. SWANSON, L. MCMAHON, S. SOM, *G. S. BLOOM;
Univ. of Virginia, Charlottesville, VA

Abstract: Tau is microtubule-associated protein enriched in the axons of neurons of the central nervous system, where its functions include direct binding and stabilization of microtubules and regulation of axonal transport. Tau is found in the CNS in six isoforms produced by the alternative splicing of a single tau gene, MAPT, with these isoforms characterized by the presence of zero, one or two N terminal inserts, and three or four C-terminal microtubule binding

repeats. Neuronal inclusions composed of hyperphosphorylated tau are a major histopathological feature of a series of neurodegenerative disorders known collectively as tauopathies, the most common and well-known of which is Alzheimer's disease (AD). While the clinical and histological presentation of these disorders is heterogeneous, a majority share the following hallmarks: loss of the normal axonal distribution of tau; accumulation of insoluble, fibrillar tau aggregates in neurites and perikarya; synaptic dysfunction; and eventual neuron death. There is also evidence that the majority of these disorders spread through the brain by a prion-like mechanism, in which pathologically misfolded tau is taken up by neurons, confers this pathological phenotype to the endogenous "normal" protein through direct protein-protein contact, and is eventually released by the cell, perpetuating the cycle. Some evidence points to short tau fibrils as being prion-like, but scant attention has been paid to small tau oligomers. Using a new morphometric method for rigorously quantifiying tau distributions in cultured neurons, we now demonstrate that externally applied tau oligomers are much more effective than tau monomers or fibrils at disrupting the normal tau distribution in primary cortical neurons, that oligomer potency varies according to tau isoform, and that oligomers made from mixtures of all six CNS tau isoforms are much more potent than oligomers made from individual isoforms. These results raise the possibility that soluble, pre-fibrillar oligomers of mixed tau isoforms account for the prion-like spread of tau pathology *in vivo* in AD and non-Alzheimer's tauopathies.

Disclosures: **E.M. Swanson:** None. **L. McMahon:** None. **S. Som:** None. **G.S. Bloom:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Program#/Poster#: 788.10/C71

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus A2013364S (NMK)

Title: Differential oligomer formation and phosphatase-activating domain exposure in tau isoforms

Authors: ***K. K. COX**[1], D. S. HIMMELSTEIN[2], N. M. KANAAN[1];
[1]Dept. of Translational Sci. & Mol. Med., Michigan State Univ., Grand Rapids, MI; [2]Dept. of Cell and Mol. Biology, Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: Tauopathies represent a collective group of neurodegenerative diseases where the microtubule-associated protein tau is abnormally modified and forms pathological inclusions in neurons and glial cells. There are six tau isoforms in the adult human CNS and different tauopathies are characterized by the pathological accumulation of various tau isoforms. Recently, our group identified a potential mechanism of tau toxicity. Specifically, disease-related modifications of tau expose a signaling motif in the amino terminus of tau called the phosphatase-activating domain (PAD). PAD exposure leads to activation of a protein phosphatase 1/glycogen synthase kinase 3 signaling cascade that ultimately impairs axonal transport. The formation of tau oligomers is one mechanism by which PAD becomes exposed during disease pathogenesis. Little is known about whether different tau isoforms exhibit a differential ability to form oligomers and whether PAD exposure is different among aggregated tau isoforms. Using a combination of *in vitro* aggregation assays, we investigated the propensity of different tau isoforms to form oligomers and the extent to which PAD is exposed in aggregates composed of each tau isoform. Oligomer formation was determined by using our tau oligomer-specific monoclonal antibody (TOC1) and PAD exposure was measured using our PAD-specific antibody (TNT1). Different tau isoforms create distinct forms of aggregates under identical polymerization conditions. For example, electron microscopy data suggest that 4 microtubule-repeat (4R) isoforms tend to form oligomers and a mixture of short, intermediate and long filaments, while 3 microtubule-repeat (3R) isoforms form only oligomers and long filaments. In general, immuoblot results suggest that the aggregated forms of each tau isoform exhibit increased TNT1 reactivity (i.e. PAD exposure) and TOC1 reactivity (i.e. presence of oligomers) compared to monomeric proteins. Preliminary quantitation of immunoblotting for TOC1 suggests that the relative proportion of oligomer formation is greater in 4R isoforms compared to 3R isoforms. Ongoing studies will determine whether aggregates from each tau isoform are toxic to cells in culture. Collectively, this work will help distinguish important differences among tau aggregates composed of each tau isoform and help identify the most toxic isoforms of tau.

Disclosures: K.K. Cox: None. **D.S. Himmelstein:** None. **N.M. Kanaan:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.11/C72

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG14449

Title: Tau oligomer formation and phosphatase-activating domain exposure in disease-related forms of tau

Authors: *C. T. TIERNAN[1], D. HIMMELSTEIN[3], S. E. COUNTS[1,2], N. M. KANAAN[1]; [1]Translational Sci. and Mol. Med., [2]Family Med., Michigan State Univ., Grand Rapids, MI; [3]Cell and Mol. Biol., Northwestern Univ., Chicago, IL

Abstract: Tau is a microtubule-associated protein that is believed to contribute to neuronal loss and cognitive decline in Alzheimer's disease (AD) and other tauopathies. Despite our growing understanding of tau mutations, conformational changes, and aggregation, the toxic tau moiety remains elusive. Tau is the predominant protein found in neurofibrillary tangles (NFTs), and therefore it was presumed that aggregation of tau monomers into NFT-related filaments was causal in AD pathogenesis. However, tau oligomers appear prior to NFT formation and are accompanied by abnormal unmasking of the tau amino terminus [i.e., the phosphatase-activating domain (PAD, aa 2-18)]. PAD exposure activates a signaling pathway that impairs anterograde axonal transport, suggesting that prefibrillar tau oligomers and associated PAD exposure mediate tau toxicity upstream of NFT formation. The present investigation sought to characterize the effects of two posttranslational modifications in the tau protein that are hypothesized to produce toxic forms of tau by facilitating oligomer formation and subsequent PAD exposure. Phosphorylation of serine 422 (pS422), which modulates caspase proteolysis at aspartic acid 421 (D421), is an early event in tangle evolution that correlates with cognitive decline. In contrast, caspase cleavage of tau at D421 is a later pre-tangle event in the evolution of tau pathology in AD. Preliminary data indicate that *in vitro* aggregation of recombinant tau pseudophosphorylated at S422 (S422E) produces more oligomeric species of tau as compared to full-length wild-type tau. These results corroborate *in situ* data demonstrating that the appearance of pS422 tau correlates better with cognitive decline than D421 tau during the progression of AD, and suggest the existence of a separate toxic pool of oligomeric tau that is independent of NFT formation. Ongoing studies will compare the extent of oligomer formation and PAD exposure in recombinant D421 and S422E tau aggregates formed *in vitro*. Our tau oligomer-specific monoclonal antibody (TOC1) will be used to measure oligomer formation and our PAD-specific antibody (TNT1) will be used to measure PAD exposure. Finally, S422E and D421 aggregate toxicity will be determined in cultured cells. Collectively, these experiments will provide a link between two important disease-related modifications of tau, oligomer formation, PAD exposure and tau-mediated toxicity.

Disclosures: C.T. Tiernan: None. **S.E. Counts:** None. **N.M. Kanaan:** None. **D. Himmelstein:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Program#/Poster#: 788.12/D1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Jean P. Shultz Biomedical Research Endowment (NMK)

Saint Mary's Doran Foundation (NMK)

Title: Characterization of tau aggregation induced by arachidonic acid and eicosanoids

Authors: *B. COMBS[1], A. S. ALBERTS[2], N. M. KANAAN[1];
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Abstract: Aggregation of the tau protein is a predominant pathological marker of tauopathies, a group of neurodegenerative disorders including Alzheimer's disease (AD). Tau aggregates are closely linked to toxicity and neuronal pathology in these diseases. Recent evidence suggests that smaller aggregates, known as oligomers, may be particularly toxic. Despite its clear role in the propagation of these diseases little is known about the initiation of tau aggregation *in situ*. Unmodified tau is normally soluble and resistant to aggregation but changes in the conformation can enhance the propensity for aggregation. Phosphorylation of tau is commonly found in tau aggregates but it does not appear to be sufficient to induce aggregation and the possibility remains that a cofactor or inducing agent may be involved. For many years, investigators have used arachidonic acid (ARA) to induce tau aggregation *in vitro*, but whether ARA or ARA metabolites (i.e. eicosanoids) are the physiological inducers of tau aggregation *in situ* is unknown. Eicosanoids are naturally occurring metabolites of ARA and are mediators in inflammatory and other signaling pathways. Alterations in the levels of particular eicosanoids have been noted in many tauopathies, including AD and traumatic brain injury. Therefore, it is possible that changes in endogenous levels of ARA or eicosanoids could lead to increased direct interactions with tau that induce its aggregation and promote a neurodegenerative cascade. This study is aimed at testing ARA and eicosanoids as potential inducers of tau aggregation both *in vitro* and in cultured cells. We tested several eicosanoids to determine their relative abilities to induce tau aggregation *in vitro* and characterize the aggregates. Unmodified ARA was clearly the most potent aggregation inducer of any of the tested molecules. Ongoing studies are using microinjection to deliver ARA and eicosanoids directly into cultured cells expressing tau in order to characterize its effects in a cellular environment. Ultimately, this work may help to identify

whether modifying ARA production/metabolism represent a viable strategy aimed at mitigating the aggregation of tau in the brain.

Disclosures: B. Combs: None. **N.M. Kanaan:** None. **A.S. Alberts:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.13/D2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Japan Foundation for Aging and Health

the Research Funding for Longevity Sciences (26-32) from the National Center for Geriatrics and Gerontology (NCGG)

Title: Identification and characterization of extracellular tau

Authors: *H. YOSHIDA;
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Abstract: Alzheimer’s disease and other tauopathies are pathologically characterized by intracellular filamentous inclusions of abnormally hyperphosphorylated tau. Recent work suggests that tau pathology is likely to spread in a prion-like manner. Some experimental models of this hypothesis using cultured cells and mouse indicated that primary seeds of the identical isoform are required and trigger the formation of the inclusions and following propagation of them. However, it is unclear what actually induces tau pathology in tauopathy and that the characteristics of tau inducing the following chain propagation of tau pathology in the model systems. In this study, extracellular tau, which is thought to be one of the candidates to mediate or induce the propagation, was identified in conditioned media of cells stably expressing human tau and fractions collected by mouse brain microdialysis, and then biochemically characterized. Tau was detected in both conditioned media of SHSY5Y cells expressing human tau and fractions collected by mouse brain microdialysis on immunoblot. It was also labeled by phosphorylation-dependent anti-tau antibody AT270, indicating phosphorylation at T181. In addition, tau in these fractions was present as multiple sizes of oligomer, while tau in the cell and mouse brain extracts as relatively smaller sizes of oligomer. These results indicated the presence

of extracellular tau as multiple sizes of oligomer, and suggest a significance of contribution of oligomer formation of tau to produce extracellular tau.

Disclosures: H. Yoshida: None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Program#/Poster#: 788.14/D3

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JSPS (Grant-in-Aid for Young Scientists (B)) 26860052

Tokyo Biochemical Research Foundation (Grants-in-Aid for Scientific Research)

ONO Medical Research Foundation (Grants-in-Aid for Scientific Research)

Title: Tau pathology regulated by membrane lipid

Authors: *A. SUMIOKA, A. GOTO, Y. SOEDA, A. TAKASHIMA;
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Abstract: Alzheimer's disease (AD) is one of the most common forms of dementia. It is required to establish their therapy. Senile plaques and neurofibrillary tangles are pathological features of AD. Neurofibrillary tangles formation is well involved with AD development (Braak staging). A major component of neurofibrillary tangle is aggregate of hyper-phosphorylated Tau protein. So aggregation of Tau protein would be one of the causative factors of AD and it is important to elucidate their molecular mechanism. Interestingly, purified recombinant Tau protein is known to be stable, and heparin as an anionic inducers, is required for *in vitro* Tau aggregation. These finding suggest an endogenous regulator for Tau aggregation in brain. In this study, we addressed a role of membrane lipid for Tau aggregation. We examined an interaction between Tau and lipids. We found lipid X1 as a specific interactor of Tau protein and confirmed their interaction on lipid bilayers by liposome floatation assay. Then we examined a mechanism of the interaction. We identified a specific region of Tau protein as a lipid interacting domain by using Tau deletion mutants. Furthermore we examine the effect of lipid X1 on Tau aggregation. We will discuss recent progress in its roles.

Disclosures: **A. Sumioka:** None. **A. Goto:** None. **Y. Soeda:** None. **A. Takashima:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 788.15/D4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 0759 DoD

Title: Temporal sequence of TBI-dependent post translational modifications of Tau in a mouse model of concussion

Authors: *R. C. PELOT[1,2,3], J. REED[1,3], G. CRYNEN[1,2], C. BACHMEIER[1,2,3], J. EVANS[1], L. ABDULLAH[1], F. CRAWFORD[1,2,3];
[1]Roskamp Inst., Bradenton, FL; [2]The Open Univ., Milton Keynes, United Kingdom; [3]The Veteran's Admin., Tampa, FL

Abstract: Traumatic Brain Injury (TBI) is a condition that has recently come to the forefront of the media's attention due to the prevalence of mild to severe head injury in combat veterans and athletes. In the United States alone, there are between 1.6 -3.8 million mild to moderate TBIs per year in connection with organized sport and recreation. In the TBI condition Chronic Traumatic Encephalopathy CTE (formerly dementia pugilistica) there appears to be a key role for the microtubule associated protein tau, but the nature of tau's role in this neurodegenerative process is as yet unknown. The use of quantitative proteomics in the characterization of disease states by Mass Spectrometry (MS) is becoming widespread, but has not yet been fully applied to tau biochemistry. While analysis of tau by western blot utilizes several well known antibodies to identify pathology, it cannot cover the entire range of PTMs, even when trying to identify phosphorylation alone. In this study, we present an MS based characterization of tau pathobiology following repetitive mild TBI in hTau mice. Mouse brain tissue was analyzed at 24hr, 1 week, 1 month, & 3 months post injury using quantitative mass spectrometry to map as many PTMs as possible, including Phosphorylation, O-GlcNAc, Deamidation, Acetylation, & Methylation. To determine the TBI-dependent changes in tau PTM status we used XCMS to measure the differences in peak area against the total ion current in injured versus sham mice over time. We have thus established a protocol to quantitatively determine the level of modification of several types of PTMs on tau, which may be further exploited to investigate pathological changes in many different tauopathies.

Disclosures: **R.C. Pelot:** None. **J. Reed:** None. **G. Crynen:** None. **C. Bachmeier:** None. **J. Evans:** None. **L. Abdullah:** None. **F. Crawford:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Title: Calorie restriction, age, and genotype modulate the intestinal microbiota in a mouse model of Alzheimer's disease

Authors: *L. M. COX[1], M. J. SCHAFER[2,4], S. D. GINSBERG[3,4], M. J. BLASER[1,5]; [1]Departments of Med. and Microbiology, [2]Dept. of Cell Biol., [3]Departments of Psychiatry and Physiol. & Neurosci., NYU Langone Med. Ctr., New York, NY; [4]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY; [5]New York Harbor Dept. of Veterans Affairs Med. Ctr., New York, NY

Abstract: Alzheimer's disease (AD), a late onset neurodegenerative disorder, is associated with accumulation of β-amyloid in senile plaques and tau in neurofibrillary tangles. Animal model studies indicate that AD-like pathology can be influenced by changes in diet, metabolism, and immunity, indicating that factors distant from the brain play a role in AD pathogenesis. The intestinal microbiota, composed of trillions of microbial cells, influences host metabolism, immunity, and cognitive function, and has been hypothesized to be linked mechanistically to AD pathobiology, but it's role remains to be adequately tested. We sought to determine whether there

were specific microbial signatures associated with calorie restriction (CR)-mediated protection against AD pathology in the well-established mouse model of cerebral amyloid overexpression, the Tg2576 mouse, which exhibits age-dependent increases in Aβ plaque load and concomitant memory deficits driven by overexpression of a human APP variant (APPswe) responsible for early-onset AD. To determine whether intestinal microbiota changes occur in Tg2576 mice, and whether or not they are responsive to CR, wild-type or Tg2576 mice were randomized to ad-libitum (AL) or CR dietary regimens, with 30% carbohydrate reduction starting at 3 months of age. As we have previously shown, CR significantly reduced amyloid plaque burden in Tg2576 mice at 15 months of age, particularly in females. Fecal microbiota samples were collected 2 months and 1 year after the dietary intervention was begun, and microbial communities were surveyed by sequencing the 16S rRNA gene V4 region, with a mean (± standard deviation) depth of coverage of 30,148 ± 4,933 sequences per sample. Both CR and mouse genotype alter the microbiota early (at age 5 months) and late (at age 15 months), with progressive changes over time. CR selected for particular bacterial taxa previously associated with prolonged longevity and reduced systemic inflammation, including Lactobacillus and Allobaculum, and the effect was greatest in the aged Tg2576 mice, suggesting that in this model system, CR may have therapeutic value with potential translational benefits. This study characterizes key taxonomic changes and identifies particular intestinal microbiota as modifiable therapeutic targets for AD. Further investigation of the interaction between the intestinal microbiota and age-related neurodegenerative disorders in animal model systems is warranted to provide mechanistic insight into AD pathology, and to reveal novel therapeutic approaches for preventing and treating AD and related dementing disorders.

Disclosures: **L.M. Cox:** None. **M.J. Schafer:** None. **S.D. Ginsberg:** None. **M.J. Blaser:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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AG014449

AG10124

Alzheimer's Association IIRG-12-237253

Penn ADCC

Title: qPCR analysis of 3-repeat tau (3Rtau) and 4-repeat tau (4Rtau) isoforms in Alzheimer's disease

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Abstract: The microtubule-associated protein tau is the main component of neurofibrillary tangles (NFTs). Six distinct isoforms of tau are produced in the adult human brain via alternative mRNA splicing. Three isoforms contain 3 microtubule binding domain tandem repeats {3-repeat tau (3Rtau)} with amino terminus inserts (0 inserts, 1 insert, or 2 inserts) termed 3R0N, 3R1N, and 3R2N, respectively. Similarly, three isoforms contain 4 microtubule binding domain tandem repeats {4-repeat tau (4Rtau)} with amino terminus inserts (4R0N, 4R1N, and 4R2N). Assessment of the mRNAs encoding the 6 isoforms of human tau have been difficult to determine unequivocally due to their high sequence homology. Therefore, specific alterations within individual tau isoforms have remained elusive, notably in vulnerable regions and cell types within the brain where tauopathic processes are thought to be initiated. We have developed a qPCR-based assay with associated computational methodology to quantitate the 6 isoforms of tau using human postmortem brain tissues as sources of input RNA. Combinatorial pairs of qPCR primers are employed to detect 3Rtau (3R0N, 3R1N, or 3R2N) and 4Rtau (4R0N, 4R1N, or 4R2N), either directly or via computational analysis. Individual tau isoform expression is represented relative to the total expression of all 6 isoforms. This experimental design allows for statistical comparisons of each tau isoform in AD and age-matched normal controls. Preliminary results from entorhinal cortex of normal control subjects indicate that 3N0R and 4R0N are most abundant, accounting for approximately 45% and 27% of the total expression of tau isoforms, respectively. 3R1N (~15%) and 4R1N (~10%) and the 2 insert tau isoforms 3R2N (~2%), and 4R2N (~1%) are expressed at lower concentrations. Preliminary qPCR assessment in AD brains (n = 14) and nondemented age-matched control (CTR) subjects (n = 11) indicate selective changes within individual tau isoforms. Specifically, downregulation of 3Rtau isoforms 3R1N and 3R2N was observed concomitantly with upregulation of the 4Rtau isoform 4R0N, indicating a complex alteration of tau isoforms in AD within an early vulnerable area. These preliminary assessments will be validated in a larger cohort of AD and CTR subjects along with additional regional evaluations including frontal cortex, hippocampus, and brainstem. Additional evaluation of AD cases as well as subjects with mild cognitive impairment (MCI) and tauopathies is being

performed to test the hypothesis of differential vulnerability of specific tau isoforms, as well as assessing whether the ratio of 3Rtau/4Rtau expression changes in neurodegenerative disorders.

Disclosures: **S.D. Ginsberg:** None. **S.H. Lee:** None. **D.J. Irwin:** None. **J.Q. Trojanowski:** 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.; Collaborator and lecturer at Johnson & Johnson. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Support from Bristol Myers Squibb and GSK. F. Consulting Fees (e.g., advisory boards); Associate Editor of Alzheimer's & Dementia. **E. Petkova:** None. **S. Che:** None.

Poster

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Program#/Poster#: 788.18/D7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Characterization of tau and phosphorylated tau (ptau) in brains from human Alzheimer's disease and tauopathy subjects

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Abstract: Alzheimer's disease (AD) and related tauopathies are associated with an increase in brain insoluble tau and ptau based on post-mortem biochemical and histological analysis. In this study, we utilized a panel of tau and ptau ELISA assays, spanning the entire tau sequence, to systematically evaluate changes in tau levels from extracts derived from brains of multiple neurodegenerative disorders. Brain samples were provided by the Center for Neurodegenerative Disease Research (CNDR) at the Perelman School of Medicine of the University of Pennsylvania. We compared soluble and insoluble brain extracts from AD, Corticobasal Degeneration (CBD), Pick's disease (PiD), Progressive Supranuclear Palsy (PSP), Parkinson's disease (PD), Frontotemporal Lobar Degeneration with TDP-43 pathology (FTLD-TDP) and age-matched control subjects (n=10/group). A panel of 5 total tau assays, which interrogate the mid-domain, amino-terminal, microtubule repeat domain and carboxy-terminal regions of tau

and 6 different ptau assays (pT181, pS202-pT205, pT231, pS396, pS404 and pS422) were used to assess disease-specific changes. In AD and CBD subjects, significant increases in amino-terminal and carboxy-terminal tau levels were evident in brain insoluble extracts, without any change in mid-domain tau. Interestingly, brain soluble tau levels were unchanged across this panel of tau assays. In contrast, brain ptau levels were significantly elevated in AD and tauopathies, in both soluble and insoluble extracts with a rank order of AD > CBD > PiD > PSP. PD and FTLD-TDP brains did not show any tau changes compared to controls. A similar pattern of tau and ptau changes was evident in mouse models that overexpress mutant tau - both aged (6-8 month) Tg4510 mice and in PS19 mice injected intra-cerebrally with preformed synthetic tau fibrils. Taken together, the results confirm that in human disease and tau overexpression models, tau pathophysiology is associated with an increase in ptau levels. In addition, these data demonstrate greater antibody accessibility of the amino and carboxy-terminal regions of tau suggesting a change in the conformation of these epitopes when tau becomes misfolded and aggregated. These studies provide novel hypothesis with respect to tau aggregation and identify targets for tau immunotherapy.

Disclosures: **S. Sankaranarayanan:** A. Employment/Salary (full or part-time):; Bristol-Meyers Squibb. **L. Yang:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. **N. Hoque:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. **G. Cadelina:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. **D. Barten:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. **C.F. Albright:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. **J.B. Toledo:** 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.; Bristol-Myers Squibb. **K. Brunden:** 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.; Bristol-Myers Squibb. **V.M.Y. Lee:** 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.; Bristol-Myers Squibb. **J.Q. Trojanowski:** 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.; Bristol-Myers Squibb. **M. Ahlijanian:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb.

Poster

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Title: Cross-talk between PI3K/AKT/GSK-3beta and PP2A pathways determines tau hyperphosphorylation

Authors: *F. LIU[1,2], Y. WANG[2], R. YANG[2], X. YIN[2], J. GU[1,2], N. JIN[1,2], K. IQBAL[1], C.-X. GONG[1], C. CHENG[2];
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Abstract: Intraneuronal aggregation of abnormally hyperphosphorylated tau is a hallmark of a family of neurodegenerative diseases called tauopathies. Hyperphosphorylation of tau disrupts microtubules and promotes its self-assembly into paired helical filaments. Thus, hyperphosphorylation of tau is crucial for tau pathology in Alzheimer's disease and related tauopathies. Glycogen synthase kinase-3β (GSK-3β) and protein phosphatase 2A (PP2A) are the major regulators of tau hyperphosphorylation. Both enzyme activities are regulated by post-translational modifications. GSK-3β is phosphorylated and inactivated by activation of PI3K/AKT pathway, whereas Leu309 methylation of PP2A catalytic subunit (PP2Ac) is required for its phosphatase activity toward tau. The relationship between these two enzymes and its impact on tau hyperphosphorylation are not well understood. In the present study, we determined the cross-talk between PI3K/AKT/GSK-3β and PP2A pathways. We found that former PI3K signaling regulated the methylation of PP2Ac via GSK-3β. Up-regulation of GSK-3β led to an increase in the methylation and activity of PP2Ac through phosphorylation of leucine carboxyl methyltransferase 1 (LCMT-1) and suppression of protein phosphatase methylesterase-1 (PME-1) expression, two enzymes maintaining PP2Ac methylation. PP2A also regulated GSK-3β phosphorylation. Down-regulation of PP2A enhanced Ser9 phosphorylation of GSK-3β and inhibited its kinase activity. Thus, GSK-3β and PP2A regulate each other and control tau phosphorylation both directly and indirectly through each other. Reduction of tau phosphorylation by inhibition of GSK-3β may be more than offset by inhibition of PP2A through

a shift in PME-1/LCMT-1 balance; PP2A regulates phosphorylation of tau at Ser262/356, a required site for tau pathology. These findings suggest targeting PP2A and not GSK-3β to inhibit tau pathology.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NCGG Institutional Grant 23-38

Japan Foundation for Aging and Health

Title: Effects of methylene blue and another oxidant on human tau transgenic fly models

Authors: S. SHEIK MOHIDEEN[1], Y. YAMASAKI[1], Y. OMATA[2], L. TSUDA[1], *Y. YOSHIIKE[1];
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Abstract: Objective: Neurofibrillary tangles (NFT) formed by aggregation of tau are one of the pathological hallmarks of Alzheimer's disease. Methylene blue (MB) has been reported to suppress tau aggregation *in vitro* by oxidizing cysteine residues of tau. Expression of human wild type tau (2N4R) in neurons of *drosophila* causes phenotypic abnormalities such as climbing deficits "without" NFT. We investigated the effects of MB and another compound that promotes oxidization by the common mechanism on tau fly models. Methods: Human tau-expressing flies were treated with 1mM compound for one month. In prior to biochemical examination of tau accumulation in fly heads, their climbing activity was analyzed. Results: Although it is known that no NFT is formed in tau flies, we were able to detect tau in the sarkosyl insoluble fraction of the fly heads. As a result of 1-month MB treatment, the sarkosyl insoluble tau was reduced. We also found that MB treatment had reduced not only tau in TBS soluble fraction but also the total concentration of TBS soluble proteins. These results suggest that MB may not simply work as a tau aggregation inhibitor but may play other roles for reducing tau accumulation. MB treatment on flies expressing tau in neurons ameliorated their climbing deficits. We also found that those

flies that were able to climb high had less sarkosyl-insoluble tau than those flies that did not climb high, suggesting a correlation between the climbing deficits and the insoluble tau accumulation. Interestingly, the median survival rate of the flies treated with MB decreased to almost half of the control. In search of a compound that may work similarly as MB without substantial aversive effects, we looked for one that has a common oxidizing property with good safety profile. Treatment of tau flies with this compound resulted in the reduction of tau accumulation as well as improvement of climbing activity even though the extent of these efficacies was smaller than MB. Unlike MB, no significant effect on fly lifespan was observed by treating with this compound. Conclusion: Treatment with MB or another oxidizing compound reduced tau accumulation in human tau-expressing fly models and improved their climbing deficits.

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Poster

788. Alzheimer's Disease: Tau Biochemistry

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Program#/Poster#: 788.21/D10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Age-dependent changes of beta-amyloid and tau protein in two mouse models of Alzheimer’s disease

Authors: *E. SCHENKER[1], G. ROLLIN-JEGO[1], F. IOP[1], R. BILLIRAS[1], J.-M. RIVET[1], D. FAVALE[1], V. PASTEAU[1], S. DIX[2], C. CZECH[3], L. OZMEN[3], J. C. RICHARDSON[4], A. GOBERT[1];
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Abstract: Background Alzheimer Disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, with the pathology progressing from the entorhinal cortex to hippocampal subregions and the cortical area via anatomical connections. The process involved in disease progression is still poorly understood. However, the spreading of the pathology through neuronal pathways can be evaluated using microdialysis. The present study monitors the different forms of beta-amyloid, tau, monoamine, acetylcholine and amino-acid levels in the

interstitial fluid (ISF) of two different transgenic mice strains considered representative of the pathology. **Method** Male TauPS2APP ($APP^{695\ (K670N-M671L)}$/ $PS2^{N141I}$/ Tau^{P301L}**)** and TASTPM ($APP^{695(K670N-M671L)}$/ $PS1^{M146V}$) mice were obtained via the PharmaCog consortium and evaluated between 3 and 23 months. A 2mm, 300kDa, cut off membrane, (Brainlink, Netherland) was stereotaxically implanted (Lat, -0.4, AP, +2.0, DV, -3.0) in the frontal cortex under a ketamine/xylazine anaesthesia. Two days later, freely-moving mice were perfused at a flow rate of 0.5µl/min with a Ringer solution and samples collected during 7 hours. For whole brain extracts parallel cohorts were evaluated. Simultaneous ELISA quantification of $Abeta_{1-40}$ and $Abeta_{1-42}$ as well as total tau was performed in ISF and brain extract samples using MesoScale Discovery's electrochemiluminescence detection. **Results** Frontocortical ISF levels of total tau increases with age in both strains. $Abeta_{1-40}$ and $Abeta_{1-42}$ ISF levels rapidly increase in the TASTPM mice whereas the increase seems to be delayed in the TauPS2APP mice. Brain tissue levels of soluble and insoluble $Abeta_{1-40}$ and $Abeta_{1-42}$ showed an increase in both strains, whereas no change in total tau was noted. The increase observed in TauPS2APP mice was in favour of the $Abeta_{1-42}$ form. No significant differences were observed in the levels of the neurotransmitters tested in either strain. **Conclusions** Monitoring beta-amyloid, tau, monoamine, acetylcholine and amino-acid levels using microdialysis provides a useful tool to assess the spreading of the AD pathology and neuronal dysfunction *in vivo*. The research leading to these results was conducted as part of the PharmaCog consortium funded by the European Community's Seventh Framework Programme for the Innovative Medicine Initiative under Grant Agreement n°115009. All procedures using these animals conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Disclosures: **E. Schenker:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **G. Rollin-Jego:** A. Employment/Salary (full or part-time):; IdR Servier. **F. Iop:** A. Employment/Salary (full or part-time):; IdR Servier. **R. Billiras:** A. Employment/Salary (full or part-time):; IdR Servier. **J. Rivet:** A. Employment/Salary (full or part-time):; IdR Servier. **D. Favale:** A. Employment/Salary (full or part-time):; IdR Servier. **V. Pasteau:** A. Employment/Salary (full or part-time):; IdR Servier. **S. Dix:** A. Employment/Salary (full or part-time):; Eli Lilly. **C. Czech:** A. Employment/Salary (full or part-time):; Hoffmann-La Roche. **L. Ozmen:** A. Employment/Salary (full or part-time):; Hoffmann-La Roche. **J.C. Richardson:** A. Employment/Salary (full or part-time):; GSK. **A. Gobert:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH UL1 TR000165

NIH R01NS075487

NIH T32 NS061788

Title: Targeting Tau-SH3 Interactions in Alzheimer's Disease

Authors: *J. COCHRAN[1], P. DIGGS[1], M. NEBANE[3], T. RUSH[1], J. YAN[3], I. PADMALAYAM[3], L. RASMUSSEN[3], E. CAPRIOTTI[2], B. BOSTWICK[3], L. WHITE[3], J. MADDRY[3], M. SUTO[3], E. D. ROBERSON[1];
[1]Ctr. for Neurodegeneration and Exptl. Therapeut., [2]Pathology Informatics, Univ. of Alabama at Birmingham, Birmingham, AL; [3]Southern Res. Inst., Birmingham, AL

Abstract: New therapies for Alzheimer's Disease (AD) are critically needed. Many therapies have focused on Aβ, which is an important cause of AD. However, Tau is a critical mediator of the detrimental effects of Aβ and Tau abnormalities can propagate independently of Aβ. Because of this, Tau-directed therapies will be an important complement to Aβ-directed therapies for AD. After showing robust benefits of Tau reduction in multiple AD models, we began investigating how Tau reduction may provide benefit in order to identify druggable targets. A variety of evidence from our lab and others implicates Tau-Fyn interactions in the pathogenesis of AD. Preventing the Tau-Fyn interaction, either by removing Tau, removing Fyn, or expressing a dominant negative Tau, has strong beneficial effects in models of AD. Because of this, we conducted a high-throughput screen and identified a variety of chemically tractable small molecule inhibitors of the Tau-Fyn interaction. We are continuing development of Tau-Fyn interaction inhibitors by analog development and screening in a variety of secondary and liability assays. As SH3 domains are enriched at the synapse compared to overall proteomic prevalence, we are investigating other Tau-SH3 interactions using both candidate and unbiased approaches using techniques in animal tissue, in reductionists assays, and in silico. These efforts will allow us to identify both liability SH3 domains and SH3 domains that may serve as other mediators of Aβ-induced synaptic abnormalities. These ongoing efforts highlight the importance of Tau-SH3 interactions as potential mediators of Aβ-induced abnormalities which are therapeutically targetable.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG017753

Title: Tau: an interactor and substrate for protein tyrosine phosphatase SHP-2

Authors: Y. KIM, M. B. FRANCIS, C. J. LEUGERS, *G. LEE;
Univ. Iowa, Iowa City, IA

Abstract: The microtubule-associated protein tau is involved in regulating microtubule assembly and function in neurons. During early brain development, tau is normally phosphorylated at several serine/threonine residues such as Thr231, with phosphorylation levels decreasing in adult. Previously, Leugers and Lee reported that in NGF-induced differentiation of PC12-derived cell lines, MAPK activation was potentiated by tau and that phosphorylation at Thr231, which reduces its affinity for microtubules, was required. To investigate the mechanism by which tau enhances the NGF-stimulated MAPK activity in PC12 cells, we examined immunoprecipitates of phospho-Thr231-tau, looking for associated proteins. We found SHP-2, a protein tyrosine phosphatase that is also required for NGF-induced MAPK activation during PC12 cell differentiation. The interaction was confirmed in COS7 cells, where tau, expressed by transfection, co-immunoprecipitated with endogenous SHP-2. To determine if phosphorylation at Thr231 of tau was required for the association with SHP-2, we expressed T231A-tau and found an association with SHP-2, suggesting that phosphorylation on Thr231 was not required for the association with SHP-2. We found that other Ser/Thr to Ala tau mutants also co-immunoprecipitated with SHP-2. However, when tau was immunoprecipitated by antibodies that did not bind to highly phosphorylated tau, SHP-2 did not co-immunoprecipitate. This suggests that tau phospho-sites other than those tested may be important for the association with SHP-2. In addition, *in vitro* phosphatase assays, using either full-length tau or tau peptide, showed that SHP-2 dephosphorylated phospho-Tyr18-tau, suggesting that phospho-Tyr18-tau was a substrate for SHP-2. Taken together, our data suggest that tau associates with SHP-2 and is a potential substrate for SHP-2 in cells. The role of the tau-SHP-2 interaction in NGF-induced MAPK activation remains to be shown. Given the regulation of phospho-Tyr18-tau seen in Alzheimer's disease (AD) brain, SHP2 might be active during neurodegeneration. Also, since MAPK activity is augmented in AD and SHP-2 is critical for MAPK activation in developing neurons, this also suggests that SHP-2 may have a role in AD.

Disclosures: **Y. Kim:** None. **M.B. Francis:** None. **G. Lee:** None. **C.J. Leugers:** None.

Poster

788. Alzheimer's Disease: Tau Biochemistry

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the American Federation for Aging Research Grant

Title: Critical role of death-associated protein kinase 1 in regulating tau protein accumulation in Alzheimer’s disease

Authors: ***T. LEE**, M.-H. YOU, B. KIM;
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Abstract: Background The neuropathological hallmarks of Alzheimer’s disease (AD) are intracellular neurofibrillary tangles made of hyperphosphorylated tau and extracellular senile plaques consisted of Aβ deposits. Interestingly, the formation of neurofibrillary tangles in AD is preceded by increased phosphorylation of tau and other proteins on specific serine or threonine residues. However, it is not fully understood how these aberrant tau phosphorylation events lead to neurodegeneration and how to prevent this phosphorylation-induced neurodegeneration in AD. Death-associated protein kinase 1 (DAPK1) is a Ser/Thr kinase and functions as a positive mediator of apoptosis and is genetically linked to AD. However, the association between DAPK1 and late onset AD has not yet been explored since little is known about the downstream targets of DAPK1 involved in AD. In this study, therefore, we aimed to elucidate the mechanisms of DAPK1 action on tau protein regulation and tau pathology using various cell lines and mouse model. Methods We investigated the effects of DAPK1 on tau protein regulation by examining tau protein stability and phosphorylation status using comprehensive approaches, including gene knockout, knockdown in cell culture models and mouse, and human patient tissues. Moreover, since DAPK1 inhibits the catalytic activity of Pin1 by phosphorylating Ser71 residue, we also assessed the correlation of DAPK1 expression and Ser71 phosphorylation of Pin1 and assessed the involvement of Pin1 inhibition in DAPK1 action. Results We

demonstrated that DAPK1, but not kinase dead mutant form DAPK1 K42A, significantly enhance tau protein stability using cycloheximide chase experiment. DAPK1, but not K42A, also triggered tau phosphorylation in cell lines and DAPK1 knockout reduced tau phosphorylation in mouse model. We also demonstrated that DAPK1 knockout significantly reduced total expression and phosphorylation of tau in sarkosyl-insoluble fraction in an age-dependent manner in mouse brains. Moreover, the expression of DAPK1 was increased more than 2 folds in hippocampus of AD patients compared to normal alone with tau hyperphosphorylation. In addition, Ser71 phosphorylation of Pin1 was increased in DAPK1-expressing neuroblastoma cells. Moreover, DAPK1 did not exert its effect on tau protein regulation in Pin1 knockout MEF cells, and did not affect T231Atau protein stability. Conclusions These results indicate that DAPK1 regulates protein stability and phosphorylation status of tau and might regulate tau-related pathology. Our results also suggest that Pin1 inhibition is critical in DAPK1-mediated tau regulation.

Disclosures: **T. Lee:** None. **B. Kim:** None. **M. You:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Program#/Poster#: 789.01/D14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Mitchell Center for Neurodegenerative diseases

Title: Removal of tau oligomers by immunotherapy decreases Aβ56* and improves cognition in an AD mouse model

Authors: *D. L. CASTILLO[1], M. J. GUERRERO-MUÑOZ[2], U. SENGUPTA[2], C. HERNANDEZ[2], K. DINELEY[2], R. KAYED[2];
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Abstract: **Background** In Alzheimer’s disease (AD) the misfolding of functional tau monomer seems to be coupled upstream to the aggregation Aβ a more potent amyloidogenic peptide. Soluble Aβ oligomeric species can drive the formation of neurotoxic tau oligomers *in vitro* and *in vivo*. However, the relationship between these two proteins and neuronal loss lacks a mechanistic explanation. Furthermore, evidence from animal models suggests that amyloid beta toxicity is mediated by tau]. We hypothesize that tau oligomers formation plays crucial role in

driving AD pathogenesis. Thus, tau oligomers represent an ideal therapeutic target for the treatment of AD. In order to study the removal of toxic tau assemblies in an animal model of AD (Tg2576), we generated a tau oligomer specific antibody (TOMA). This antibody does not recognize the functional monomeric tau or oligomers from other amyloidogenic proteins. **Methods** Here we used the Tg2576 mouse model which overexpress the human APP with the Swedish double mutations (K670N, M671L) under the control of a hamster prion protein promoter.14-month old Tg2576 mice, received a single iv injection of 30 µg of the TOMA antibody. Control group received 30 µg of non-specific IgG. Cognitive function was assessed by novel object recognition test, 15 days after injection. In addition, western blot, ELISA and Immunostaining were performed to evaluate the response to treatment. **Results** Our results indicate that single iv-injection of the TOMA antibody, reduce endogenous tau oligomers and improve cognition in the Tg2576 mouse. Interestingly, removal of tau oligomers by immunotherapy decreases amyloid beta-56* and increases deposition of plaques in immunized mice. **Conclusion** Our results support the findings that tau oligomers mediate Aβ toxicity *in vivo*. Moreover, removal of tau oligomers by immunotherapy may induce Aβ aggregates to assembly into inert and perhaps protective plaques. Thus, targeting tau oligomers by immunotherapy may represent a novel strategy for the treatment of AD and other neurodegenerative tauopathies.

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Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.02/D15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Bexarotene reduces intraneuronal APP/Aβ and neuronal loss in 5XFAD mice

Authors: *M. HOLLEY, T. JAY, B. CASALI, T. MALM;
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Abstract: Alzheimer’s disease (AD) is the most common form of dementia, and is characterized by accumulation and deposition of soluble forms of amyloid beta (Aβ), gliosis, and extensive neuronal loss. We have recently demonstrated that an agonist of the nuclear receptor RXR bexarotene (bex), promoted the clearance of amyloid from the brain and improved cognition in

several amyloidogenic AD mouse models. To extend this work we chose to examine 5XFAD mice, a mouse model that exhibits neuronal loss. 5XFAD mice exhibit intraneuronal amyloid deposition at 1-2 months of age, mild cognitive deficits at 4 months, and extensive neuronal death. The first wave of neuronal death occurs in the subiculum between 2-4 months of age, and the second wave occurs in layer V of the cortex between 6-8 months. Therefore, we hypothesized that bex treatment in 5XFAD mice will improve cognition before and after neuronal loss and attenuate neuronal death through reduction in soluble Aβ and insoluble Aβ. We treated 4 and 8 month 5XFAD mice with bex for 14 days. Bex treatment increased ApoE, Abca1, and Abcg1 levels in 5XFAD mice at all ages indicating increased lipidation of ApoE. Although bex treatment significantly reduced amyloid plaque burdens, bex treatment did not significantly reduce soluble or insoluble Aβ. Importantly, in both 4 and 8 month 5XFAD bex treated mice, intraneuronal APP/Aβ was reduced in the cortex. The reduction in intraneuronal APP/Aβ, in bex treated mice, correlated with a significant preservation of subicular and cortical neurons during their respective die-off periods. Moreover, bex treatment improved long term memory in fear conditioned mice aged 4 and 8 months, and improved olfaction cross habituation in 8 month 5XFAD mice. There is evidence that autophagy and lysosomal acidification are impaired in 5XFAD mice. Western analysis indicates that bex treatment alters protein expression of Beclin-1 and LC3II which are critical for initiation of autophagy and autophagosome formation, respectively. Collectively, we provide evidence that Bex treatment reduces amyloid species and improves behavior in an aggressive model of AD which exhibits neurodegeneration.

Disclosures: **M. Holley:** None. **T. Jay:** None. **B. Casali:** None. **T. Malm:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Title: Specific alterations of tau phosphorylation and neuronal signaling induced by the amyloid-β oligomer Aβ*56

Authors: *F. AMAR[1,2,3], M. A. SHERMAN[1,2,3], T. J. RUSH[4], M. LARSON[1,2,3], J. A. SCHNEIDER[5], D. A. BENNETT[5], K. H. ASHE[1,2,3,6], A. BUISSON[4], S. E. LESNE[1,2,3]; [1]Grad. Program in Neurosci., [2]Bud Grossman Ctr. for Memory Res. and Care, [3]Inst. for Translational Neurosci., Univ. of Minnesota, Minneapolis, MN; [4]Inst. des Neurosciences, Univ. Joseph Fourier- Site Santé, La Tronche Cedex, France; [5]Rush Alzheimer's Dis. Ctr., Rush Univ. Med. Ctr., Chicago, IL; [6]Geriatric Res. Educ. Clin. Ctr., VA Med. Ctr., Minneapolis, MN

Abstract: In many neurodegenerative disorders including Alzheimer's disease (AD), oligomeric forms of amyloid proteins are believed to be the principal bioactive species. However, our understanding of these disorders is currently limited by insufficient knowledge about the specific molecular pathways activated by different assemblies. Here, we demonstrate that the amyloid-β (Aβ) oligomer Aβ*56 associated with preclinical AD forms a complex with NMDA receptors (NMDAR) resulting in an aberrant increase in intracellular calcium, driven by synaptic NMDARs, and the specific activation of the Ca2+-dependent calmodulin kinase CaMKIIα. Active CaMKIIα induced selective pathological changes in tau *in vivo* and *in vitro*, involving phosphorylation and missorting. Importantly, other forms of endogenous Aβ oligomers did not trigger these effects. Our results indicate that distinct Aβ oligomers activate neuronal signaling pathways in a highly selective manner, leading to AD pathology, and suggest a general strategy for dissecting the molecular events caused by endogenous oligomeric misfolded proteins.

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Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Program#/Poster#: 789.04/D17

Topic: C.02. Alzheimer's Disease and Other Dementias

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DGAPA Posdoctoral Fellow to L.F.H.Z.

Title: Chronic exposition to ozone produces β amyloid 1-42 accumulation in mitochondria of rat hippocampal neurons

Authors: *L. F. HERNANDEZ, E. RODRÍGUEZ MARTÍNEZ, S. RIVAS-ARANCIBIA; Phisiology, UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, Mexico, Mexico

Abstract: Oxidative stress is a major risk factor for Alzheimer´s Disease that has been suggested to be the trigger of AD pathology. However, whether oxidative damage precedes and contributes directly to the intracellular accumulation of beta amyloid 1-42 peptide is still a matter of debate. Nevertheless, it has been recently shown that free radicals cause exacerbation of the amyloid pathology in AD. Environmental, dietary and pathological factors have been related with oxidative stress generation. The chronic exposure to low doses of ozone similar to a day of high pollution in México City causes a state of oxidative stress producing progressive neurodegeneration in hippocampus of rats exposed to this gas. Several reports have demonstrated that mitochondria are one of the first organelles affected by oxidative stress and beta amyloid 1-42 toxicity. However the mechanisms related with neurodegeneration process and mitochondrial damage under chronic exposure to low doses of ozone have not been demonstrated. The aim of this study was to analyze the effect of ozone chronic exposure on the changes in the accumulation of beta amyloid 1-42 peptide in mitochondria of hippocampal neurons of rats exposed to low doses of ozone. Method: each group of 10 rats received one of the following treatments; control group received only air, and groups 2, 3, 4, 5 y 6 received ozone doses of 0.25 ppp. for 4 h daily, during 7, 15, 30, 60 and 90 days respectively. Groups were processed for immunohistochemical and double immunofluorescence analysis against the following proteins: beta amyloid 1-42 peptide and cytochrome C. Cellular fractionation of hippocampal cells for recover mitochondrial fraction was performed and mitochondrial protein extract was analyzed also by gel electrophoresis and Western Blot to detect beta amyloid 1-42. The results show a significative accumulation of beta amyloid peptide in 30, 60 and 90 days of ozone exposition. This indicates that there is a correlation between the time of exposure to ozone and accumulation of beta amyloid peptide 1-42 in mitochondria of hippocampal neurons of rats. Conclusions: Accumulation of beta amyloid 1-42 peptide may promote mitochondrial dysfunction and cell death. Although our findings do not explain all components of the puzzle regarding how amyloid beta causes neuronal damage, they do provide another potentially important pathogenic mechanism that may contribute to the onset and progression of AD. Finally, all this indicates that the chronic state of oxidative stress induces the neurodegeneration process probably through of beta amyloid 1-42 accumulation in mitochondria.

Disclosures: L.F. Hernandez: None. **E. Rodríguez martínez:** None. **S. Rivas-arancibia:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.05/D18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Gsk antisense improves learning and memory and reduces active gsk in samp8 mice

Authors: *S. A. FARR[1], V. B. KUMAR[1], K. WITT[2], K. E. SANDOVAL[2], J. E. MORLEY[3]; [1]Geriatrics/GRECC, St. Louis University/VA Med., St Louis, MO; [2]Pharm., SIUE, Edwardsville, IL; [3]Geriatrics/Endocrinology, St. Louis Univ. Sch. of Med., St. Louis, MO

Abstract: Glycogen synthase kinase (GSK) -3β is involved in the phosphorylation of Tau and binds and phosphorylates presinilin-1 (PS-1). Human brains from Alzheimer's patients have increased GSK-3 associated with neurofibrillary tangles, neurodegeneration and increased tau phosphorylate. The SAMP8 have a natural mutation which results in age-related impairment in learning and memory, elevated Aβ, oxidative tissue damage and elevated hyperphosphorylated Tau. We have previously shown that reducing Aβ in 12 month old SAMP8 mice with an antisense directed at APP improves learning and memory. Here, we examined the effect a phosphorothionated GSK-3β antisense in SAMP8 mice on learning and memory in T-maze foot shock avoidance. The GSK-3β antisense had a sequence that orresponds to 94-113 nucleolides downstream from the initiation codon of GSK-3 mRNA. This is an internal sequence with high probability of being away from any loop formation in the mRNA. As an internal site it should not block 100% of GSK mRNA. SAMP8 mice were injected IV with GSK antisensen 5 times at one-week intervals. Acquisition was tested 5 days after the third injection in an aversive T-maze. Retention was tested one-week later. The day after T-maze retention testing mice were tested in novel object recognition with a 24 hour retention delay. Anti-GSK improved T-maze acquisition and retention and novel object recognition retention in 12 month old SAMP8 mice compared to the mice that received a random antisense. The 12 month-old SAMP8 mice that received anti-GSK took significantly fewer trails to reach criterion of one avoidance (10.1±0.57) compared to the 12 month-old SAMP8 mice which received random antisense (112.56±0.50). On the one week retention test mice which received anti-GSK took significantly fewer trails to reach criterion of 5 avoidances in 6 consecutive trials (8.5± 0.76) compared to the mice which received random antisense (15.88±2.2). On the 24 hour retention test, the mice that received GSK antisense spent significantly greater amount of time with the novel object (63.89±2.44) than the mice that received random antisense (52.86 ± 3.45). Assays of brain tissue indicate that GSK antisense significantly reduced active GSK compared to the mice that received random antisense.

GSK-3 is thought to play a role in amyloid-β effects in the brain. Here we demonstrate that reducing GSK-3 with a phosphorothinoated GSK-3β antisense improves learning and memory. GSK-3β antisense is a potential treatment for Alzheimer's disease.

Disclosures: S.A. Farr: None. **V.B. Kumar:** None. **K. Witt:** None. **K.E. Sandoval:** None. **J.E. Morley:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Title: Comparative study of biochemical markers in cerebrospinal fluid in dog after intracerebroventricular injection of streptozotocin

Authors: *G. KIM, H. OH, M. KIM, Y. JO, J. CHOI, B. LEE;
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Abstract: Dogs naturally develop to cognitive dysfunction like Alzheimer disease (AD) in human. Several studies indicate that cerebrospinal fluid (CSF) composition depends on the disease stage and reflects the brain pathology in AD patients. The goal of this study is to investigate the concentration of various biochemical markers in CSF of dogs after icv-STZ to propose a new AD animal model. In icv-STZ group, STZ was injected into CSF via the cerebro-medullary cistern using 25G needle on day 1 and 7 under general anesthesia. They received a

single infusion of various doses of STZ (0.5 mg/kg, 1 mg/kg or 1.25 mg/kg). In the control group, same volume of saline solution was injected. CSF drainage was also performed at pre-injection (day 1) and on days 7, 14 and 21. Collected CSF was used for analysis of glucose, sodium, chloride, potassium, pyruvate and lactate concentrations. After the procedure, dogs were replaced on a cage at room temperature and were carefully observed until recovery from anesthesia. After 1 and 7 days of STZ injection, there was no significant change in the biochemical markers among the dogs infused with all varius doses of STZ compared to control dogs. However, 14 days after STZ injection, icv-STZ dogs that was injected with 1.25mg/kg showed significant high concenetrations of lactate, pyruvate and potassium in CSF samples ($P < 0.05$) compared to other dogs with doses of 0.5 mg/kg and 1mg/kg. After 21days, icv-STZ dogs injected of 1mg/kg and 1.25 mg/kg had higher concentration of lactate, pyruvate and pottasium ($P < 0.05$) than those of control and 0.5mg/kg. No significant differences in the levels of sodium, chloride and glucose of all treated groups were observed. These biochemical markers alteration in icv-STZ dogs is similar to those observed in human AD patients. These result suggests the possibility of using icv-STZ dogs as human AD models because of alterations in CSF composition after icv-STZ.

Disclosures: **G. Kim:** None. **H. Oh:** None. **M. Kim:** None. **Y. Jo:** None. **J. Choi:** None. **B. Lee:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This work was supported by the DGIST R&D Program of the Ministry of Science, ICT and Future Planning (14-BD-06).

Title: The effects of agmatine on alzheimer’s disease induced by brain insulin resistance

Authors: S. KANG[1,2], *J. SONG[1], W. LEE[1], K. PARK[1], J. LEE[1,2];
[1]Anat., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]BK21 Plus Project for Med. Science, and Brain Res. Inst., Seoul, Korea, Republic of

Abstract: Type 2 diabetes mellitus (T2DM) has detrimental effects in various organs including the brain. Many studies reported that patients with T2DM suffer from cognitive decline and

severe process of Alzheimer's disease due to brain insulin resistance. Insulin, the hormone secreted from beta-cells in pancreas, regulates glucose uptake in peripheral tissues and modulates also cognitive and memory dysfunction in brain. Agmatine is an endogenous aminoguanidine compound made from arginine producted by arginine-decarboxylase (ADC) gene. It shows positive effects on a variety of diseases including diabetes, stroke, Alzheimer's disease, depression. In present study, we investigated the agmatine's neuroprotective effect in type 2 diabetes induced Alzheimer's disease mouse model. Male ICR mice were fed a 60% high fat diet (HFD) for 8 weeks and intraperitoneally injected streptozotocin (100mg/kg) at 4th week. Then, mice were intraperitoneally administered with agmatine (100mg/kg) for 2 weeks. Following agmatine injection, mice were subjected to perform Morris Water Maze for identify their cognitive decline and its recovery. After that, mice were sacrificed and brains were prepared for immunohistochemistry (IHC) and western blot assay. To determine biochemical changes, we checked blood glucose level, body weights once a week, and serum insulin level at the end of experiment. For confirm insulin resistance state, we conducted glucose tolerance test and insulin tolerance test, and calculate the HOMA-R index using blood glucose level and serum insulin level. The treatment of agmatine alleviated insulin resistance and improved cognitive decline in Alzheimer's disease mouse model induced by high fat diet. Thus, we concluded that agmatine may alleviate the pathologies of Alzheimer's disease through improving the insulin resistance in brain.

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Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The presenting and the last authors' personal monetary contributions

Title: Intra-cerebro-ventricular erythropoietin reverses morphological and cognitive deterioration in a streptozocin-induced Alzheimer's disease rat model

Authors: ***G. O. PEKER**[1], V. SOLMAZ[2], T. CAVUSOGLU[3], G. YIGITTURK[3], O. ERBAS[4]; [1]Physiol., [2]Neurol., [3]Histology-Embryology, Ege Univ. Fac. of Med., Izmir, Turkey; [4]Physiol., Osmangazipasa Univ. Fac. of Med., Tokat, Turkey

Abstract: Background & Aim: Alzheimer's (AD) is the most devastating disease with highest prevalence rate in late middle-old aged population universally. Progressive aggregation of amyloid plaques and neuro-fibrillary tangles, and resultant neuronal death characterize this incurable, but poorly and only palliatively manageable disease. Erythropoietin (EPO) is the essential tropic/local hormone stimulating primarily red blood cell generation and maturation, and overall blood cellular elements plus several other tissues. We aimed to simulate AD in relatively older adult rats by inducing "acute brain diabetes" using intra-cerebro-ventricularly (ICV) administered streptozocine (STZ) and then comparing shuttle box performance of those chronically treated with and without systemic EPO (Epprex, Janssen). Material & Methods: With the approval of the Ege University Experimental Animal Ethics Committee, we handled 24 male (16 m.o.) Sprague Dawley rats; then, administered 12 rats STZ (3mg/kg in saline, total V=5µL, ICV, bilaterally); administered 6 rats saline (Group III) in equal V, through the same route; and spared 6 rats as naive controls (Group IV). Twelve STZ rats were then distributed randomly as Group I and Group II (n=6, each). Group I received EPO (5000IU/kg/d, ip) and Group II (placebo) received saline (1ml/kg/d,ip) for 15 days. Passive avoidance learning (PAL) task and endurance of its memory were assessed (as described in the authentic literature) in every rat starting from the 16th day on. On the 18th day, all rats were euthanized, and their brains were fixed and sectioned for Nissl staining for neuronal counts, especially in their respective, corresponding CA1 and CA3 hippocampal regions and levels. Findings & Results: Both hippocampal CA1 and CA3 neuronal counts and PAL task and memory scores showed very significant ($p<0.000$) differences between the STZ-treated (Groups I and II) and saline-treated (Group III) and naive control (Group IV) rats. STZ + EPO-treated Group I showed significant ($p<0.05$) improvements both histologically and behaviorally, compared to STZ + saline-treated Group II. Conclusion & Future Plan: Our "acute brain diabetes" model has worked out. ICV EPO has definitely favored histology, at least in the quantity and gross outlook of the neurons of interest, and more importantly, the PAL paradigm which is an index of a simple behavioral homeostasis-related cognitive function. We foresee enlightening differentially and more specifically whether this remarkable neuro-protective/neuro-restorative effect of EPO has been direct (genomic), indirect (metabolic) or both.

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Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Program#/Poster#: 789.09/D22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant AG34103

Title: Interactive effects of high fat diet and testosterone on neuroinflammation and Alzheimer's-related pathways

Authors: *V. A. MOSER, A. JAYARAMAN, J.-W. LEE, C. J. PIKE;
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Abstract: Despite the complex etiology of Alzheimer's disease (AD), a number of risk factors for the onset and progression of this neurodegenerative disorder have been identified. Among these are obesity and age-related decline in testosterone levels in men. Interestingly, obesity and testosterone are reciprocally interactive, with low testosterone promoting obesity and vice versa. Although both of these risk factors have been independently linked with AD, their potential interactions in regulating AD pathogenesis and the underlying mechanisms have thus far not been explored. A pathway common to both obesity and testosterone that is also associated with AD is inflammation. Thus, the goal of the current study was to examine the independent and combined effects of testosterone and obesity on AD outcomes and to explore neuroinflammation as a potential mechanism underlying these effects. To this end, we experimentally manipulated testosterone and obesity in both wild-type rodents and a mouse model of AD, then evaluated indices of inflammation and AD-related pathways. Male mice were maintained on either a control or a high fat diet for four months under varying levels of testosterone: sham orchidectomized (ORX), ORX, and ORX + testosterone replacement. Mice on high fat diet developed metabolic syndrome, as indicated by insulin and glucose resistance, had significantly higher levels of soluble β-amyloid, and also showed a marked increase in gene expression of pro-inflammatory cytokines. Moreover, the effects of high fat diet were exacerbated under conditions of testosterone depletion, whereas testosterone replacement therapy improved AD and neuroinflammatory outcomes, in both the presence and absence of high fat diet. In summary, we find that testosterone and diet-induced obesity independently and cooperatively impact metabolic, inflammatory, and AD-related endpoints, suggesting that neuroinflammation may be an important underlying mechanism in the process of these diseases.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant EB000768

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Title: *In vivo* imaging of smooth muscle cell loss during the progression of cerebral amyloid angiopathy

Authors: M. ARBEL-ORNATH[1], A. KIM[1], J. RAMOS-RODRIGUEZ[2], L. ZHAO[1], M. GARCIA-ALLOZA[2], S. M. GREENBERG[1], M. P. FROSCH[1], *B. J. BACSKAI[1]; [1]Dept Neurol., Mass Gen. Hosp., CHARLESTOWN, MA; [2]Biomedicine, Inst. of Biomolecules (INBIO), Univ. of Cadiz, Cadiz, Spain

Abstract: Cerebral amyloid angiopathy (CAA) is an age-related disease that frequently co-occurs in Alzheimer disease (AD) patients. Like the senile plaques of AD, the cerebrovascular deposits in CAA are comprised primarily of the β-amyloid peptide. The pathological manifestations of CAA include vascular smooth muscle cell (VSMC) degeneration and hemorrhagic and ischemic stroke. CAA-related hemorrhages are not readily preventable, and represent a serious and debilitating outcome in affected patients, highlighting the need to understand the vascular components of amyloid deposition. The concomitant accumulation of amyloid deposits along arteries and VSMC loss is established and play a crucial role in the collapse of the vessel wall and subsequent hemorrhage; however the temporal and spatial relationship between the two phenomena remains poorly understood. This is largely because the meningeal vessels, which are the ones affected by CAA in the available mouse models, are difficult to study post mortem. To better understand the close interaction between accumulation of amyloid at the arterial wall and degeneration of VSMC we established a colony of AD mouse model (APPSW/PS1dE9) in which the VSMC express EGFP (smMHC/Cre/eGFP)and took advantage of advanced imaging techniques to perform longitudinal *in vivo* imaging during the progression of CAA. This methodology allowed us to closely inspect individual VSMC over months and ask how the amyloid insult affects their structure/presence. Longitudinal *in vivo* imaging of the cerebrovasculature of these mice along CAA progression revealed an overall age-dependent loss of VSMC which correlated with the amyloid progression rate. Importantly, wild type littermates showed preserved VSMC viability. A closer inspection of both VSMC and CAA in the APP/PS1 mice at small vessel segments showed that amyloid burden itself does not correlate with VSMC loss and cannot be used to predict cell loss. This can be due to unknown factors that affect VSMC in these animals or that vessel physiology may be affected by CAA up- or downstream to it and not necessarily by this within the segment itself. Altogether, amyloid deposition on vessel walls, and the subsequent loss of VSMC may contribute to the arteries

thickening, decreased contractility and eventual collapse of the vessel wall and hemorrhage evident in CAA brains. Understanding the intimate interaction of amyloid deposition and VSMC loss and vessel dysfunction throughout the course of the disease may elucidate novel underlying mechanisms. Moreover, the ability to quantify VSMC loss during CAA progression highlights the potential of this model for evaluation of therapeutic interventions.

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Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Title: Comparative genome-wide analysis of RXR binding and expression profiling in Bexarotene treated APOE4 mice

Authors: *D. GEORGIEV, A. MOUNIER, K. NAM, N. F. FITZ, A. A. CRONICAN, I. LEFTEROV, R. KOLDAMOVA;
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Abstract: Retinoid X receptors (RXRs) are ligand-activated transcription factors that form permissive heterodimers with Liver X Receptors (LXR). Recently we have shown that RXR ligand Bexarotene improves memory and Aβ clearance in amyloid precursor protein (APP) mice expressing human apolipoprotein E (APOE) isoforms. To identify direct RXR targets genome-wide we applied chromatin immunoprecipitation followed by high-throughput sequencing

(ChIP-seq). We chose mice expressing endogenous APP to avoid possible effects of Aβ pathology on transcription factor binding. Since we were particularly interested in determining the effect of RXR binding in APOE4 and APOE3 expressing mice we chose 6 months old targeted replacement mice (WT/E4). Functional annotation clustering using Database for Annotation, Visualization and Integrated Discovery (DAVID) revealed unique Biological Process (BP) Gene Ontologies that were significantly enriched in samples from Bexarotene treated animals. The highest significantly enriched clusters in Bexarotene treated samples only were transcription regulator activity, vascular development and neuron differentiation. To determine how Bexarotene affects mRNA levels and potentially gene expression in brain, we applied high-throughput mRNA-seq technology with RNA isolated from cortices of the same mice. We identified 1440 differentially affected genes. Of those, 1020 were up- and 420 down-regulated. Among the up-regulated genes the first BP categories identified as significantly enriched were: ion transport, neuron differentiation and intracellular signaling cascade; among the down-regulated: neuron differentiation, regulation of transcription from DNA and RNA polymerase. By comparing the list of genes identified by ChIP-seq as unique binding sites in Bexarotene treated mice (27 genes) and genes differentially affected as identified by RNA-seq (55 genes; GO:0030182~neuron differentiation) we revealed differentially affected genes which are direct RXR targets. We further validated the effects of RXR-specific ligand treatment on neuron differentiation/neurogenesis in cell culture systems and *in vivo* using Next Generation Sequencing and immunohistochemistry. Conclusion: these preliminary data demonstrate the applicability of new and powerful sequencing technologies for research in Alzheimer's disease model mice and for addressing questions with significant therapeutic implications.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT 155242

DGAPA IN-209413

Title: Chronic sucrose intake is related with early cognitive impairments in 3xTg-AD and non transgenic mice

Authors: *K. R. GUZMAN-RAMOS[1,2], L. AYALA-GUERRERO[1], P. GARCIA-DELATORRE[3], F. BERMUDEZ-RATTONI[1], G. PACHECO -LOPEZ[2];
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Abstract: High sugar diets are among the most popular within the western societies and their consumption have produced health detriments at the central nervous system level. It is known that high caloric diets and type 2 diabetes are risk factors for the development of dementias, and particularly Alzheimer's Disease (AD). However, the molecular and metabolic events that lead to cognitive impairments are not clear. We studied the effect of a sucrose rich diet in two types of mice: a murine model of AD harboring three mutations related to the amyloid beta and p-tau accumulation (3xTg-AD) and non-transgenic strain where the transgenic was developed (WT). As a consequence of high sucrose diet, we found an early deterioration of object and taste recognition and spatial memories in the 3xTg-AD mice that underwent sucrose diet for 5 months (7 months of age at the time of tests), comparable to the deterioration found in the 12 months-old 3xTg-AD mice group that had a normal diet. Additionally, the high sucrose diet induced in the WT mice a cognitive impairment in the taste recognition and spatial memories also comparable to 12 month-old 3xTg-AD mice. Metabolic and molecular dysfunctions of glucose biochemistry are also related to the cognitive performance of the affected groups. This data suggest that an early exposure to high caloric food has a powerful impact on the acceleration or production of cognitive impairments.

Disclosures: K.R. Guzman-Ramos: None. **L. Ayala-Guerrero:** None. **P. Garcia-delaTorre:** None. **F. Bermudez-rattoni:** None. **G. Pacheco -Lopez:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.13/D26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

NSERC

Title: Learning and memory is largely intact in an idiopathic rat model of Alzheimer's Disease that pairs Aβ injections with either stress or cholinergic depletions

Authors: *S. H. DEIBEL, N. S. HONG, R. J. KEELEY, R. J. BALOG, C. M. BYE, S. MACINNIS, S. M. HIMMLER, R. J. MCDONALD;
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Abstract: Alzheimer's disease (AD) is likely a disease of complex etiology, which involves multiple risk factors. When these risk factors are presented concomitantly, cognition and brain pathology are more severely compromised than if those risk factors were presented in isolation. Reduced cholinergic tone, stress, and elevated beta-amyloid (Aβ) load are all risk factors for AD. The present studies sought to investigate alterations in learning and memory in a variety of tasks when Aβ was paired with either stress or cholinergic depletions. Male Long-Evans rats received either sham surgeries, stress (2 week variable stress paradigm), cholinergic depletions of the medial septum, Aβ25-35 injections, both cholinergic depletion and Aβ25-35 injections (Aβ + ACh group), or both stress and Aβ25-35 injections (Aβ + stress group). Both the Aβ + ACh and Aβ + stress rats had impaired acquisition in the standard version of the Morris water task during the first half of training but displayed normal retention and no impairment in acquisition of a novel platform location during a single massed training session. Similarly, the Aβ + ACh and Aβ + stress rats were not impaired in the discriminative fear-conditioning to context task or the stimulus-response radial arm maze task. Despite minor cognitive deficits, the Aβ + ACh rats had reduced cholinergic tone in the hippocampus as indicated by a reduction in dorsal hippocampal acetylcholinesterase staining. These data suggest that certain components of memory in the Morris water task are impaired by pairing Aβ with either cholinergic depletions or stress, when behavioural testing is conducted shortly after the presentation of these factors. The pattern of effects also suggests that only subcircuits of the hippocampus are dysfunctional.

Disclosures: S.H. Deibel: None. **N.S. Hong:** None. **R.J. Keeley:** None. **R.J. Balog:** None. **C.M. Bye:** None. **S. MacInnis:** None. **S.M. Himmler:** None. **R.J. McDonald:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG042513

NIH P01 NS074969

NIH R21 AG045691

Knight Alzheimer's Disease Research Center at Washington University

Title: Using microimmunoelectrodes to study rapid mechanisms of Aβ clearance from the brain interstitial fluid of mice

Authors: *C. M. YUEDE[1,2,3], C.-Z. LI[4], J. R. CIRRITO[1,2,3];
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[4]Nanobioengineering/Bioelectronics, Florida Intl. Univ., Miami, FL

Abstract: The accumulation of amyloid-β peptide (Aβ) in the brain plays a central role in the pathogenesis of Alzheimer's disease (AD). The balance between Aβ generation and elimination determines the steady-state concentration of Aβ in the brain. Human studies strongly suggest that a key factor leading to Aβ accumulation is a defect in clearing the peptide from the brain. Several mechanisms involved in Aβ clearance have been identified, and some of these mechanisms may be fast-acting and are unavailable to measure using currently available tools. We have recently developed a novel microimmuno-microelectrode (MIE) to detect brain ISF Aβ every 30-60 seconds in living mice, using square wave voltammetry. This new technique provides the temporal resolution necessary to assess very rapid changes in Aβ elimination in living mice. In our design, specificity is achieved by using anti-Aβ antibodies immobilized to the electrode surface. Anti-Aβ antibodies capture/bind Aβ molecules to the MIE surface and decrease the electron tunneling distance between the electrode and Aβ molecules. MIEs are prepared similar to our previously described methods (Prabhulkar et al., 2012). Activation of carboxylic groups on the carbon fiber surface is achieved by application of EDC/NHSS to form a semi-stable reactive amine NHS ester. The activated microelectrodes are placed in a solution of anti-Aβ antibody. Following antibody attachment, MIEs are incubated with 0.1 % ethanolamine to block reactive NHS sites then 0.15% BSA to block any non-specific protein binding sites. We are using MIEs to determine the rapid kinetics of protein elimination in mice that have suppressed clearance mechanisms. By using a combination of pharmacological and genetic inhibition of proteases and transporters we can assess how these different clearance pathways act in synergy on Aβ. Using the MIEs, we can demonstrate that the elimination half-life of ISF Aβ *in vivo* is very short ($t_{½}$ = 37.5 minutes). This tool can be used to assess the fast-acting clearance mechanisms in the brain of living AD transgenic mice. This study was funded by: NIH R01 AG042513, P01 NS074969, R21 AG045691, and the Knight Alzheimer's Disease Research Center at Washington University.

Disclosures: C.M. Yuede: None. **C. Li:** None. **J.R. Cirrito:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

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Program#/Poster#: 789.15/D28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Colciencias; contrato RC 498-2012.

Title: Restoration of cognitive function in the triple transgenic mouse model of Alzheimer´s diseases by the LXR agonists GW3965: Potential molecular mechanism

Authors: *A. G. SANDOVAL HERNANDEZ, SR[1], G. P. CARDONA-GÓMEZ[2], G. ARBOLEDA[1];
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Abstract: Alzheimer's disease (AD) is the major cause of dementia. The pharmacological activation of nuclear receptors (Liver X receptors: LXRs or Retinoid X receptors: RXR)has been showed to induce overexpression of the ATP-Binding Cassette A1 (ABCA1) and Apolipoprotein E (ApoE), changes that are associated to the improvement in cognition, reduction of neuroinflammation. This treatment has showed to increase the clearance of amyloid load soluble and insoluble in some specific AD models, but the mechanism on cognitive improvement is not completely understood yet. Our may aim is to analyze the potential mechanism of LXR activation involved in the improvent of the cognitive function in old 3xTg-AD mice model. We used de LXR agonist GW3965 (33mg/kg/day) administered i.p. during 12 weeks (long-term treatment) in 3xTg-AD mice at 12 months of age. We realized spatial learning and memory analysis by Morris Water Maze test, evaluated amyloid load by immunohistochemistry and soluble Aβ by ELISA. Also, we used ApoE immunoreactivity and its correlation with Glial Fibrilary Acidic Protein (GFAP), Neuronal Stem Cells marker Nestin and proliferating cells Phospho histone H3, neurons markersNeuN and Doublecortin (DCX), oligodendrocytes markers (O1 and O4) and myelin marker myelin Basic Protein (MBP) by Laser Confocal Microscopy. Our finding confirmed that LXRs agonist GW3965-treated 3xTg-AD mice showed better performance in the learning and memory tasks, correlated whith an increase in the ApoE and ABCA1 immunoreactivities in the hippocampus and cerebral cortex of 3xTgAD treated mice. Soluble and insoluble amyloidosis labeling did not present any changes. Novelty, we found that ApoE overexpressing are mostly localized in neurons (86±12%) particularly in the GCL of hippocampus and entorhinal cortex of GW3965-treated 3xTg-AD mice, accompanied by an

increase in the number of stem and proliferating cell, a significant increase in oligodendrocytes markage (O1 and O4) and myelin markers in the Dentate Gyrus without changes in mature neurons and a significant reduction of astrogliosis (GFAP+). Together our data suggest an alternative molecular mechanism by which LXRs agonist could exert control on lipid transport in neurons, generating neuroprotection, neurogenesis and remyelinization, changes that are of potential benefit as a therapeutic strategy against AD.

Disclosures: A.G. Sandoval Hernandez: None. **G.P. Cardona-Gómez:** None. **G. Arboleda:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.16/D29

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Rat P3 ERP in behavioral chains as a function of cue duration, inter-event interval, and predictability of reinforcement

Authors: *W. D. KLIPEC[1], A. PAJSER[2], R. LEWIS[1], T. GRAY[1], K. ELDER[1];
[2]Psychology, [1]Drake Univ., Des Moines, IA

Abstract: The human P300 Event Related Potential (ERP) is a time locked, averaged EEG to task relevant stimuli. Previous research in our laboratory has shown that a robust P3 ERP, recorded in freely behaving rats, is decremental function of its proximity to the primary reinforcer in backward chaining, suggesting that the P3 ERP is a correlate of conditioned reinforcement. Because many of the stimuli in behavioral chains occur in close temporal proximity, the ERPs may overlap and interfere with each other. Previous research in our laboratory has established the optimal delays for lever insertion and food delivery. In this paradigm, a 4.0 kHz, 80 dB tone cues the insertion of a lever, following a 1-sec delay. Responding on the lever delivers reinforcement on a VR-6, schedule of reinforcement, cued by a 5.5 kHz, 80 dB tone where food reinforcement is delivered after a 1.3-sec delay. As a control, a random non-target 2.5 kHz, 80 dB tone was presented on an 8:1 non-target to target ratio. Here we manipulated tone duration and found that the P1, P2 and P3 ERP latencies were a function of tone onset and unaffected by tone duration. We found no systematic changes in amplitude. By triggering lever insertion and pellet delivery by tone offset, we found that P3 ERP latencies to lever insertion and food delivery moved proportionate to the tone duration, confirming that these

P3 ERPs were a function of those events. While holding tone duration constant we manipulated the density and predictability of the reinforcers by using fixed (FR) and variable (VR) ratio schedules of reinforcement. Here we found that the P3 amplitude was greater when the reinforcer was unpredictable (VR) compared to predictable (FR). This suggests that the P3 ERP is dependent on the informational value of the tone cue rather than just the occurrence of, or effort to produce the reinforcer. The data replicated the finding that P3 ERP amplitude is a decremental function of its proximity to the primary reinforcer in backward chains. We have now established the optimal parameters in this paradigm for producing a robust and reliable P3 ERP in rat model of the human P300 ERP paradigm. Our data strongly suggest that the rat P3 ERP is the equivalent of the human P300 ERP. Accordingly, this animal model of the human P300 ERP would be an excellent tool for conducting pharmacological investigations on the substrates of the P300 ERP. Moreover, since alterations in the human P300 ERP are trait markers for schizophrenia and early onset Alzheimer's disease, and since rat models of schizophrenia and Alzheimer's disease exist, the rat P3 ERP may also be a useful tool for research on these diseases.

Disclosures: W.D. Klipec: None. **A. Pajser:** None. **R. Lewis:** None. **T. Gray:** None. **K. Elder:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.17/D30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fundamental science for medicine

RFBR 12-04-00776-a

Title: NMR analysis of cerebrospinal fluid: Animal model of Alzheimer disease

Authors: *E. MUGANTSEVA, S. PASKEVICH, M. MOLCHANOV, M. TIMCHENKO; Inst. of Theoretical and Exptl. Biophysics RAS, Pushchino, Russian Federation

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive loss of cognitive functions. According to the amyloid cascade hypothesis, the aggregated forms of the amyloid-beta peptides initiate pathological processes in neurons of the

hippocampus, the cortex, and some other structures of the brain. Finding biomarkers for diagnosis of Alzheimer's disease in early stage is one of the most important neuroscience problems. The aim of this study was to investigate metabolite profiles of cerebrospinal fluid using NMR-spectroscopy. In the present work, Aβ25-35 or vehicle was bilaterally injected into the cerebral ventricles of rats. The concentrations of main metabolites in cerebrospinal fluid (CSF), hippocampus, frontal cortex in control rats and rats with administration of beta amyloid were received. We studied over 30 metabolites. Adenosine triphosphate level in CSF was decreased. Glucose level reduced as well as pyruvate and creatinine concentration. Decreased levels of N-acetyl aspartate have been interpreted to indicate neuronal/axonal loss, or compromised neuronal metabolism. NMR analysis of the brain and spinal fluid major metabolites revealed altered metabolic profile.

Disclosures: E. Mugantseva: None. **S. Paskevich:** None. **M. Molchanov:** None. **M. Timchenko:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer’s Association 133086

Carraway Foundation Grant

Title: Allopregnanolone rescues rotenone induced cognitive impairment in mice with apoe4 domain interaction

Authors: *J. WANG, X. HOU, S. ADEOSUN, Q. ZHANG, B. ZHENG;
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Abstract: Although the causes of Alzheimer’s disease (AD) are not fully understood, it is accepted that AD is caused by multiple risk factors rather than a single one. Besides the greatest risk factor of advancing age, several known genetic variations and environmental toxins contribute to the development of AD. These include the apolipoprotein E4 (ApoE4) allele, which is the major genetic risk factor for sporadic Alzheimer’s disease (AD), due to the higher prevalence and earlier onset of AD in apoE4 carriers. Our previous works and those of others

indicated that the interaction between the N- and the C-terminal domains in the protein may be the main pathologic feature of ApoE4**.** Rotenone, a wildly used broad-spectrum pesticide, is reported a potential neurotoxin for some neurodegenerative disease. Here we sought to investigate the cognitive and neural pathology interactions between the apoE4 domain interaction and rotenone exposure. We further tested the therapeutic potential of a neurogenic neurosteroid, allopregnanolone, in the restoration of cognitive function and brain neuronal circuits in these mice. In this study, the domain interaction in apoE4 is modeled via a point mutation (Thr-61 to Arg-61) from that introduces the domain interaction feature of human apoE4 into native mouse ApoE. The hippocampal dependent spatial and working memory, as assessed by radial arm water maze and novel arm discrimination, was impaired in Arg-61 mice when compared to C57BL/6J mice. The systematic-administration of rotenone caused the learning and memory impairment in C57BL/6J mice and further worsened the impairment in Arg61 mice. After the treatment of allopregnanolone, the short term and long term memory in Arg61 mice was improved. Allopregnanolone was also able to rescue the rotenone induced cognitive deficits in both C57BL/6J mice and Arg61 mice. We are in the process of characterizing cellular and molecular mechanisms that may explain these behavior findings. Our study demonstrated that ApoE4 domain interaction interacts with a neurotoxin, rotenone, and accelerate the cognitive impairment in AD. And in addition, allopregnanolone promotes the cognitive function and may serve as a therapeutic strategy for sporadic AD.

Disclosures: J. Wang: None. **X. Hou:** None. **Q. Zhang:** None. **B. Zheng:** None. **S. Adeosun:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Program#/Poster#: 789.19/D32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CTI

Title: Adult mesenchymal stem cells have a potential to lower cerebral Abeta amyloidosis in Alzheimer’s disease mouse model

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder pathologically characterized by extracellular senile plaques, primarily composed of polymerized amyloid-beta (Abeta) peptides. There is currently no cure for this devastating disease that represents one of today's major healthcare challenges due to its severe socio-economic burden. The huge potential offered by adult ischemic-tolerant mesenchymal stem cells (itMSC) for the treatment of various neurodegenerative disorders including AD is still not fully exploited. Safety phase I clinical trials with peripheral itMSC delivery have been successfully completed, however the impact of these cells on Abeta amyloid pathology have not been yet investigated in a pre-clinical study. To this end, we evaluated the impact of a repeated intravenous itMSC delivery on amyloid pathology in a mouse model of Alzheimer's disease. Ten weeks of itMSC treatment safely reduced cerebral Abeta plaques (~ - 30 %) in amyloid-depositing Alzheimeric mice. Microglial activation was quantified to evaluate neuroinflamation changes in transgenic brains following itMSC treatment. The beneficial effect on plaques was accompanied by a significant diminution of neuroinflammation markers without appearance of cerebral amyloid angiopathy (CAA) or microhemorrhages. An innovative imaging technology was developed to enable the three-dimensional visualization of individual Abeta plaques *in vivo* as well as quantitative blood flow imaging (Bolmont et al., 2012, Bouwens et al.,2013, 2014), giving an unprecedented opportunity for investigation at the microscopic scale of functional brain alterations related to amyloid deposition and clearance by itMSC in living Alzheimer mice.Our pre-clinical results indicate that adult itMSC can decrease Abeta amyloid pathology in a mouse model of Alzheimer's disease. As revealed in the current study, the combination of safety and efficacy to remove amyloid plaques offered by itMSC, together with successfully completed safety phase I clinical trials, may put itMSC as a perspective candidate for treating AD.

Disclosures: **T. Bolmont:** A. Employment/Salary (full or part-time):; Stemedica Int. **A. Lukashev:** A. Employment/Salary (full or part-time):; Stemedica Int. **T. Lasser:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DoD/W81XWH-13-1-0253

Roskamp Foundation

Title: Exploiting the pathogenic inter-relationship between TBI and AD in mouse models using proteomic and lipidomic technology

Authors: *J. O. OJO[1], J. M. REED[1], J. EVANS[1], G. CRYNEN[1], L. ABDULLAH[1], M. MULLAN[1], F. CRAWFORD[1,2];
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Abstract: Traumatic brain injury (TBI) is a major cause of disability in the military and civilian population, and for many years has been known to be a major risk factor for Alzheimer's disease (AD). Although the existence of this relationship is well recognized, and the overlap and distinction between pathological features of AD and TBI, have long been the subject of reporting and discussion, the precise nature of how TBI leads to or precipitates AD pathogenesis is currently not understood. To address this problem we are generating time-dependent molecular profiles of response to TBI and AD pathogenesis in mouse models, using proteomic and lipidomic analyses. We are using the well-validated PSAPP (PS1(M146L), APP(K670N,M671L) and hTau mouse models of AD that develop human amyloid and tau pathological features, and our well established model of single and repetitive mild TBI (mTBI) in C57BL/6 mice. Brain tissue and plasma from these animals are being collected at different ages (for AD models), or at different timepoints after single or repetitive mTBI. Liquid chromatography/mass spectrometry (LC-MS) and in source collision induced dissociation (SCID) approaches will be applied to develop molecular profiles of proteins and lipid species that are significantly differentially expressed as a consequence of AD or mTBI. We anticipate that the exploration of molecular profiles in brain and plasma samples from these animal models of mTBI and AD will enable us to identify cellular pathways that have pathogenic significance in human conditions. Moreover, we aim to further explore these identified pathways as potential targets for therapeutic intervention. Preparation of tissue and plasma samples and Omic analyses are currently ongoing and comparisons of TBI profiles at 24hrs and 3 months post-injury with profiles at 3 months of age in the AD models will be presented. This study is funded by a CDMRP award to FC (DoD/W81XWH-13-1-0253), and by the Roskamp Foundation.

Disclosures: J.O. Ojo: None. **J.M. Reed:** None. **J. Evans:** None. **G. Crynen:** None. **M. Mullan:** None. **F. Crawford:** None. **L. Abdullah:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.21/D34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR RPP

Innovation PEI

Title: The galantamine prodrug, Memogain®, reverses deficits in hippocampal neurogenesis associated with the loss of basal forebrain cholinergic neurons

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Abstract: Loss of basal forebrain cholinergic innervation of the hippocampus and severe neuronal loss within the hippocampal CA1 region are early hallmarks of Alzheimer's disease (AD), and are strongly correlated with cognitive status. This loss of cholinergic innervation is a key factor underlying alterations in hippocampal neurogenesis, which are also characteristic of AD. We have previously reported the effects of various cholinergic compounds on hippocampal neurogenesis indicating that acetylcholine serves as a potent neurogenic regulator. Memogain® (GLN 1062) is an inactive galantamine pro-drug with 15 fold higher brain availability than galantamine. It is designed to provide improved blood brain barrier penetration, greater potency, and fewer side effects than the cholinesterase inhibitors currently used for the treatment of Alzheimer's dementia. This would serve both to promote patient adherence and permit the use of higher doses. Galantamine is unique among the cholinesterase inhibitors in that it also has allosteric actions at α-7 nicotinic receptors, activation of which has been linked to both disease-modifying and cognitive enhancing effects, as well as effects on hippocampal cell proliferation. Here, we describe the neurogenic actions of Memogain® in a rodent model of cholinergic depletion. Infusion of the immunotoxin, 192IgG saporin (SAP), used to induce selective basal forebrain cholinergic cell loss reminiscent of that found in AD, resulted in a pronounced loss of basal forebrain cholinergic neurons and hippocampal ChAT fiber density. Consistent with earlier reports, SAP-lesioned animals had significantly fewer BrdU+ and PCNA+ cells in both the dentate gyrus and CA1 region of the hippocampus, when compared to sham-operated control animals. These animals also displayed significant impairments in spatial working memory, as assessed by a T-maze and the radial arm maze. By contrast, animals treated with Memogain® displayed a restoration of hippocampal cell proliferation, increased neuronal cell counts, normalized neuronal migration, and improvements in cognitive function. Thus, the beneficial effects of Memogain® may extend beyond acute cognitive enhancement, to include disease modification through support of hippocampal neurogenesis.

Disclosures: **J.M. Van Kampen:** A. Employment/Salary (full or part-time):; Neurodyn Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Galantos Pharma. **D.G. Kay:** A. Employment/Salary (full or part-time):; Neurodyn Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Neurodyn Inc. **A. Maelicke:** A. Employment/Salary (full or part-time):; Neurodyn Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Inc..

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.22/D35

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Involvement of DNA polymerase beta in the regulation of neurogenesis in aging and Alzheimer’s disease

Authors: *M. M. MISIAK[1,2], P. SYKORA[1], D. CROTEAU[1], V. A. BOHR[1], M. P. MATTSON[2];
[1]Lab. of Mol. Gerontology, NIH/National Inst. On Aging, Baltimore, MD; [2]Lab. of Neurosciences, NIH/National Inst. on Aging, Baltimore, MD

Abstract: Alzheimer’s disease (AD) involves the progressive degeneration of neurons critical for learning and memory. In addition, findings from animal models and postmortem human brains suggest that neurogenesis in the hippocampus and olfactory bulb (OB) is impaired during aging and in AD. Olfactory deficits occur during normal aging, and a more profound olfactory deficit is an early symptom of AD. Because DNA base excision repair (BER) is reduced in aging and AD, we tested the hypothesis that inefficient BER due to reduced DNA polymerase-β (Polβ) levels can disrupt proliferation, differentiation and cellular metabolism in neural stem cells (NSC) in the adult brain. In the absence of Polβ, neurons in the brains of mice undergo massive perinatal apoptosis. Polβ levels in the brain decrease with normal aging, and in Down syndrome patients who invariably develop AD-like pathology, cognitive impairment and olfactory deficits. We noticed that Polβ-/- mouse embryos lack a discernible OB. To study the role of Polβ in neurogenesis during aging and in AD, Polβ+/- mice were crossed with 3xTgAD mice (an AD mouse model). We injected Polβ+/+, Polβ+/-, 3xTgAD and Polβ+/-/3xTgAD mice (cohorts of 6 and 14 month-old mice)with bromodeoxyuridine (BrdU) to monitor neurogenesis. The analysis demonstrates that Polβ+/- and 3xTgAD, have significantly decreased neuronogenesis in the hippocampus and in the OB, relative to wild type age-matched controls. The extent of reduced neurogenesis in the hippocampus was not substantially different between the Polβ+/-, 3xTgAD and the Polβ+/-/3xTgAD mice. The decline in BrdU-positive cells was correlated with an

increase in apoptosis in each strain. However, the Polβ+/-/3xTgAD mice showed the most substantial level of apoptosis suggesting that the DNA damage caused by lack of Polβ compounded by the stress of the amyloid and Tau accumulation in 3xTgAD mice converge on the apoptotic pathway. Consistent with the biochemical findings, the Polβ+/-/3xTgAD mice showed the most severe memory impairment in behavioral tests. Currently we are testing olfaction in the mice using multiple behavioral tests. We are also performing studies aimed at establishing the molecular mechanisms by which deficiency in a DNA repair enzyme adversely affects cellular energy metabolism and neurogenesis.

Disclosures: M.M. Misiak: None. **P. Sykora:** None. **D. Croteau:** None. **V.A. Bohr:** None. **M.P. Mattson:** None.

Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CNPq

FAPESC

UNESC

Title: Can the d-galactose administration by oral route in Wistar rats mimics the Alzheimer disease?

Authors: *J. BUDNI[1], R. PACHECO[1], S. DA SILVA[1], M. L. GARCEZ[1], F. MINA[1], J. DE MEDEIROS[1], S. M. FREITAS[1], D. V. VITTO[1], S. S. VALVASSORI[1], G. SCAINI[1], E. L. STRECK[1], J. QUEVEDO[1,2];
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Abstract: Background: Chronic systemic d-galactose administration using as a model of aging and Alzheimer disease in rodents, d-galactose is a reducing sugar at high levels, that can form advanced glycation endproducts, induce neurological impairments, mitochondrial dysfunctions and oxidative stress. The use of D-galactose administration by oral route is still controversial, if mimetic neurodegenerative disorders and brain aging or neuroprotective alternative, the

alterations d-galactose administration by oral route are still unknown, especially the changes in energy metabolism. **Objective:** The purpose of this study was to investigate the effects of on acquisition of short- and long-term memories through the inhibitory avoidance test and habituation to a novel environment, measure respiratory chain enzyme activities and levels of blood glucose in rats treated with d-galactose chronic administration by oral route. **Methods:** Wistar male adult rats received water or d-galactose (100 mg/kg) by oral gavage, once a day, during 8 weeks. Short-term and long-term memories were tested 1.5 and 24 h after training, respectively, in the inhibitory avoidance and 24 h after training in open field. Activity of mitochondrial respiratory chain complexes (I, II, III, and IV) was measured in prefrontal cortex and hippocampus and levels of blood glucose was measured the end of 1, 2, 4, 6 and 8 weeks after treatment. **Results:** Was observed cognitive impairment in open-field test and inhibitory avoidance tests in 4 weeks after initial treatment, in addition, rats showed cognitive impairment after 6 weeks of treatment with d-galactose observed by the decrease of the crossings in open field test. In biochemical analysis was observed increased activity of respiratory chain complexes I, II, II-III e IV in pre frontal cortex and hippocampus since the first week to the eighth week of treatment and only after the second week of treatment was observed increase in glucose concentration in animals treated with d-galactose. **Conclusion:** The administration of d-galactose by oral route caused cognitive impairment mainly 4 and 6 weeks of treatment, possibly this model mimics the mitochondrial abnormalities in early stages of Alzheimer disease. More studies should be conducted to elucidate the mechanisms by which d-galactose administrated via orally generates cognitive changes observed in this model.

Disclosures: J. Budni: None. **R. Pacheco:** None. **S. da Silva:** None. **M.L. Garcez:** None. **F. Mina:** None. **S.S. Valvassori:** None. **G. Scaini:** None. **E.L. Streck:** None. **J. Quevedo:** None. **J. de Medeiros:** None. **S.M. Freitas:** None. **D.V. Vitto:** None.

Poster

789. Alzheimer's Disease: APP Abeta In Vivo Models II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 789.24/D37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer’s Association Investigator Initiated Research Grant (133086)

Carraway Foundation Grant

Title: Neurotoxicity of alumina nanoparticles in transgenic mice bearing susceptible genes of Alzheimer's disease

Authors: *Q. ZHANG[1,2], B. ZHENG[1], R. KAUR[1], J. WANG[1];
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Abstract: Increasing exposure to environmental pollutants, such as nanoparticles (NPs) may be responsible for the prevalence of neurodegenerative diseases, including Alzheimer's disease (AD). NPs have by now habitually used in a vast range of industrial and medical applications, including hi-tech materials, plastics, paint, orthopaedic implants, paper derivatives, cosmetics and sun cream. Due to such a widespread use the toxicity of these NPs has been widely studied, proving the evidence for causing genotoxicity, cytotoxicity and oxidative damage. Among the NPs, the effects of alumina NPs on the central nervous having been received the attention. Aluminum (Al) is a vital etiopathogenic agent responsible for the incidence of neurodegenerative diseases. Many reports have demonstrated that nano-sized alumina particles have an enhanced capacity to produce reactive oxygen species and consequently have widespread toxic properties. Our previous work reported that the intranasal instillation of nano-sized alumina caused learning and memory impairment and brain inflammation in a size-dependent manner. In the present work, we are investigating the neurotoxicity of alumina nanoparticles in transgenic mice bearing familial and sporadic susceptible genes of Alzheimer's disease. We characterize the permeability of alumina-NPs in the nasal olfactory pathway and the hippocampal distribution after intranasal infusion, the cognitive performance and brain neuropathology in Tg and non-Tg mice. We concluded that alumina NPs could path through blood-brain barrier and deposit in the brain, and induce the decline of the learning and memory capacity of the in size-dependent and time-dependent manners. The present study may establish a path for studying the interaction of metal NPs toxicity with genetic mutations for AD progression.

Disclosures: Q. Zhang: None. **B. Zheng:** None. **R. Kaur:** None. **J. Wang:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.01/D38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ACPHS intramural start-up funds

Western Alliance to Expand Student Opportunities grant

Title: Why is not everybody getting Alzheimer's? Mammalian blood contains small molecules that prevent Aβ peptide aggregation

Authors: *A. V. KRIVOSHEIN[1], F. M. MUSTEATA[2];
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Abstract: How can we affect the equilibrium between monomeric and oligo/polymeric forms of Aβ peptides? Answering this question would hold a promise to novel therapeutics for Alzheimer's disease and other diseases involving intrinsically disordered proteins. It has been known for some time that both blood serum and cerebrospinal fluid (CSF) prevent and reverse aggregation of Aβ(1-40) and Aβ(1-42) peptides *in vitro*. Yet the chemical nature of substance(s) responsible for this amyloid-disaggregating effect remains unknown. To our surprise, we found that the disaggregating activity largely resides in the low-molecular-weight, protein-free fractions of human and mammalian blood serum and CSF, suggesting the existence of endogenous small molecules that inhibit aggregation of Aβ peptides. We report separation using a combination of activity-guided size exclusion chromatography and reversed-phase HPLC and structure elucidation using tandem mass spectrometry of some of these small molecules. We believe that these molecules can serve as therapeutic leads as well as biomarkers for early diagnosis of Alzheimer's disease. We also present preliminary data on structurally related amyloid-disaggregating small molecules found in plants of *Psychotria* genus. Some of these plants are used medicinally by the indigenous people in the Peruvian Amazon. Address correspondence to Arcadius V. Krivoshein, Albany College of Pharmacy & Health Sciences, arcadius.krivoshein@acphs.edu

Disclosures: A.V. Krivoshein: None. **F.M. Musteata:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.02/D39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS055088

NIH Grant NS065319

Title: Bax-interacting factor 1 (Bif-1) expression is decreased in human Alzheimer's disease and modulates beta-amyloid related neuropathology in APP/PS1 mice

Authors: *D. B. WANG[1], Y. KINOSHITA[1], C. KINOSHITA[1], T. UO[2], B. L. SOPHER[3], G. A. GARDEN[3], Y. YANG[4], C. KEENE[4], T. BILOUSOVA[5], K. GYLYS[5], H.-G. WANG[6], R. S. MORRISON[1];
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Abstract: Bax-interacting factor 1 (Bif-1) is a tumor-suppressor gene that promotes apoptosis and autophagy. Previously, we identified a novel neuroprotective function for Bif-1 related to expression of neuron-specific isoforms. Here, we report that protein and mRNA levels of neuron-specific Bif-1 isoforms are selectively decreased in the cerebral cortex of patients with late-stage (Braak VI) Alzheimer's disease (AD). Bif-1 protein in beta-amyloid (Abeta)-positive synapses was also decreased in AD patients, and there was a negative correlation between Abeta load and Bif-1 expression. Additionally, symptomatic AD mice expressing mutant amyloid precursor protein and presenilin 1 (APPswe/PS1dE9; Jankowsky et al., 2004) displayed lower levels of neuron-specific Bif-1 isoforms in both the cortex and hippocampus compared to wild-type controls and younger pre-symptomatic AD mice. To further evaluate the role of Bif-1 in AD pathology, we crossed Bif-1 knockout mice (Bif-1 KO; Takahashi et al., 2005) with AD mice. After 6 months, AD/Bif-1 KO mice showed a larger plaque burden, increased astrogliosis, and increased mortality compared to AD mice. AD/Bif-1 KO mice also displayed synaptic degeneration and cognitive impairment, which was not yet detected in AD mice at this early age, suggesting that loss of Bif-1 hastens disease onset. Although Bif-1 KO mice had no distinguishable phenotype at 6 months, they developed synaptic degeneration and cognitive and motor impairment by 12 months. Finally, overexpression of neuron-specific Bif-1 isoforms in mouse primary cortical neuron cultures protected against Abeta-induced apoptosis and mitochondrial dysfunction. Taken together, these results suggest that reduced expression of neuron-specific Bif-1 isoforms in human AD contributes to neuropathology and that Bif-1 may be a potential biomarker and/or drug target for AD.

Disclosures: D.B. Wang: None. **Y. Kinoshita:** None. **C. Kinoshita:** None. **T. Uo:** None. **B.L. Sopher:** None. **G.A. Garden:** None. **Y. Yang:** None. **C. Keene:** None. **T. Bilousova:** None. **K. Gylys:** None. **H. Wang:** None. **R.S. Morrison:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.03/D40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: European Research Council (E.R.C.) award given to M.S. (Grant no. 232835)

EU Seventh Framework Program HEALTH-2011 given to M.S. (Grant no. 279017)

Title: Activation of the brain's choroid plexus for monocyte trafficking to the CNS mitigates pathology in a mouse model of Alzheimer's disease

Authors: *N. ROSENZWEIG, K. BARUCH, A. KERTSER, G. KUNIS, A. DECZKOWSKA, A. SAREL, L. CAHALON, M. SCHWARTZ;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disorder and the leading cause of senile dementia worldwide; its pathophysiology is associated with unresolved chronic neuroinflammation. In this disease, blood-borne monocyte-derived macrophages (mo-MΦ) were shown to play an important role in mitigating the neuroinflammatory response, though their entry to the CNS appears to be insufficient, and the signals which regulate their trafficking are poorly understood. Our group recently pointed to the brain's choroid plexus (CP), which forms the blood-cerebrospinal fluid-barrier (BCSFB), as a selective gateway through which mo-MΦ are recruited to the CNS following acute injury. Here we show, in 5XFAD transgenic mouse model of AD (AD-Tg), that mo-MΦ trafficking to the CNS is suppressed due to CP dysregulation of IFN-γ signaling, needed for transepithelial migration of leukocytes across the CP. Pharmacological as well as genetic manipulations in AD-Tg mice, which led to increased levels of IFN-γ at the CP, resulted in epithelial upregulation of leukocyte trafficking determinants, which was followed by mo-MΦ recruitment via the CP-CSF migratory pathway to cerebral sites of amyloid-beta (Aβ) accumulation, plaque removal in the hippocampus and the cortex, and reversal of cognitive decline. Collectively, our results suggest that lacking mo-MΦ infiltration to the CNS, due to CP dysfunction, takes part in AD pathophysiology, and thus point to the BCSFB as a target amenable for immunomodulation as a potential therapy for AD. N.R. and K.B. contributed equally to this study.

Disclosures: **N. Rosenzweig:** None. **K. Baruch:** None. **A. Kertser:** None. **G. Kunis:** None. **A. Deczkowska:** None. **A. Sarel:** None. **L. Cahalon:** None. **M. Schwartz:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.04/D41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Scottish Rite Charitable Foundation

Title: Age-dependent decline in aerobic glycolysis correlates with memory loss in a transgenic mouse model of Alzheimer's disease

Authors: *R. A. HARRIS[1], J. T. NEWINGTON[1], R. BARTHA[2], G. TAGLIALATELA[3], R. C. CUMMING[1];
[1]Biol., Univ. of Western Ontario, London, ON, Canada; [2]Robarts Res. Inst., London, ON, Canada; [3]The Univ. of Texas Med. Br., Galveston, TX

Abstract: The majority of glucose consumed by the adult brain is fully oxidized to carbon dioxide and water in the mitochondria of neurons to supply the large amounts of ATP required for synaptic transmission. However, a certain percentage of glucose in the brain is exclusively metabolized by glycolysis, even in the presence of oxygen, with the generation of lactate as a by-product. This process is known as aerobic glycolysis. Emerging evidence now suggests that aerobic glycolysis in the brain plays a critical role in generating biosynthetic metabolites during early CNS development and persists in certain regions of the adult brain to support synaptic plasticity, learning and memory. However, aerobic glycolysis steadily declines with age and virtually disappears in the elderly. Our lab has recently demonstrated that metabolic reprogramming toward aerobic glycolysis confers a survival advantage to nerve cells against the toxic effects of amyloid beta, a key pathogenic peptide in Alzheimer’s disease (AD). Neuronal cells with elevated aerobic glycolysis exhibited a marked reduction in mitochondrial-derived reactive oxygen species and a reduced propensity to undergo apoptosis. In this study, we demonstrate that a progressive decline in aerobic glycolysis occurs in the mouse brain with age, an event which correlates with a loss of spatial learning and memory. Proton magnetic resonance spectroscopy revealed an age-dependent decline in cortical lactate levels in wild-type mice. Western blot analysis of cortical extracts revealed a decline in key regulatory proteins of aerobic glycolysis in both control and, to a greater extent, transgenic AD mice at 12 months of age when compared to tissue from younger mice. The decline in aerobic glycolysis regulators correlated with the onset of memory loss in transgenic AD mice as measured by the Morris water maze. In addition, immunoblot analysis of post-mortem cortical tissue from non-demented individuals with AD neuropathology (NDAN) exhibited elevated markers for aerobic glycolysis as

compared to age-matched tissue from AD patients or control individuals. These data indicate that aerobic glycolysis in the brain declines normally with age, which may contribute to neurodegeneration and memory loss associated with aging and AD.

Disclosures: R.A. Harris: None. **J.T. Newington:** None. **R. Bartha:** None. **G. Taglialatela:** None. **R.C. Cumming:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.05/D42

Topic: C.05. Aging

Support: Louise & André Charron

NSERC

RQRV

Title: Werner mice displays higher oxidative stress, tau phosphorylation and apoptotic/autophagic levels than wild type mice in hippocampus

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[1]INRS-Institut Armand-Frappier, LAVAL, QC, Canada; [2]INAF, Université Laval, QC, Canada; [3]Ctr. de Recherche sur le Cancer, Université Laval, QC, Canada

Abstract: Werner syndrome (WS) is an autosomal recessive disorder associated with mutations in a gene encoding for a RecQ-type DNA helicase. This enzyme is involved in different aspects of DNA repair, replication, and transcription. Clinically, WS is one of the most intriguing disorders characterized by an accelerated aging phenotype. Interestingly, Alzheimer's disease (AD)-associated neuropathology in the brains of individuals with WS has been reported with greater amyloid (Aß) load and plaque counts in the temporal lobe and an elevation of tau-immunoreactivity than normal cases. However, the association of mutations in the WS DNA helicase with CNS pathology and particularly AD-neuropathology remains to be elucidated. We generated a WrnΔhel/Δhel mouse model that lacks part of the DNA helicase domain of the WS gene homologue (WrnΔhel/Δhel). These mice recapitulate most of the WS phenotypes including premature visceral obesity, hypertriglyceridemia, insulin-resistant type 2 diabetes and

cardiovascular diseases, and increased genomic instability resulting in a 15%-17% decreased mean life span. Moreover, these mice also exhibit increased heart and liver tissue reactive oxygen species (ROS) and oxidative DNA damage. The objectives of this study were to compare the levels of oxidative markers/pathways and AD-neuropathology (Tau phosphorylation) on different regions of the brain from 12 months WrnΔhel/Δhel and age matched wild type C57Bl/6 animals. We have also analyzed the levels of apoptotic (caspase 3) and autophagic (eIF2, 46/48kDa) markers in order to point out one possible mechanism of cells death during this disorder. Our results showed that the basal levels of reactive oxygen species (ROS), as measured by DCFDA, were higher in hippocampus from WrnΔhel/Δhel than in control mice and similar in both groups in other brain structures. Glutathione and Nrf2 levels were also similar in both groups while levels of the antioxidant thioredoxine and of the protein carbonyls were lower in WrnΔhel/Δhel mice. Interestingly, p66Shc, the lifespan determinant and the primary source of ROS, and phosphorylated tau levels were higher in WrnΔhel/Δhel mice. Finally, we found an elevated level of EIF2 and caspase 3 in the hippocampus of mutant mice. These results are in accordance with previous studies demonstrating that WS patient fibroblasts displayed higher autophagy. To conclude, the WrnΔhel/Δhel mouse model displays some AD-related neuropathology and autophagy/apoptotic cell death and support the potential therapeutic use of mTORC1 inhibitors in the treatment of WS.

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Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.06/D43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MDA Grant 217526

University of Toledo Biomedical Innovation Award

Veteran's Administration Merit Award

Title: A derivative of the neurochemical lanthionine ketimine affects autophagy by increasing beclin-1 protein expression in cell culture and in the 3xTg-AD mouse model of Alzheimer’s disease

Authors: *K. HENSLEY[1], K. VENKOVA[2], A. HRISTOV[2], P. ESLAMI[3], A. POTESHKINA[4], M. HARRIS-WHITE[5];
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Abstract: Autophagy is a tightly-regulated cellular process for recycling proteins, macromolecular aggregates and damaged organelles. Autophagy is dysregulated in a number of conditions including Alzheimer's disease (AD), wherein the autophagy-regulating protein beclin-1 is significantly decreased. Genetic over-expression of beclin-1 reduces amyloid beta peptide (A-beta) burden in AD models, raising the possibility that beclin-1 regulation could be a novel route to AD treatment. The present study demonstrates that XN-001, a derivative of a natural brain sulfur amino acid metabolite that slows cognitive decline and reduces pathology in the 3xTg-AD mouse, can induce beclin-1 protein accumulation in brains of 3xTg-AD mice and also in human SH-SY5Y neuroblastoma cells. The beclin-1 increase is time and dose-dependent in cell culture and parallels alterations in other autophagy markers including LC3-II and p62/SQSTM1. Previous studies indicated that neurotrophic effects of XN-001 are mediated through the microtubule-associated protein, CRMP2 (DPYSL2), which collects in AD-associated neurofibrillary tangles. Beclin-1 from human brain lysates bound GST-rhCRMP2 and conversely, CRMP2 co-immunoprecipitated with beclin-1. Depleting cellular CRMP2 with shRNA caused a corresponding decrease in beclin-1, confirming a functional relationship between these two proteins. These findings further clarify the mechanism-of-action for XN-001; uncover a heretofore unappreciated relationship between beclin-1 and CRMP2; and suggest a novel role for brain sulfur amino acid metabolites in regulating neural autophagy pathways.

Disclosures: K. Hensley: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); XoNovo Inc.. **K. Venkova:** None. **A. Hristov:** None. **P. Eslami:** None. **A. Poteshkina:** None. **M. Harris-White:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

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Program#/Poster#: 790.07/D44

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grants AG032755

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The Alzheimer's Art Quilt Initiative

The Alzheimer's Association

The Foundation for Medical Research

Title: Corticotropin-releasing factor receptor antagonism prevents onset of cognitive deficits and impacts amyloid pathology in an alzheimer's disease mouse model

Authors: *C. ZHANG[1], C.-C. KUO[2], S. H. MOGAHDAM[1], S. NUBER[1], L. MONTE[1], K. C. RICE[3], E. MASLIAH[1], R. A. RISSMAN[1];
[1]Dept. of Neurosciences, UCSD, La Jolla, CA; [2]NeuroInformatics Ctr., Univ. of Oregon, Eugene, OR; [3]Chem. Biol. Res. Br., NIH, Bethesda, MD

Abstract: Alzheimer's disease (AD) is the most common form of dementia, characterized by progressive cognitive impairment and two neuropathological hallmarks: β-amyloid plaques (Aβ) and neurofibrillary tangles (NFT). Recent work has implicated environmental factors, prominently including stress, as promoting AD pathogenesis. Anatomical and biochemical study also supports an involvement of corticotropin-releasing factor (CRF) and its components involved in stress signaling system in AD. Data from our lab has demonstrated that chronic stress exposure can induce accumulation of tau phosphorylation (tau-P) aggregates in the rodent hippocampus, process dependent on signaling through the type 1 CRF receptor (CRFR1). Here, we demonstrate that CRFR1 antagonism can also impact production and accumulation of Aβ and prevent onset of cognitive impairment in an AD mouse model. To explore the impact of CRFR1 antagonist treatment on AD cognitive and neuropathological outcomes, we treated 30-day old Alzheimer's transgenic (PSAPP) mice and wild type (WT) littermates with daily administration of a small molecule CRFR1 antagonist (R121919). PSAPP female mice receiving R121919 had significantly better performance with less time and shorter distance spent in the spatial learning test using water maze, which demonstrates that the R121919 treatment significantly reduces both long-term and short-term memory deficits of Tg-female mice. Furthermore, the percentage of amyloid accumulation was significantly reduced in animals treated with R121919 regardless of gender and brain region. Therefore, we hypothesize that CRFR1 antagonism may present a viable option for preventative and/or disease-modifying therapy for AD.

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Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.08/D45

Topic: C.05. Aging

Support: NSERC Grant A7441

Title: Use of the frailty index to compare the stages of aging in the 3x-Tg and 5xFAD mouse models of Alzheimer's disease

Authors: *R. E. BROWN[1], A. A. WONG[2], K. R. STOVER[2];
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Abstract: All mice show neural and behavioural changes as they age, but most murine aging data are based on the C57BL6 mouse, which has a long lifespan. The 3x-Tg and 5xFAD mouse models of AD, have much shorter lifespans than the C57BL6 mouse and within the 3x-Tg mice, males have shorter lifespans than females. All mice go through the same stages of age-related physical and cognitive changes, but at different chronological ages. In order to compare the stages of aging across different strains and sexes of mice, a measure is required which is independent of chronological age. The Frailty Index (FI) appears to meet this criterion (Parks, RJ, et al. 2012. J Gerontol A Biol Sci 67, 217-227; Whitehead, JC. et al. 2014, J Gerontol Biol Sci, in press). We used the FI to compare age-related changes in behaviour and physiology of male and female 3x-Tg and 5xFAD mouse models of AD and their wildtype controls. At 12 months of age, the 5xFAD mice showed a higher FI score than their WT controls, while the 3x-TG mice had a lower FI score than their WT controls. These FI scores correlated with measures of motor control, which decline more rapidly in 3x-Tg than in 5xFAD mice. We are determining whether the FI predicts differences in longevity of males and females in these two mouse models. The use of such a measure, which is independent of chronological age, will be important in the comparative studies of age-related neurodegenerative diseases in mice and in determining the appropriate age at which to initiate therapeutic procedures.

Disclosures: R.E. Brown: None. **A.A. Wong:** None. **K.R. Stover:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.09/D46

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG031846

VFW research funds from the College of Pharmacy of the University of Minnesota

Title: Triptolide preserves cognitive function and reduces neuropathology in a mouse model of Alzheimer's disease

Authors: *S. CHENG, K. J. LEBLANC, L. LI;
Experimental/Clin Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease and a major form of dementia characterized clinically by progressive cognitive impairment. Pathological hallmarks of AD include deposits of aggregated amyloid-β protein (Aβ), neurofibrillary tangles (NFTs), and gliosis in the brain. Mounting evidence indicates an involvement of inflammation in the pathogenesis of AD and anti-inflammatory and immune-modulatory agents have emerged as potential therapeutics for AD. Triptolide, a major bioactive ingredient of a widely used herbal medicine, has been shown to possess multiple pharmacological functions, including potential neuroprotective effects pertinent to AD *in vitro*. However, the therapeutic potential of triptolide for AD *in vivo* has not been thoroughly evaluated. In the present study, we investigated the impact of peripherally administered triptolide on AD-related behavior and neuropathology in $APP_{swe}/PS1_{\Delta E9}$ (APP/PS1) mice, an established model of AD. Our results showed that two-month treatment with triptolide rescued cognitive function in APP/PS1 mice. Immunohistochemical analyses indicated that triptolide treatment led to a significant decrease in amyloid-β (Aβ) deposition and neuroinflammation in treated mice. In contrast to previous findings *in vitro*, biochemical analyses showed that triptolide treatment did not significantly affect the production pathway of Aβ *in vivo*. Intriguingly, further analyses revealed that triptolide treatment upregulated the level of insulin-degrading enzyme, a major Aβ-degrading enzyme in the brain, indicating that triptolide treatment reduced Aβ pathology by enhancing the clearance pathway of Aβ. Our findings demonstrate that triptolide treatment ameliorates key behavioral and neuropathological changes found in AD, suggesting that triptolide may serve as a potential therapeutic agent for AD.

Disclosures: S. Cheng: None. **K.J. LeBlanc:** None. **L. Li:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.10/D47

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG042890

Title: Increased synaptic sensitivity to Aβ-oligomer binding after traumatic brain injury in the rat

Authors: *W. FRANKLIN[1], M. PARSLEY[2], M.-A. MICCI[1], G. TAGLIALATELA[3]; [2]Anesthesiol., [3]Neurol., [1]UTMB At Galveston, Galveston, TX

Abstract: Compelling evidence indicates that traumatic brain injury (TBI) is a risk factor for later development of Alzheimer's disease (AD). However, the molecular mechanisms and changes that link the two conditions have not been yet established. Understanding such mechanism(s) is critical to develop effective interventions to reduce the incidence of AD in TBI subjects. Synaptic binding of amyloid beta (Aβ) oligomers (the most toxic form of Aβ) is an early dysfunctional event of the pathology of AD, driving initial cognitive decline through disruption of affected synapses. Notably, it has also been shown that there is a transient increase of Aβ levels after TBI in both humans and laboratory animals. Based on this evidence, we hypothesized that after TBI central synapses may remain vulnerable to the dysfunctional binding of Aβ oligomers, thus increasing the risk of triggering an Aβ-driven disruptive cascade later on in life. In the present work, we began testing this hypothesis by subjecting rats to traumatic brain injury using an established fluid percussion protocol. 3μL of preformed Aβ-oligomers at a concentration of 0.8μg/μL were then stereotaxically injected bilaterally into the CA1 and CA3 hippocampal areas, 2 or 7 days after TBI. The animals were then sacrificed 2 hrs after the Aβ injection and several brain areas (including the hippocampus) dissected and snap frozen for later isolation of synaptosomes. An ELISA was performed on isolated hippocampal synaptosomes to determine the presence of Aβ-oligomers comparing TBI versus sham animals. We found an increase in Aβ-oligomer in hippocampal synaptosomes from TBI rats as compared to sham injured animals. Increased Aβ was observed both ipsilaterally and contralaterally to the injury side, suggesting that the increased susceptibility of hippocampal synapses to Aβ binding is a global event and not limited to the immediate injury site. Overall, our results indicate that TBI causes synapses to become more vulnerable to Aβ binding. Although further ongoing studies are needed to better characterize this phenomenon, these initial results provide a novel insight into the molecular changes that may link TBI and increased risk of AD in humans.

Disclosures: **W. Franklin:** None. **G. Taglialatela:** None. **M. Parsley:** None. **M. Micci:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

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Program#/Poster#: 790.11/D48

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Age-dependent decrease of amyloid β toxicity in dentate gyrus granule cells of mice

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common form of dementia in the world. The disease involves formation of plaques and tangles, which are seen as hallmarks for AD. Plaques are insoluble protein aggregates composed of amyloid β (Aβ). Although Aβ plaques have been regarded to be critical in the neuropathology of AD, it was recently demonstrated that the appearance of soluble Aβ oligomers correlates better with the memory decline observed in AD patients. Although AD mostly affects elderly people of the age over 65, many investigations are done in cell culture or organotypic slices from newborn mice. Young neurons are known to be more vulnerable to stress and show different protein expression patterns (for example NMDA receptor subunit compositions) compared to adult neurons. Thus, we wondered whether there are differences in the Aβ toxicity in young and adult mice. To this end, we established a recombinant adeno-associated virus (rAAV) based mouse model, in which Aβ is specifically overexpressed in virus-infected neurons. To investigate age-dependent differences in Aβ toxicity we stereotactically injected rAAVs into the hippocampus of young and adult mice and analyzed infected cells several weeks post-infection. The frequency of miniature excitatory postsynaptic currents (mEPSC) frequencies was significantly reduced in infected dentate gyrus granule cells 9 weeks after injection of Aβ overexpressing rAAVs into the hippocampus of P7 mice, suggesting that Aβ reduces the number of functional synapses. However, overexpression of Aβ for 9-12 weeks did not affect mEPSC frequency or amplitude when rAAVs were injected into the hippocampus of P70 mice. Finally, overexpressing a mutated Aβ form (I716V), which leads to production of significantly more Aβ, induced a decreased mEPSC frequency in granule cells of P70 mice, indicating that higher Aβ-levels are required to induce toxicity in adult mice when compared to newborn mice.

Disclosures: **J. Von Engelhardt:** None. **M. Mueller:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.12/D49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant RMF-72554

Title: GPR84 deficiency reduces microgliosis and accelerates dendritic degeneration and cognitive decline in a mouse model of Alzheimer’s disease

Authors: L. VALLIÈRES, J. AUDOY-RÉMUS, A. DUMAS, M. FILALI, *S. LACROIX, S. RIVEST, M.-E. TREMBLAY;
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Abstract: Microglia surround the amyloid plaques that form in the brain of patients with Alzheimer’s disease (AD), but their role is controversial. These cells can express GPR84, an orphan receptor whose pathophysiological role is unknown. Here, we show that GPR84 is upregulated in microglia of APP/PS1 transgenic mice, a model of AD. Genetic deletion of GPR84 in these mice accelerated cognitive decline and reduced the number of microglia, especially near plaques. The lack of GPR84 did not affect the formation of the latter, but promoted dendritic degeneration. Furthermore, GPR84 did not seem to influence the progression of other diseases in which its expression has been reported, i.e., experimental autoimmune encephalomyelitis (EAE) and endotoxic shock, as the clinical signs of these diseases were comparable in mice expressing or not GPR84. We conclude that GPR84 plays a beneficial role in amyloid pathology by acting as a sensor for a yet unknown ligand that promotes the recruitment of microglia, a response affecting dendritic degeneration and required to prevent further cognitive decline.

Disclosures: L. Vallières: None. **S. Lacroix:** None. **J. Audoy-Rémus:** None. **A. Dumas:** None. **M. Filali:** None. **S. Rivest:** None. **M. Tremblay:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

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Program#/Poster#: 790.13/D50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation

C.A.R.T Fund

the Maurice Marciano Family foundation

The National Center for Advancing Translational Sciences through CTSI Grant UL1TR000124

Title: ACE-overexpressing macrophages exhibit a pro-healing phenotype in murine AD models

Authors: *A. RENTSENDORJ[1], J. SHEYN[2], D.-T. S. FUCHS[2], Y. KORONYO[2], E. Y. HYDEN[5], K. L. BLACK[2], S. FUCHS[6], D. B. TEPLOW[5], K. E. BERNSTEIN[3], M. KORONYO-HAMAOUI[4];
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Abstract: Introduction: A key pathology of Alzheimer's disease (AD) is the accumulation of amyloid-β protein (Aβ). Angiotensin-converting enzyme (ACE) is known to degrade neurotoxic Aβ1-42 to less pathogenic alloforms. Our group and others have demonstrated a direct role for monocyte-derived macrophages (Mo/MΦ) in the clearance of fibrillar Aβ in transgenic murine models of AD. It has been recently reported that MΦ cell elongation by itself can promote polarization from the pro-inflammatory towards the "therapeutic" anti-inflammatory phenotype. To study the role of ACE on Mo/MΦ and ability to resist AD, we previously introduced targeted ACE overexpression in myelomonocytic cells (ACE10) to the double-transgenic APP/PS1 murine models (ADtg); these mice exhibited enhanced immune responses coupled with a remarkable prevention of AD-associated neuropathology and cognitive decline. Aims: To investigate the therapeutic potential of ACE10-Mo/MΦ *in vivo* and *in vitro* on AD-like progression. Methods: ACE10 vs. WT bone marrow-derived CD115+-Mo/MΦ were adoptively transferred to post-symptomatic ADtg mice, and then assessed for cognitive function and pathological outcomes. Further, primary cultures of ACE10 vs. WT Mo/MΦ were examined in response to fibrillar and non-fibrillar Aβ1-42 assemblies. Results: Our studies indicated that ACE10- Mo/MΦ have a significant 2.5-fold cell processes elongation relative to WT-Mo/MΦ *in vitro*, possibly entailing the macrophage-polarization towards a pro-healing and anti-inflammatory phenotype. While phagocytosis of fibrillar Aβ1-42 seems unchanged between ACE10- and WT-Mo/MΦ, our findings clearly indicated an accelerated rate of Aβ1-42 extracellular degradation by the ACE10-Mo/MΦ. Further, our data showed an enhanced cell

survival following exposure to toxic Aβ1-42. *In vivo* studies demonstrated that adoptive transfer of ACE10- vs. WT-Mo/MΦ CD115+-Mo/MΦ into symptomatic ADtg mice resulted in retained behavioral functions (Barnes maze test) and attenuation of neuropathology, especially in mice receiving the ACE10 Mo/MΦ. Blood-borne infiltrating ACE10-MΦ relative to WT-MΦ was increasingly present surrounding Aβ plaques and had a decreased TNF-α expression. Biochemical analysis of cerebral Aβ1-42 and Aβ1-40 levels in ACE10-ADtg mice further indicated greater enzymatic degradation of Aβ1-42. Conclusions: These studies provide both *in vivo* and *in vitro* evidences to support a potential therapeutic role for the ACE-overexpressing inflammatory cells in resisting Aβ-induced toxicity in AD murine models.

Disclosures: **A. Rentsendorj:** None. **J. Sheyn:** None. **D.S. Fuchs:** None. **Y. Koronyo:** None. **E.Y. Hyden:** None. **K.L. Black:** None. **S. Fuchs:** None. **D.B. Teplow:** None. **K.E. Bernstein:** None. **M. Koronyo-Hamaoui:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.14/D51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA RO1 AG040092 to CAL

Title: *In vivo* effects of pyroglutamate-3 amyloid-beta seeding in APP/PS1ΔE9 mice

Authors: *H. CREHAN[1,2], H. CYNIS[1,2,3], J. L. FROST[1], K. LE[1], S. SCHILLING[3,4], H.-U. DEMUTH[3,4], C. A. LEMERE[1,2];
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Abstract: Alzheimer's disease (AD) is neuropathologically characterized by neurofibrillary tangles composed of hyperphosphorylated tau and plaques composed of amyloid-β (Aβ). Pyroglutamate-3 Aβ (pGlu-3 Aβ) is an N-terminally truncated and pyroglutamate-modified Aβ species, which has been shown to be a major component of Aβ deposited in the plaques and blood vessels in AD and Down syndrome brain (Saido et al., 1995; Lemere et al., 1996). This pGlu-3 modified Aβ is formed following the cyclization of an exposed glutamate residue at the N-terminus by glutaminyl cyclase and has been shown to be highly neurotoxic with an increased

aggregation propensity (Russo et al., 2002; Schilling et al., 2006; Nussbaum et al., 2012). It is unclear as to whether pGlu-3 Aβ is initially present in the plaques and blood vessels or if it is modified at a later stage. We investigated whether pGlu-3 Aβ acts as a seed for Aβ aggregation and enhances Aβ deposition, neuroinflammation and neurodegeneration in APP/PS1ΔE9 transgenic mice. Female APP/PS1ΔE9 Tg mice (avg. 3.8 mo; C57BL/6J) received bilateral intrahippocampal stereotaxic injections of 2µl of brain extract (10% w/v protein) from aged mice overexpressing pGlu-3 Aβ peptides (APP-NLQ Tg) (n=7), brain extract from aged wildtype (WT) B6 mice (n=7) or PBS (n=7). Quantitative image analysis revealed a trend towards an increase in Aβx-42 immunoreactivity in hippocampus of NLQ extract-injected Tg mice compared with WT extract-injected Tg mice. No differences were observed in Thioflavin S fibrillar plaque load. Further analyses are underway to determine Aβ levels biochemically by ELISA, as well as neuroinflammatory and neurodegenerative changes using immunohistochemical markers of gliosis and synapses. We suggest that pGlu-3 Aβ may enhance AD-like pathogenesis in APP/PS1ΔE9 transgenic mice.

Disclosures: **H. Crehan:** None. **H. Cynis:** Other; Holger Cynis is a former employees of Probiodrug and possess stock options in the company.. **J.L. Frost:** None. **K. Le:** None. **S. Schilling:** Other; Stephan Schilling is a present employee of Probiodrug and possess stock options in the company. **H. Demuth:** Other; Hans-Ulrich Demuth is an employee of Probiodrug and possess stock options in the company. **C.A. Lemere:** Other; Cynthia A. Lemere serves on the scientific advisory board of Probiodrug..

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.15/D52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH (NIA) AG044213-01

ADDF

VA

Title: Oral delivery of a small molecule TNFa inhibitor improves cognition and reduces AD pathology and neuroinflammation in the 3xTg AD mouse

Authors: *P. GABBITA[1], P. ESLAMI[3], M. JOHNSON[3], N. KOBRITZ[3], F. ZEMLAN[2], S. POTESHKINA[3], S. VARADARAJAN[4], J. TURMAN[4], M. HARRIS-WHITE[3];
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Abstract: Neuroinflammation is widely accepted as one of the etiologic events in Alzheimer's Disease (AD). Increased production of the cytokine tumor necrosis factor-alpha (TNFa) has been proposed to contribute to the onset and progression of AD. Increased TNFa is found in the brains of patients with mild cognitive impairment (MCI) and may be important for the conversion of MCI to AD. However, TNFa is a multifunctional cytokine used by multiple cells types including hematopoietic and central nervous system glia and neurons. Although many studies suggest a negative impact of excess TNFa signaling on AD pathology, a number of studies demonstrate the neuroprotective benefits of TNFa. A recent study of long-term global inhibition of TNFa receptor signaling revealed enhanced AD pathology in the 3xTgAD mouse and that the absence of TNFa signaling resulted in CNS immune cells unresponsive to developing AD pathology. These findings highlight the importance of modulation, and not complete abolition, of TNFa signaling in the CNS. Orally bioavailable, small molecule TNFa inhibitors are an attractive approach to treating AD-related neuroinflammation. In the current study, we focus on the central role of TNFa modulation and resulting changes in neuroinflammation, amyloid pathology and cognitive function in the 3xTgAD mouse model. Pharmacokinetic studies show that oral administration of a small molecule TNFa inhibitor resulted in good systemic exposure in rodents. Six month old 3xTgAD mice were randomly allocated into treatment groups (Control diet, and three doses of the small molecule TNFa inhibitor formulated in the diet). The small molecule TNFa inhibitor delivered orally to 3xTgAD mice for 10 months significantly improved learning and memory function as assessed by Barnes maze. Small molecule TNFa inhibitor treatment also modulated brain cortical TNFα protein levels in 3xTg AD mice indicating that the compound engages and modulates the drug target, brain TNFa. AD pathology was assessed by amyloid (6E10 antibody) and phosphorylated tau (AT8 antibody) immunohistochemistry and revealed a reduction in both amyloid and phospho-tau. Long term oral treatment with the inhibitor had no apparent adverse effects. Taken together, these data strongly indicate that small molecule TNFa modulators are safe, orally bioavailable candidates for AD treatment. This work was supported by the Veterans Administration, Alzheimer's Drug Discovery Foundation and the National Institutes of Health.

Disclosures: P. Gabbita: A. Employment/Salary (full or part-time):; P2D Bioscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); P2D Bioscience. **P. Eslami:** A. Employment/Salary (full or part-time):; VA-GLAHS. **M. Johnson:** A. Employment/Salary (full or part-time):; VA-GLAHS. **N. Kobritz:** A. Employment/Salary (full or part-time):; VA-GLAHS. **F. Zemlan:** A. Employment/Salary (full or part-time):; P2D Bioscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

funds); P2D Bioscience. **S. Poteshkina:** A. Employment/Salary (full or part-time):; VA-GLAHS. **S. Varadarajan:** A. Employment/Salary (full or part-time):; Univ. of North Carolina - Wilmington. **J. Turman:** Other; Univ. of North Carolina. **M. Harris-White:** A. Employment/Salary (full or part-time):; VA-GLAHS and David Geffen School of Medicine at University of California Los Angeles (UCLA).

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

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Program#/Poster#: 790.16/D53

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT CB2011-169023

Title: Spirulina maxima reduces the neurotoxicity and improves memory in rats with Aß25-35 in the hippocampus

Authors: *I. LIMON PEREZ DE LEON[1], L. ORTEGA[1], E. RÁMIREZ[1], I. MARTÍNEZ[2], L. PÉREZ-JIMÉNEZ[1];
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[2]Lab. de Neuroquímica, Benemerita Univ. Autonoma De Puebla FCQ, Puebla, Mexico

Abstract: The Spirulina maxima (Sp max), cyanobacteria have a high protein content and its biochemical composition has a high content of vitamin E, phycocyanin, β-carotene, γ-linoleic acid, components that have been shown to increase antioxidant enzymes such as SOD , CAT and GPx and theirs properties as alkoxides scavenger radicals, hydroxyl and peroxyl. Because of this it is proposed that Spirulina maxima may be effective in the adjuvant treatment for neurodegenerative diseases such as Alzheimer's disease. The aim of this study was that the sub-chronic administration of Spirulina maxima reduces nitrosative stress, the damage in spatial memory and neurodegeneration in lesioned rats with peptide Aβ25-35 in the CA1 region of the hippocampus. The administration of Spirulina maxima by 50 days after injection of the Aβ25-35 peptide into hippocampal CA1 region improves learning and memory in rat . Also in these conditions the Spirulina maxima decreases levels of nitrites and protein nitration in injured rats with Aβ25-35 peptide in the CA1 region of the hippocampus . Finally we found that the maximum of Spirulina maxima decreased neurodegeneration lesioned rats in the Aβ25-35 peptide in the CA1 region of the hippocampus. These data suggested that the alls components of the Spirulina maxima help in the treatment of neurodegenerative diseases.

Disclosures: **I. Limon Perez De Leon:** None. **L. Ortega:** None. **E. Rámirez:** None. **I. Martínez:** None. **L. Pérez-Jiménez:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Norampac-Alzheimer fundation

CREMOGH scholarship

Title: Hypercholesterolemia aggravates Alzheimer's disease-related pathology in APP/PS1 mice

Authors: ***P. THÉRIAULT**, A. ELALI, S. RIVEST*;
Dept. of Mol. Medicine, Fac. of Med., Lab. of Neurosci. Res. Ctr. of CHUQ, Québec, QC, Canada

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation and aggregation of the neurotoxic amyloid-β peptide (Aβ) within the brain parenchyma and vasculature. It was suggested that the dysfunction of the blood-brain barrier (BBB) accompanied by impaired immune responses contribute to AD pathogenesis. Age and hypercholesterolemia are two major risk factors associated to AD. Although suspected, their impact on BBB's characteristics and the immune responses remains elusive. This study aims to characterize the molecular and cellular mechanisms underlying the implication of these two risk factors in AD pathogenesis. For this purpose, 3 and 12 months old APP/PS1 transgenic mice were fed with a high-fat diet for 4 months. Afterwards, cognitive functions were assessed by T-water maze and new object recognition (NOR) neurobehavioral tests. Aβ deposits were quantified in the brain parenchyma by stereological analysis. Moreover, parenchymal and vascular Aβ deposition kinetics were visualized by two-photon intravial microscopy. Soluble Aβ levels were measured by ELISA in total brain homogenates and in isolated microvessels. Moreover, flow cytometry analyses were performed to investigate circulating monocyte responses. Finally, protein interaction (WB, IP, IF) and enzyme activity (zymographies) studies were used to assess BBB characteristics. Interestingly, our behavioral analyses revealed that the high-fat diet significantly worsens the cognitive functions of APP/PS1 mice. We also observed a significant increase in Aβ deposition in the cortex and hippocampus of animals fed with high-fat

diet, which correlated with the neurobehavioral data. Moreover, we observed that age increased the frequency of pro-inflammatory monocyte subset ($Ly6C^{high}$), which was exacerbated by the high-fat diet. Our results indicate that hypercholesterolemia accelerates AD-related pathology in APP/PS1 mice, by increasing the accumulation of soluble and insoluble Aβ in the brain, thus worsening the cognitive impairments of these mice. The mechanisms involved in these observations are presently under investigation. The increased oxidation of lipids in the circulation - as a direct consequence of age and the high-fat diet - triggers the activation of matrix metalloproteinases (MMPs) and promotes an exacerbated proinflammatory environment, both of which are involved in BBB destabilization and dysfunction. These orchestrated responses caused by age and hypercholesterolemia abolish the protective role of the BBB in detoxifying the brain, thus contributing in AD pathology.

Disclosures: P. Thériault: None. **A. ElAli:** None. **S. Rivest*:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA P50 AG16573

AG00538

UCI-UROP

Title: Restoration of lipoxin A_4 signaling reduces Alzheimer's disease-like pathology in the 3xTg-AD mouse model

Authors: *H. DUNN[1], R. R. AGER[2], D. BAGLIETTO-VARGAS[2], D. CHENG[2], M. KITAZAWA[3], D. H. CRIBBS[2], R. MEDEIROS[2];
[1]Univ. of California, Irvine, Irvine, CA; [2]UC Irvine Inst. for Memory Impairments and Neurolog. Disorders, Irvine, CA; [3]Univ. of California, Merced, Merced, CA

Abstract: The initiation of an inflammatory response is critical to the survival of an organism. However, when inflammation fails to reach resolution, a chronic inflammatory state may occur, potentially leading to bystander tissue damage. Surmounting evidence suggest chronic inflammation contributes to the progression of Alzheimer's disease (AD), and identifying

mechanisms to resolve the pro-inflammatory environment stimulated by AD pathology remains an area of active investigation. Previously, we found that treatment with the pro-resolving mediator Aspirin-Triggered Lipoxin A_4 (ATL), improved cognition, reduced Aβ levels, and enhance microglia phagocytic activity, in Tg2576 transgenic AD mice. Here, we evaluated the effect of aging on brain lipoxin A_4 (LXA_4) levels using non-transgenic and 3xTg-AD mice. Additionally, we investigated the effect of ATL treatment on tau pathology in 3xTg-AD mice. We found that LXA_4 levels are reduced with age, and significantly more impacted in 3xTg-AD mice. Moreover, ATL (15 μg/kg) enhanced the cognitive performance of 3xTg-AD mice, reduced Aβ levels, as well as decreased the levels of phosphorylated-tau (p-tau). The decrease in p-tau was due in part to an inhibition of the tau kinases GSK-3β and p38 MAPK. In addition, microglial and astrocyte reactivity was inhibited by ATL treatment. Our results suggest that the inability to resolve the immune response to AD pathology is partially due to a reduction in LXA_4 levels. Furthermore, we demonstrate that activation of LXA_4 signaling could serve as a potential therapeutic to target AD related inflammation and cognitive dysfunction.

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Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 790.19/D56

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Chronic LPS-induced inflammation and insulin signaling disruption in a diabetic model of Alzheimer’s Disease

Authors: *A. S. MURTISHAW[1], C. F. HEANEY[2], M. M. BOLTON[2], K. D. BELMONTE[2], P. M. HAGINS[2], M. A. LANGHARDT[2], J. W. KINNEY[2];
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Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by cognitive and behavioral impairments, in addition to pathological hallmarks that include amyloid plaques, neurofibrillary tangles, and neuronal loss. While the etiology of AD is largely unknown, the vast majority of AD cases are late onset and sporadic in origin (sAD), likely arising from interactions between genetic susceptibilities and external factors. Among the top risk factors associated with

sAD are Diabetes Mellitus (DM) and neuroinflammation. Specifically, disruptions to insulin signaling in patients with DM have been implicated as a leading mechanism for DM being an AD risk factor. While considerable research has been conducted to understand the role of neuroinflammation in transgenic models of AD, less progress has been made to understand the interaction of neuroinflammation in the non-transgenic diabetic model of AD. Utilizing streptozotocin (STZ) to disrupt insulin signaling in the brain, we have examined the effects of inflammation in this diabetic animal model of AD. Our lab previously demonstrated that an acute, one-time inflammatory response (LPS) in the STZ animals produced subtle improvements in spatial learning and reduced elevations of oligomeric beta-amyloid compared to the STZ alone group. This current investigation is directed at understanding the interactions of chronic inflammation and insulin signaling disruption, specifically, whether chronic immune activation exacerbates the STZ deficits. One week following STZ infusion, LPS was administered twice per week for 7 weeks, in order to chronically activate the immune system. Spatial learning and memory was examined in the Morris water maze and hippocampal tissue was analyzed for oligomeric beta-amyloid, phosphorylated tau, and other proteins relevant to AD. Our data indicate that both the STZ and STZ+LPS groups exhibited deficits in spatial learning consistent with AD animal models.

Disclosures: A.S. Murtishaw: None. **C.F. Heaney:** None. **M.M. Bolton:** None. **K.D. Belmonte:** None. **P.M. Hagins:** None. **M.A. Langhardt:** None. **J.W. Kinney:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

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Topic: B.11. Glial Mechanisms

Support: 2R01NS036692

5R01NS031234

1F3NS074597

1R01NS082851

Title: Vascular amyloid and gliovascular dysfunction in a mouse model of Alzheimer’s disease

Authors: *I. KIMBROUGH[1], S. ROBEL[2], E. ROBERSON[2], H. SONTHEIMER[2];
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Abstract: The brain is vitally dependent on blood flow alterations due to high energy demands during neuronal activity. This regulation is governed by astrocytes whose endfeet appose blood vessels and vasoregulate, thus acting as liaisons between neurons and the vasculature. Cerebral blood flow is reduced in Alzheimer's disease (AD), a devastating disease associated with the massive loss of neurons resulting in dementia and ultimately death. AD is characterized by amyloid plaque deposited throughout the brain and often lining blood vessels. In this study, we investigated the effects of this vascular amyloid on astrocyte-vascular coupling in a hAPP J20 mouse model of AD. Analysis of electron micrographs revealed morphological changes of the intimate vascular endfoot relationship in AD mice. Using immunohistochemistry, we detected strong astrogliosis as well as loss of endfeet or their polarity even at early stages of the disease. To investigate how these anatomical changes affected the blood-brain barrier (BBB), we injected vascular dyes of varied molecular weights retro-orbitally and performed *in vivo* two-photon imaging through a chronic cranial window, thus allowing us to observe and evaluate changes in blood-brain barrier (BBB) integrity over time. We found BBB breaches and leakage specifically where plaques were deposited. Finally, we induced calcium spikes both pharmacologically and via focal uncaging, allowing us to specifically activate the astrocytic component of the neurovascular unit (NVU) and explore changes in vascular regulation as a result of Aβ deposition in murine cerebral arteriole vessel walls. We found that AB deposition disconnected the functional control of astrocytic endfeet from the vasculature. In conclusion, through fixed tissue, acute slice, and *in vivo* analysis, we found anatomical disruption of astrocyte-vascular coupling that functionally translated to impaired vessel response in a mouse model of AD.

Disclosures: I. Kimbrough: None. **S. Robel:** None. **E. Roberson:** None. **H. Sontheimer:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

Location: Halls A-C

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Program#/Poster#: 790.21/D58

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UNLV Internal Faculty Opportunity Award

Title: Facilitation of GABAB receptor function modulates chronic inflammatory effects

Authors: M. A. LANGHARDT, A. S. MURTISHAW, C. F. HEANEY, M. M. BOLTON, K. C. D. BELMONTE, P. M. HAGINS, *J. W. KINNEY;
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Abstract: Chronic inflammation in the brain (neuroinflammation) has been implicated in numerous degenerative disorders, in particular Alzheimer's disease (AD). Substantial data indicate that persistently activated microglia contribute to AD pathology; including amyloid plaque deposition, the formation of neurofibrillary tangles, and behavioral deficits. The recent identification of greater risk for AD in individuals with the TREM2 mutation further supports a role for inflammatory processes contributing to AD. Numerous investigations have evaluated the effects of neuroinflammation utilizing chronic administration of Lipopolysaccharide (LPS), which is capable of mounting an immune response through the activation of Toll-like receptor 4 (TLR4). Additional investigations have evaluated mechanisms to ameliorate the inflammation, most frequently the use of NSAIDs. In the present study we capitalized on recent findings of an anti-inflammatory role associated with GABA signaling. Data indicating that astrocytes synthesize and release GABA to regulate immune responses and that microglia express GABAB receptors suggest a role for GABAB in mediating inflammation. A growing literature suggests that activation of GABAB on microglia serve to reduce the activation status and diminish the release of pro-inflammatory cytokines. In the below studies we investigated if administration of the GABAB receptor agonist baclofen would lessen both the immune response evoked by LPS, as well as behavioral deficits associated with chronic inflammation. Our data indicate that the administration of baclofen initially attenuated the pyrogenic effects of LPS, however, this effect was lost after the first 2 weeks of LPS administration. Our data further demonstrate that the administration of baclofen rescued spatial learning and memory deficits seen in animals chronically administered LPS alone. These data provide evidence that modulation of GABAB receptor function altered the immune response evoked by TLR4 activation. These data also suggest a potential role for GABAB in modulating the immune response in AD.

Disclosures: M.A. Langhardt: None. **A.S. Murtishaw:** None. **C.F. Heaney:** None. **M.M. Bolton:** None. **J.W. Kinney:** None. **K.C.D. Belmonte:** None. **P.M. Hagins:** None.

Poster

790. Modulation of Disease Pathology in Alzheimer's Disease Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ADDF Grant #20131214

Brightfocus Grant #A2011081

Title: Atherogenic diet-induced accelerated amyloid deposition in PDAPP Tg but not in APP-PS1 Tg mouse model of AD

Authors: *N. R. BHAT, K. SAMBAMURTI, M. KINDY, T. ZHU, S. MOHANTY; Neurosciences, Med. Univ. South Carolina, CHARLESTON, SC

Abstract: Dietary fat/cholesterol is known to induce amyloid deposition in different animal models of AD albeit to varying degrees. In this report, we compared two AβPP Tg mouse models of AD i.e., PDAPP (PDGFB-APPSwInd) and APP-PS1 (APPswe, PSEN1dE9) for amyloid accumulation in response to an atherogenic diet (Western diet, Research Diets). While 9 month-old PDAPP mice on a normal diet (ND) developed sparse amyloid plaques mostly confined to the hippocampus, those fed WD between 6 and 9 months showed increased amyloid in the hippocampus as well as in the cortical areas. The amyloid plaques were associated with clusters of activated microglia and astrocytes as revealed by immunohistochemistry. Increased accumulation of Aβ was confirmed by ELISA and Western blot analyses of the brain extracts. Western blot also showed the presence of increased PHF-1 reactive p-tau in WD-fed mice relative to ND-fed control PDAPP Tg mice. The above changes were accompanied by increased expression of VCAM1, P-Selectin, TNFα and iNOS, and reduced levels of phospho (Ser1177)-eNOS indicating adverse cerebrovascular and neuroinflammatory changes. Behaviorally, WD-fed PDAPP Tg mice performed worse on a water-version of T-maze relative to ND-fed controls. In contrast to the findings with the PDAPP Tg mice, the atherogenic diet failed to induce amyloid accumulation over basal (ND-fed) level and was overall less effective in the APP-PS1 Tg mice. The findings have implications for the correct choice of AD mouse models for the study of diet-induced β-amyloidosis.

Disclosures: N.R. Bhat: None. **K. Sambamurti:** None. **M. Kindy:** None. **T. Zhu:** None. **S. Mohanty:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Colciencias grant 1115-545-31478

Title: Beta-secretase 1 regulates the lipid profile of ca1 neurons in 3xtg-ad mouse model of Alzheimer’s disease

Authors: *J. G. VILLAMIL, A. BARRERA OCAMPO, D. PIEDRAHITA, J. CRUZ JIMENEZ, P. CARDONA GOMEZ;
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Abstract: Deposits of β-Amyloid peptides (βA) and hyperphosphorylated tau are histopathologic hallmarks of Alzheimer’s disease (AD). βA is product of the cleavage of the amyloid precursor protein by the enzyme β-secretase 1 (BACE1) through the amyloidogenic pathway, making BACE1 a therapeutic target against AD. Alterations in lipid metabolism are a risk factor for AD and the lipid composition of neural membranes seems to determine BACE1 activity by an unknown mechanism. The objective of this study is to determine the effect of RNA interference against BACE1 (shBACE-miR) on the lipid profile of hippocampal CA1 neurons in 3xTg-AD mice at 6 and 12 months of treatment. The results obtained by mass spectrometry show increased levels of DAG in AD mice compared with healthy animals ($p<0.05$). The treatment with shBACE-miR restores the basal levels of lyso-phosphatidylethanolamine ($p<0.05$) and ether-phosphatidylethanolamine ($p<0.05$) in 3xTg-AD mice treated for 6 and 12 months. Additionally, gas chromatography analysis evidences a tendency towards increase of stearic acid and diminution of polyunsaturated fatty acids like eicosatrienoic acid in AD mice compared to healthy animals, without changes by the shBACE1miR treatment. Our preliminary results suggest that silencing BACE1 induces changes in the composition of neural lipid profile that could favor the recovery of cellular homeostasis in the hippocampus of triple transgenic mice of AD.

Disclosures: J.G. Villamil: None. **A. Barrera Ocampo:** None. **D. Piedrahita:** None. **J. Cruz Jimenez:** None. **P. Cardona Gomez:** None.

Poster

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NIH Grant P30NS055077

NIH Grant P50AG005136

Title: U1snRNP pathology identified by proteomics analysis of Alzheimer's disease links aberrant alternative splicing to elevated beta-amyloid levels

Authors: *P.-C. CHEN[1], B. BAI[1], C. M. HALES[2], Y. M. GOZAL[2], E. DAMMER[3], D. M. DUONG[2], H. D. REES[3], N. T. SEYFRIED[3], A. I. LEVEY[3], J. J. LAH[3], J. PENG[1]; [1]Structural Biol., St Jude Children Research's Hosp., Memphis, TN; [2]2Department of Neurology, Ctr. for Neurodegenerative Dis., [3]Dept. of Neurology, Ctr. for Neurodegenerative Dis., Emory Univ., Atlanta, TN

Abstract: Alzheimer's disease (AD), the most popular dementia, affects over 15 million people worldwide and it is expected to double in the next 20 years. However, there are currently no cures or treatments to slow down memory loss. Also clinical trials targeting on reducing amyloid-beta levels gains disappointing results. Therefore, it is urgent to find new targets for developing prevention or therapy for AD. With using large-scale proteomic analysis by liquid chromatography-mass spectrometry, we have identified accumulation of U1snRNP components in the detergent-insoluble proteome specifically in AD brain but not in Parkinson's or other degenerative diseases. In AD, these U1snRNP components, U1-70K and U1-A forms into cytoplasmic tangle-aggregates and eliminates from nuclear. As splicing happens in the nuclear, loss of nuclear U1snRNP indicates that loss-of function may play an important role in AD etiology. We found knockdown of U1-70K increases secreted amyloid-beta in both 293 and differentiated SH-SY5Y cells. Further, knockdown of U1-70K enhances exon skipping in *APP*, *BACE1*, *Presenilin-1, Presenilin-2*, in which the exon 5 skipping of *Presenilin-2* has been linked to sporadic AD. These data suggest aberrant splicing induced by loss of U1-70K may contribute to beta-amyloid elevation. We proposed elimination of nuclear U1snRNP in neurons may impair alternative splicing of genes and promotes the amyloid pathology and toxicity in AD.

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Poster

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Support: National Natural Science Foundation of China (81171091 to XXY, and 81171160 to XGL)

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XYSM-PUMC Human Brain Banking Consortium (CM and XXY)

Title: Vascular BACE1 elevation and cerebral amyloid angiopathy in Chinese subjects: Cholestrol as a stress factor for endothelial Aβ overproduction

Authors: *X.-X. YAN[1], Z.-Q. XUE[1], X.-G. LUO[1], X. ZHOU[2], J.-J. YU[2], W.-P. GAI[3], H. CAI[4], C. MA[5];
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Abstract: Cerebral amyloidosis is a common brain pathology associated with aging and age-related neurological diseases. Extracellular β-amyloid (Aβ) deposition in the parenchyma of the brain, especially in the form of neuritic or senile plaques, represents a hallmark lesion in Alzheimer's disease (AD). Amyloid deposition also occurs in the wall of blood vessels in the brain, referred to as cerebral amyloid angiopathy (CAA). CAA has been linked to vascular and overall cerebral dysfunctions during aging and in AD, while its pathogenic mechanism is poorly understood. Therefore, understanding its pathogenic mechanism may provide valuable information for developing effective therapeutic strategies for age-related dementia. Upregulation of the amyloidogenic enzyme β-secretase-1 (BACE1) has been shown to play a key role in Aβ overproduction and deposition in brain parenchyma. Here, we investigate a potential involvement of BACE1 in CAA by examination of postmortem human brains and human vascular cell models. Postmortem brains (n=27) from subjects aged 22-101 years were examined. BACE1 immunoreactivity (IR) was increased at local cerebral vasculature among 13 cases with extracellular plaques and/or CAA. Among the brains (n=14) lacking cerebral amyloidosis, vascular BACE1 IR was detectable at rare sites in some cases (n=4). BACE1 labeled vasculature appeared to be predominantly capillaries and arterioles. The BACE1 IR was localized mainly in the endothelium and partially coexisted with the vascular Aβ IR. Biochemical analysis confirmed the presence of BACE1 and other amyloidogenic proteins as well as their enzymatic activity in preparations of surgically removed peripheral human blood vessels, while primary cell culture indicated the expression of these proteins mainly in the endothelial cells. Notably, BACE1

protein expression and enzymatic activity as well as Aβ levels were elevated in cultured human umbilical endothelial cells in response to high cholesterol exposure. Together, these results suggest that Aβ overproduction by vascular cells may be involved in the development of CAA. Vascular Aβ overproduction may be mediated by BACE1 in response to circulatory/metabolic abnormalities including hypercholesterolemia.

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Poster

791. APP Cleavage and Metabolism Processes

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Program#/Poster#: 791.04/D63

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of kallikrein-related peptidase 7 as a novel amyloid-β peptide-degrading protease secreted from astrocytic cells

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Abstract: Alzheimer disease (AD) is a most common neurodegenerative disorder among dementia. AD is caused by aberrant accumulation and deposition of amyloid β peptide (Aβ), which is mainly secreted from neurons. Recently, pathophysiological roles of glial cells in AD have been highlighted, while it remains unclear how these non-neuronal cells are involved in the Aβ metabolism. To identify the Aβ-degrading enzyme derived from glial cells, we examined the degradation of Aβ in the cultured medium of human astrocytoma cell lines, U87 and CCF-STTG1. We found that these conditioned media from both cells showed the Aβ-degrading activity that was specifically inhibited by diisopropyl fluorophosphate, tosyl phenylalanyl chloromethyl ketone and zinc ion. These enzymatic characteristics suggested that kallikrein-related peptidase 7 (KLK7), which belongs to a member of tissue kallikrein-related peptidase family proteins, is involved in the Aβ degradation. Notably, low KLK7 level in CSF of AD

patients has been reported (Diamandis EP, et al. 2004). Supporting this notion, RNAi experiment revealed that KLK7 was required for the Aβ-degrading activity in the medium of CCF-STTG1 cells. Moreover, recombinant KLK7 protein was capable to degrade Aβ. Intriguingly, the conditioned media from rat primary astrocytes, but not neurons, showed the Aβ-degrading activity as well as KLK7 expression. These results suggest that KLK7 is a novel Aβ-degrading protease secreted from astrocytic cells.

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Poster

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Program#/Poster#: 791.05/D64

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Pharmacological and safety profiles of JNJ-45261957 a new γ-secretase modulator

Authors: F. P. BISCHOFF, F. J. R. ROMBOUTS, D. BERTHELOT, D. OEHLRICH, M. A. J. DE CLEYN, A. I. VELTER, M. SURKYN, S. VAN BRANDT, S. PIETERS, G. MINNE, G. J. MACDONALD, M. DESMIDT, N. BODE, *M. H. MERCKEN, H. BORGHYS, H. J. M. GIJSEN;
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Abstract: There is to date no efficacious treatment for patients suffering from Alzheimer's disease (AD), the most common form of dementia. With an increasingly ageing population worldwide, AD represents a huge burden to society. Genetic mutations promoting the proteolytic processing of the amyloid precursor protein (APP) by β- or γ-secretase (GS) are believed to be responsible for the rapid onset of the disease and their study has provided the genetic framework for the amyloid hypothesis. GS modulation has been proposed as a potential disease modifying anti-Alzheimer's approach. In contrast to γ-secretase inhibitors (GSIs), γ-secretase modulators (GSMs) cause a product shift from the longer amyloid isoforms to shorter, more soluble and less amyloidogenic isoforms, without inhibiting NOTCH proteolytic processing. Potent GSMs, from different chemicals classes, have been reported recently. Typically, these compounds are characterized by high lipophilicity and high molecular weight, properties that have been associated with low probability of success in clinical development. In this paper, we will discuss some aspects of the *in vitro* and *in vivo* pharmacological profiles of a new GS modulator, namely

JNJ-45261957, as well as early observations related to liver toxicity and tolerance studies conducted in rats and beagle dogs including single and repeated dose phases.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG025952

NIH R01AG033016

Title: BACE1 neuronal polarized sorting: Role of the clathrin adaptor protein GGA3

Authors: ***S. LOMOIO**, K. R. WALKER, G. TESCO;
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Abstract: BACE1 localizes at the presynaptic terminal however the mechanisms of BACE1 axonal sorting remain unknown. Polarized delivery of membrane proteins is regulated by the interaction of signals present in their CTF-fragment with specific trafficking molecules. Sorting signals include the di-leucine motifs, [DE]XXXL[LI] or DXXLL, the tyrosine-based motifs, NPXY or YXXØ, and ubiquitin. The [DE]XXXL[LI] motif is recognized by the adaptor protein complexes AP-1, 2, 3 and 4, while GGA1, 2, and 3 bind to DXXLL via the VHS domain. The BACE1-CTF contains a specific di-leucine sorting signal (495DDISLL500) and an ubiquitination site at K501. GGA1, 2, and 3, a family of monomeric clathrin adaptors, have been shown to bind to the BACE1 496DISLL500 motif via their VHS domain and the phosphorylation of BACE1-S498 appears to increase their binding. Our previous studies have shown that BACE1 is degraded via the lysosomal pathway and that depletion of GGA3 results in increased BACE1 levels and activity owing to impaired lysosomal trafficking and degradation. Moreover we have demonstrated the role of GGA3 in the regulation of BACE1 *in vivo* by showing that BACE1 levels are increased in the brain of GGA3 null mice. We recently reported

that GGA3 regulates BACE1 degradation independently of the VHS/di-leucine motif interaction but requires binding to ubiquitin. More importantly we have previously shown that GGA3 levels are significantly decreased and inversely correlated with BACE1 levels in the post-mortem temporal cortex of AD patients. In spite of the compelling evidence showing that GGA3 regulates BACE1 trafficking, previous studies have been conducted in non-polarized cells. Moreover, while increasing evidence is accumulating for a role of the AP complexes in neuronal polarized sorting, the function of GGAs in neurons remains to be clarified. Interestingly, GGA3 is expressed highest in the brain and in neurons suggesting a specific role for GGA3 in neuronal function. In order to determine the polarized sorting of BACE1, mouse hippocampal neuronal cultures were transiently transfected with BACE1-GFP. After fixation neurons were imaged with a Leica confocal microscope and the polarity index (axon/dendrites ratio) was calculated using ImageJ. Our data indicate that BACE1 is present both in the axon and dendrites 1 day after transfection with a preference for dendritic targeting. We are currently investigating the extent to which GGA3 regulates axonal sorting of BACE1 using cultured hippocampal neurons from GGA3+/+ and GGA3-/- mice. We are studying the localization of GGA3 and BACE1 with axonal and dendritic markers and calculate polarity index for the proteins of interest.

Disclosures: **S. Lomoio:** None. **K.R. Walker:** None. **G. Tesco:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

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IIRG from Alzheimer’s Association

NIH Grant R01 AG027924

Title: Heparan sulphate proteoglycan negatively modulates brain Aβ clearance

Authors: C.-C. LIU[1], J. CIRRITO[2], X. LI[1], T. KANEKIYO[1], Y. YAMAGUCHI[3], D. HOLTZMAN[2], *G. BU[1];
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Abstract: Accumulation of amyloid-β (Aβ) peptide in the brain is the first critical step in the pathogenesis of Alzheimer's disease (AD). Studies in humans suggest that Aβ clearance from the brain is frequently impaired in late-onset AD. Aβ accumulation leads to the formation of Aβ aggregates which disturb synaptic functions and lead to eventual neurodegeneration. Cell surface heparan sulfate proteoglycan (HSPG), abundantly expressed in neurons, have been implicated in several features in the pathogenesis of AD, including its co-localization with amyloid plaques and its modulatory role in Aβ aggregation. Previous studies have shown that heparin, which antagonizes HSPG, suppresses the interaction of Aβ to neurons and reduces Aβ neuronal toxicity. However, the mechanisms by which neuronal HSPG regulates brain Aβ clearance and related amyloid pathology *in vivo* remains unclear. Here, we show that HSPG negatively regulates Aβ clearance *in vivo*. Deletion of neuronal HSPG by conditionally disrupting the HS polymerizing enzyme Ext1 in postnatal neurons in adult amyloid model APP/PS1 mice significantly increased brain interstitial fluid (ISF) Aβ clearance when analyzed by *in vivo* microdialysis. A deficiency of neuronal HSPG also led to a dramatic reduction in the deposition of amyloid plaque, suggesting that Aβ binding to HSPG and related cellular uptake either inhibit or represent an inefficient pathway for Aβ clearance. Our findings have implications for AD pathogenesis and may provide insights into therapeutic intervention targeting Aβ-HSPG interaction.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS034467

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Title: Pericyte loss influences Alzheimer-like neurodegeneration cascade in mice

Authors: *A. P. SAGARE[1], R. D. BELL[2], Z. ZHAO[1], Q. MA[1], E. A. WINKLER[2], A. RAMANATHAN[1], B. V. ZLOKOVIC[1];
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Abstract: Pericytes are vascular mural cells embedded within the basement membrane of blood microvessels. Pericytes are uniquely positioned within the neurovascular unit between endothelial cells of brain capillaries, astrocytes, and neurons. Recent studies have shown that pericytes regulate key neurovascular functions including blood-brain barrier formation and maintenance. Neurovascular dysfunction, microvascular reductions and pericyte degeneration and loss have been demonstrated in Alzheimer's disease (AD), a neurodegenerative disorder associated with abnormal elevation of amyloid β-peptide (Aβ), tau pathology and neuronal loss. Whether pericytes can influence the natural course of AD-like neurodegeneration and contribute to disease pathogenesis and accumulation of AD pathology remains, however, unknown. Utilizing mice which overexpress Aβ-precursor protein, we show that pericyte loss elevates brain Aβ40 and Aβ42 levels and accelerates amyloid angiopathy and cerebral β-amyloidosis by diminishing clearance of soluble Aβ40 and Aβ42 from brain interstitial fluid prior to Aβ deposition. We show that pericyte deficiency leads to the development of tau pathology and an early neuronal loss that is normally absent in Aβ-precursor protein transgenic mice resulting in cognitive decline. Our data suggest that pericyte loss influences and accelerates multiple steps of AD-like neurodegeneration pathogenic cascade in Aβ-precursor protein overexperssing mice. Therefore, pericytes may represent a novel therapeutic target to modify disease progression in AD.

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Poster

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Support: National Institutes of Health (R21AG039596)

Alzheimer's Association (11RG-05-14584)

American Health Assistance Foundation (A2009045)

Title: Roles of Nicastrin, Pen-2, and Aph-1 in gamma secretase catalyzed turnover of the C-terminal fragment of APP

Authors: *X. XU, C. HU, T. LI, L. ZENG, M.-Z. CUI;
Pathobiology, Univ. Tennessee, KNOXVILLE, TN

Abstract: Based on the "amyloid cascade hypothesis", the ratio of Aβ42 verses Aβ40 plays a key role in Alzheimer's disease (AD). The ratio of Aβ42/Aβ40 is controlled by gamma secretase, which cleaves APP (Aβ precursor protein) at C terminal and release Aβ in different length: Aβ38, Aβ40, Aβ42, Aβ43, Aβ46, Aβ49 and so on. Hence, dissecting the biological and biochemical nature of gamma secretase is important in understanding the mechanism of Aβ formation. Gamma secretase is a complex composed of four components: presenilins (PS1 or PS2), nicastrin (NCT), anterior pharynx-defective 1 (Aph-1), and presenilin enhancer 2 (pen-2). The roles of these components remain unclear. It is believed that all the four components are required for gamma secretse activity. Previous studies have suggested that PS functions as the catalytic subunit; NCT may serve as a receptor for the substrate; Aph-1 is assumed to stabilize the other three components; and pen-2 was reported to be essential for the endoproteolysis of PS. However, our recent study revealed that pen-2 is dispensable for the endoproteolysis of PS, but is required for gamma secretase activity. Our data also demonstrate that NCT is also required for gamma secretase activity. Interestingly, we found that APP is processed by gamma secretase activity in the absence of Aph-1, indicating that Aph-1 is not required for gamma secreatse activity. Furthermore, our data revealed that post-translational modification of PS1 has a strong effect on gamma secretase activity.

Disclosures: X. Xu: None. **C. Hu:** None. **T. Li:** None. **L. Zeng:** None. **M. Cui:** None.

Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: FRET-based assays for the detection of amyloid degrading protease activity

Authors: *C.-Y. KO, M. ZHU, O. GURINOVICH, F. LI, R. ZHANG, J. ZHONG, V. RAKHMANOVA;
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Abstract: Amyloid degrading proteases (AβDPs) have been identified as playing a role in β-amyloid (Aβ) cleavage. The accumulation of Aβ plaques, a hallmark of Alzheimer's disease (AD) patient brain, has been hypothesized to be due to the imbalance between AβDPs activity and Aβ production resulting in AD pathogenesis. In order to confirm the role(s) of AβDPs in neuronal degeneration diseases, reliable assays for AβDPs activity detection have to be developed. We have designed several assays to detect AβDP targets using fluorescence resonance energy transfer (FRET) based peptide substrates. Substrate sequence is derived from the cleavage site of each AβDP target. The peptide substrate is coupled with an optimized fluorescent donor (HiLyte™ Fluor 488 or 5-FAM) and a quenching acceptor (QXL® 520). Upon cleavage by active AβDP, the fluorescent donor is separated from the quencher and resulted in an increased fluorescence signal. The long-wavelength fluorescence signal has minimal interference from autofluorescence derived from biological samples and testing compounds. Moreover, the perfect overlap of absorption and emission spectra of our proprietary FRET pairs allows for a greater level of sensitivity detection. Utilizing FRET technology, the assay offers a homogeneous format which can be easily adapted for high-throughput screening (HTS) for drug discovery. Several AβDPs, such as β-secretases, neprilysin, ADAMs, IDE, MMPs, and cathepsins have been proven to play roles in the regulation of Aβ clearance in AD. Using different FRET pairs and novel sequence design, assays have been developed for monitoring enzyme activity of β-secretases, neprilysin, ADAM 10, TACE (ADAM17), MMP-2, MMP-9, cathepsins B and D. The origin of peptide substrate sequence ensures that the assays can be used for either specific β-amyloid degradation detection or general enzyme activity screening. Furthermore, long wavelength, fluorescent readouts yield sensitivity in the sub-nanogram range. In order to increase specificity of AβDPs activity detection in biological samples, our future goal will aim at combining FRET technology and specific antibody to develop immunocapture-based fluorometric assay.

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Poster

791. APP Cleavage and Metabolism Processes

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Title: Curcumin prevent alzheimer's pathology through a new mechanism for the inhibition of aβ oligomer formation

Authors: Y.-Q. YANG[1], Y.-L. ZHANG[1], S.-L. LI[1], M.-M. KANG[1], Z.-B. LIANG[1], X.-Q. HU[2], *W.-J. GAO[3], F. LI[1];
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Abstract: In the last decade of studies, soluble oligomeric forms of amyloid-β (Aβ) were believed to play a larger role in the pathogenesis of Alzheimer's disease (AD) than deposited β-amyloid. However, there is still a lack of direct evidence, and its mechanism remains largely unknown. In this study, we first found a new type of senile plaques composed of soluble non-fibrillar Aβ oligomers on the posthumous brain specimens of the patients with Alzheimer's disease. We refer to as the Aβ oligomer plaques. They were distributed among the classical senile plaques comprised of insoluble fibrillar β-amyloid in the cerebral cortex and hippocampus. To some extent, the Aβ oligomer plaques were only part of classical senile plaques, but sometimes they could make senile plaques in whole by themselves, suggesting the possibility that the Aβ oligomer plaques were a critical transition state of senile plaques. We speculated that, by soluble nature of oligomers, some therapeutic agents targeting the oligomers might make the Aβ oligomer plaques dissolution. In this study, we also observed that the anti-Aβ oligomer humanized antibody had a high affinity for the Aβ oligomer plaques, but the anti-Aβ antibody that specifically binds only to the fibrillar β-amyloid could not recognize them. Curcumin, a phenolic compound extracted from the rhizome of the herb curcuma longa, could label the Aβ oligomer plaques on brain sections of AD patients and stain the same structures as antibody against Aβ oligomer. *In vitro*, we found that curcumin could inhibit the Aβ aggregation pathway from Aβ monomers to oligomers and from oligomers to Aβ deposition. We further confirmed that the effects of curcumin on Aβ aggregation were associated with its blocking a conversion of α-helical conformation of β-amynoid into the β-stranded conformation at the early step. It is

generally known that the conformational conversion can result in the exposure of the hydrophobic surface of β-amynoid which is conducive to Aβ aggregation. Our study might offer insights in better improving our understanding of oligomeric Aβ-induced toxicity. Based on the pleiotropic activity of curcumin, we might make curcumin potentially useful for treatment of Alzheimer's disease by targeting the soluble Aβ oligomers.

Disclosures: **Y. Yang:** None. **W. Gao:** None. **F. Li:** None. **S. Li:** None. **M. Kang:** None. **Z. Liang:** None. **X. Hu:** None. **Y. Zhang:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RO1 AG042513

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R21 AG045691

Knight Alzheimer's Disease Research Center at Washington University

Title: Role of NMDA in amyloid-beta generation

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Abstract: Extracellular accumulation of the amyloid-beta (Abeta) peptide as oligomers and plaques is thought to be a key event in Alzheimer's disease (AD) pathogenesis. Amyloid-beta aggregation is concentration-dependent, with higher concentrations of Abeta much more likely to form these toxic multimers. Mechanisms that reduce Abeta production and extracellular levels are therefore promising targets for influencing disease pathology. Previously, our lab found that administration of *N*-methyl-D-aspartic acid (NMDA) directly to the brain reduced Abeta levels in the interstitial fluid (ISF) of wild-type mice and the APP/PS1 mouse model of AD. We have shown that this NMDA-mediated suppression requires the phosphorylation and activation of extracellular-regulated kinase (ERK). ERK signaling then reduces Abeta production, apparently

through increased α-secretase enzymatic activity. ERK can be activated through a wide selection of receptors and signaling molecules; however, its selectivity for downstream effects is largely dependent on the cellular context in which it is activated. In order to understand the downstream effects of NMDA receptors on Abeta production, we must first determine the pathway(s) through which NMDA receptors signal to ERK. In order to define this pathway, we have inhibited key signaling molecules and measured the effect on NMDA receptor-mediated Abeta generation using a combination of *in vivo* microdialysis in the hippocampus of young PS1/APP mice as well as *in vitro* primary hippocampal neuronal culture. By detailing the components necessary for NMDA receptors to decrease brain Abeta levels, we hope to identify potential selective targets for the regulation of Abeta production.

Disclosures: J. Hettinger: None. **J.R. Cirrito:** None.

Poster

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Program#/Poster#: 791.13/D72

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Human ES cell-derived neurons as a preclinical model system for testing gamma-secretase inhibitors and modulators

Authors: *D. KARLSSON, H. MURREY, D. JOHNSON, I. SINGEC;
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Abstract: Alzheimer's disease (AD) is the most common form of dementia, accounting for an estimated 70 percent of cases. γ-Secretase (GS) is an intramembrane aspartyl-protease that cleaves amyloid precursor protein (APP) to generate Aβ species, including the neurotoxic Aβ-42. Aβ-42 is a component of β-amyloid plaques, which are histopathological hallmarks of AD. γ-Secretase modulators (GSMs) are potentially important treatment options for AD, since they decrease the production of Aβ-42 without affecting the processing of other critical γ-secretase substrates. Human neurons, for instance derived from human ES cells, hold great promise for streamlined testing of known and new drugs under defined conditions. In fact, "humanizing" the drug discovery process in early stages, could pave the way for more rapid, accurate, and economic strategies. As part of Pfizer's Humanizing Drug Discovery initiative, we studied functional human neurons (H9 line), and used clickable GSM and GSI photoaffinity probes to assess the functionality of GS. In addition, we developed γ-Secretase inhibitor-photoaffinity

probes for fluorescent imaging applications to monitor the subcellular localization of these compounds in live cells. We found that human neurons expressed functional GS and produced detectable levels of Aβ peptides. Generation of these peptides was inhibited by known GSIs, with IC_{50} values comparable to other live-cell assays. Lastly, we observed discrete subcellular localizations of GSIs in live human neurons. Together, these studies suggest that functional human neurons contain active GS thereby representing an invaluable preclinical model system for assessing compound activity and providing new mechanistic insights into molecular targets and drug response.

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Poster

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NIH T32NS061764

Title: Role of deubiquitinating enzyme ubiquitin specific peptidase 8 (USP8) in β-Site amyloid precursor protein cleaving enzyme (BACE1) degradation

Authors: *E. F. YEATES, G. TESCO;
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Abstract: Alzheimer's disease (AD) is a progressive neurological disorder that affects 1 in 9 people over age 65 in the United States. Amyloid beta (Aβ) is the chief component of the amyloid plaques characteristic of AD. β-Site amyloid precursor protein cleaving enzyme (BACE1) is the rate-limiting enzyme in the formation of Aβ from amyloid precursor protein (APP). Increases in BACE1 protein levels have been seen in post mortem samples from AD patients. We have previously shown that BACE1 is ubiquitinated on lysine 501 and is degraded in lysosomes. However, the ubiquitin ligases and deubiquinating enzymes that regulate BACE1 ubiquitination, remain to be identified. As BACE1 is degraded in the endosomal-lysosomal system, we hypothesize that ubiquitin specific peptidase 8 (USP8), an endosomal-associated

deubiquitinating enzyme, regulates the degradation of BACE1, by deubiquinating it. In particular, decreasing levels of USP8 are expected to increase BACE1 degradation, decrease BACE1-mediated APP processing and ultimately decrease amyloid beta formation. The goals of the study were to determine whether knockdown of USP8 *in vitro* increased BACE1 degradation and resulted in decreased BACE1-mediated APP processing. We used an H4 neuroglioma cell line overexpressing BACE1-GFP to determine the effects of USP8 on BACE-GFP *in vitro*. In overexpression experiments, cells were transfected either with a HA-tagged USP8 plasmid or vector. In siRNA-mediated silencing experiments, cells were transfected with either USP8 siRNA or non targeting siRNA. Total cell lysates were examined for expression of BACE-GFP by Western blotting. Preliminary evidence shows that while an overexpression of USP8 does not result in a change in BACE-GFP levels compared to empty vector, knockdown of USP8 results in decreased BACE1-GPF protein levels. Furthermore, knockdown of USP8 results in increased full length APP and decreased APP C-terminal fragment (CTF) levels, suggesting a decrease in BACE1-mediated APP processing. Conditioned media samples from siRNA treated cells were analyzed for secreted Aβ40 content using an Aβ40 ELISA. USP8 knockdown did not alter secreted Aβ40 levels in conditioned media. Future experiments will determine whether USP8 knockdown decreased APP CTFs levels independent of BACE1-mediated processing, by increasing CTF degradation. Furthermore, future experiments will determine whether USP8 knockdown affects the levels of the alpha and gamma secretases involved in APP processing. The results of our studies will aid our understanding of the regulation and degradation of BACE1, an important enzyme in the formation of Aβ.

Disclosures: E.F. Yeates: None. **G. Tesco:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DGAPA, PAPIIT, UNAM IN204212-3

CONACYT 239696

Title: APP metabolism is modulated by cholesterol in cultured astrocytes

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by extracellular deposits of amyloid β protein (Aβ) derived from the amyloid precursor protein (APP) through the amyloidogenic pathway. Studies in neurons have found that the APP cleavage enzymes, β- and γ- secretases, reside in cholesterol- and sphingolipid-rich detergent-resistant lipid raft microdomains. Cholesterol has long been associated with AD pathogenesis and different studies have found a positive correlation between the level of circulating cholesterol and Aβ load in the AD's brain. Although the majority of studies on APP processing have been performed in neurons, recent studies suggest that astrocytes can be an important cellular source of Aβ. However, it is not completely understood the mechanisms involved in APP processing in astrocytes. The aim of this work was analyze the relationships between cholesterol content and expression of APP and Aβ production in astrocytes. Primary cultured astrocytes were used trough the study and were incubated with cholesterol at different doses to analyze the expression of GFAP, APP and β-secretase. At present we have found that cholesterol induces the activation of astrocytes and the increase of APP and BACE-1 proteins. Also, cholesterol promotes the association of APP with BACE-1 and the production of C-99 fragment derived from the APP cleavage by this enzyme. Concomitant with these effects the non-amyloidogenic pathway-related fragment, sAPPα was found decreased. These results confirm that cholesterol contents may have an impact in the APP metabolism in astrocytes and may be a risk factor for amyloid production by these cells.

Disclosures: M.E. Avila-Muñoz: None. **C. Arias:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Support: National Institutes of Health (R21AG039596)

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Title: FLICE-inhibitory protein(c-Flip) is a novel substrate of gamma-secretase

Authors: *L. ZENG, C. HU, T. LI, F. ZHANG, M.-Z. CUI, X. XU;
Pathobiology, The Univ. of Tennessee, Knoxville, TN

Abstract: Mutations in the presenilin 1 (PS1) gene are responsible for the majority of familial form of Alzheimer disease. Studies suggest that PS1 functions as the catalytic subunit of the γ-secretase complex, which is a key enzyme involved in β-amyloid peptide (Aβ) formation, a hallmark of Alzheimer's disease (AD). In addition to Aβ formation, PS1 has also been implicated in apoptosis. However, the mechanism by which PS1 is involved in apoptosis remains elusive. In this study, we investigated the effect of PS1 on the turnover of cellular FLICE-inhibitory protein (c-FLIP). We found that overexpression of PS1 induced apoptosis in certain types of cells and the induction of apoptosis is associated with the cleavage of c-FLIP. Our data further demonstrated that PS1-induced cleavage of c-FLICE was inhibited by γ-secretase inhibitors, but not by caspase inhibitor, indicating that cleavage of c-FLIP is catalyzed by γ-secretase. Moreover, our data also demonstrated that addition of γ-secretase inhibitors also blocked PS1-induced apoptosis. These data suggest that c-FLIP is involved in PS1-induced apoptosis. To further elucidate the pathway, we examine the roles of some key caspase and mitochondrial apoptotic factors in PS1-induced apoptosis. To this end, our data revealed that knockdown of caspase-8, FADD, bid, and Bax, or overexpression of Bcl-2 strongly blocked PS1-induced apoptosis, while knockdown of Bak had no effect on PS1-induced apoptosis. Knockdown of caspase-9 and SMAC at the same time also significantly inhibited PS1-induced apoptosis. Taken together, our data suggest that PS1 induces a FLIP-mediated mitochondria dependent apoptosis. In addition, our study also revealed that c-FLIP is a novel substrate of γ-secretase.

Disclosures: L. Zeng: None. **C. Hu:** None. **T. Li:** None. **F. Zhang:** None. **M. Cui:** None. **X. Xu:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: WA1477/4 to J. Walter

SFB645 to J.Walter

Title: EphrinB2 processing by γ-secretase regulates migration of microglia

Authors: ***N. KEMMERLING**[1], P. WUNDERLICH[1], N. HERSCH[2], B. HOFFMANN[2], K. GLEBOV[1], B. D. STROOPER[3], H. NEUMANN[4], J. WALTER[1];
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Abstract: *Introduction*: Presenilin mutations are a major cause of early onset familial Alzheimers disease (FAD) cases. Presenilin proteins are critical components of the γ-secretase complex that cleaves many type I transmembrane proteins, including EphrinB2. Microglia, which are important immune cells in the central nervous system (CNS), also play a crucial role in the clearance of the Alzheimer-associated amyloid β peptide. The sensing of amyloid β deposits or other cellular debris and pathogens induces migration of microglia to allow efficient phagocytosis. Here, we investigated the functional role of γ-secretase dependent cleavage of EphrinB2 in microglial migration. *Materials and Methods*: Mouse embryonic stem cell derived microglia (ESdM) from wild-type (WT) and presenilin double knock (PSdKO) mice were investigated regarding their migrational behaviour. In addition, podosome morphology and characteristic signaling proteins related to the migrational processes were analyzed by cell biological and biochemical methods. *Results*: ESdM from PSdKO mice showed significantly impaired migration as compared to ESdM from WT mice. Interestingly, the migrational deficit could be rescued by expression of the EphrinB2 intracellular domain. Furthermore, cleavage of EphrinB2 by γ-secretase was also involved in the phosphorylation of key proteins in the formation of podosomes. *Conclusions*: γ-Secretase dependent cleavage of EphrinB2 is involved in the regulation of microglial migration and thus, might also affect the clearance of amyloid β in Alzheimer's disease**.**

Disclosures: **N. Kemmerling:** A. Employment/Salary (full or part-time):; Full time. **P. Wunderlich:** None. **N. Hersch:** A. Employment/Salary (full or part-time):; Full time. **B. Hoffmann:** A. Employment/Salary (full or part-time):; Full time. **K. Glebov:** A. Employment/Salary (full or part-time):; Ful time. **B.D. Strooper:** A. Employment/Salary (full or part-time):; Full time. **H. Neumann:** A. Employment/Salary (full or part-time):; Full time. **J. Walter:** A. Employment/Salary (full or part-time):; Full time.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 791.18/E5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Bioorthogonal γ-secretase inhibitor (GSI) photoaffinity probes enable determination of target-engagement and compound localization in live cells and primary rat neurons

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Abstract: γ-Secretase (GS) is an intramembrane aspartyl protease, comprised of at least four protein subunits. This complex cleaves the amyloid precursor protein to generate Aβ species, including the neurotoxic Aβ-42. Aβ-42 is a component of β-amyloid plaques, and is believed to play a causative role in Alzheimer's disease (AD) progression. γ-Secretase inhibitors (GSIs) have emerged as early potential treatments for AD. However, these compounds have often failed in clinical trials due to negative effects, likely due to cleavage inhibition of other GS substrates. We have developed a suite of bioorthogonal GSI photoaffinity probes based on the structure of BMS-708,163, an allosteric sulfonamide GSI which binds to the N-terminal fragment of presenilin (PS1-NTF). We evaluated these small-molecule probes in HeLa membranes, live HeLa cells, and primary neuronal cultures to interrogate the compound mechanism-of-action. Probes were synthesized with a benzophenone for photocrosslinking the compound to target proteins and a bioorthogonal handle for reaction with cognate bioorthogonal reporter molecules. We evaluated different bioorthogonal chemistries, including the inverse-electron demand Diels-Alder ligation and the strain promoted azide-alkyne cycloaddition chemistry, and compared these live cell labeling approaches to traditional Cu-catalyzed click chemistry. Initially, we labeled HeLa membranes to validate each bioorthogonal probe. We observed selective labeling of PS1-NTF with bioorthogonal probes in HeLa membranes, whereas a direct-fluorophore linked probe had a high-degree of non-specific labeling, demonstrating the utility of installing a smaller, bioorthogonal handle. We next investigated labeling of presenilin in live cells. In addition, we competed off binding of the probes with the parent compound to determine specificity. We were able to demonstrate specific labeling of PS1-NTF in live HeLa cells and neurons, with different kinetics depending on the bioorthogonal reactive group. Furthermore, we were able to determine the target engagement of an alkyne-modified probe in live, primary cortical neurons with an EC_{50} of ~10 nM. These compounds were then used for live cell imaging, to interrogate their distribution in different subcellular compartments. We identify a punctate distribution of BMS-708,163 photoaffinity probes in live cells, with significant localization to lysosomes and endosomes. These studies validate bioorthogonal probes for the investigation of γ-secretase inhibition in live cells, and provide a method that can be applied to characterize novel GSIs and GSMs developed to treat AD.

Disclosures: H.E. Murrey: None. **D.S. Johnson:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SFB 645 to Jochen Walter

NRW International Graduate Research School LIMES - Chemical Biology (2011-2013)

Title: A role of Alzheimer's disease associated γ-secretase activity in lipid metabolism and lipid droplet formation

Authors: *E. GUTIERREZ[1], D. LÜTJOHANN[2], L. KÜRSCHNER[3], C. THIELE[3], J. WALTER[1];
[1]Neurol., Bonn Univ. Clin., Bonn, Germany; [2]Inst. for Clin. Chem. and Clin. Pharmacol., Bonn university clinic, Bonn, Germany; [3]Life and Med. Sci. (LIMES) institute, Membrane Biol. & Biochem. Unit, Univ. of Bonn, Bonn, Germany

Abstract: ***Objectives:*** Mutations in genes encoding for Presenilins (PS) and the amyloid precursor protein (APP) are the main cause for most cases of early-onset Alzheimer's disease (EOAD). PS are the catalytically active components of the γ-secretase complex (γ-sec), which is responsible for the cleavage of C-terminal fragments (CTF) of APP, leading to the formation of Aβ. Aß is a major component of extracellular plaques found in brains of Alzheimer disease (AD) patients. While the involvement of both PS and APP in AD is well recognized, as is their role in the cellular production of Aβ, the mechanisms linking these proteins to the disease are not comprehensively understood. It has been shown that PS proteins are also involved in cholesterol metabolism. The purpose of this study is to further elucidate the role of PS on sterol, triglyceride and lipid droplet (LD) metabolism, as well as the contribution of the accumulation of the APP CTF C99 to these processes. ***Methods:*** To study the effects of PS activity we used pharmacological inhibition of γ-sec and knock-out cell models lacking PS expression. LD content was analyzed by fluorescence microscopy using the dye LD540. Sterol levels and esterification ratios where analyzed by gas-liquid chromatoraphy-mass spectrometry (GLC-MS) and the Amplex red cholesterol assay. The interaction between C99 and cholesterol was studied by fluorescence microscopy on cell models overexpressing a C99-GFP fusion protein, together with a cholesterol staining with Filipin. ***Results:*** Genetic deletion of PS or pharmacological inhibition of γ-sec activity lead to significantly increased amounts of lipid droplets and triglycerides, together with significantly higher levels of the cholesterol precursors lathosterol

and desmosterol, and significantly lower sterol esterification ratios. Moreover, following pharmacological inhibition of γ-sec, overexpressed APP C99-GFP was observed to accumulate in cholesterol-positive intracellular membrane structures, and an increased expression and accumulation of C99-GFP was shown to correlate with an increased number of cellular LDs. ***Conclusions:*** Our findings support an important role of PS and γ-sec in lipid metabolism. We identified alterations relevant to cellular sterol and lipid homeostasis upon loss of PS activity. Furthermore, the observed association of C99-GFP with cholesterol in cell-based assays, together with the correlation between C99-GFP accumulation and increased LD formation, indicates that this γ-sec substrate represents a potential link between PS activity and lipid metabolism.

Disclosures: E. Gutierrez: A. Employment/Salary (full or part-time):; Full time. **D. Lütjohann:** A. Employment/Salary (full or part-time):; Full time. **L. Kürschner:** A. Employment/Salary (full or part-time):; Full time. **C. Thiele:** A. Employment/Salary (full or part-time):; Full time. **J. Walter:** A. Employment/Salary (full or part-time):; Full time.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

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Title: PS1/γ-secretase promotes EphB4-induced angiogenic complexes and angiogenesis via ephrinB2 processing

Authors: *A. GEORGAKOPOULOS[1], N. WARREN[2], G. VOLOUDAKIS[3], N. K. ROBAKIS[3];
[2]Psychiatry, [1]Mount Sinai Sch. Med., NEW YORK, NY; [3]Psychiatry, Mount Sinai Sch. of Med., New York, NY

Abstract: Evidence in the last decade implicates cerebral microvasculature abnormalities in the genesis of Alzheimer's disease (AD) neuropathology by mechanisms that remain unknown. EphB4/ephrinB2 system is an important regulator of the vascular system in both development

and adulthood. Binding of EphB4 receptor to its transmembrane ligand ephrinB2 on the surface of endothelial cells of blood vessels stimulates angiogenesis, which is the generation of new blood vessels from pre-existing vasculature and transgenic mouse experiments indicate that the intracellular (cytoplasmic) domain of ephrinB2 is necessary for this function. We found that Presenilin1 (PS1), a component of the γ-secretase proteolytic complex which plays a central role in familial AD (FAD), interacts with ephrinB2/EphB4 system and regulates its vascular functions. More specifically we found that EphB4 stimulates processing of ephrinB2 in a PS1/γ-secretase-dependent manner producing cytosolic peptide ephrinB2/CTF2, which corresponds to ephrinB2 cytoplasmic domain, and that EphB4-induced sprouting of endothelial cells depends on γ-secretase activity. When peptide ephrinB2/CTF2 is overexpressed in endothelial cells it stimulates their sprouting and tube formation and mutations that inhibit its phosphorylation at conserved tyrosine residues or removal of its PDZ-binding domain hinder its angiogenic activity. Recent literature shows that a crucial step in angiogenic factor-induced angiogenesis is formation of complexes between Raf-1/Rok-α kinases and Vascular Endothelial cadherin (VE-cadherin), a process regulated by small G protein Rap1. We observed that treatment of endothelial cell cultures with EphB4 increases these angiogenic complexes in a γ-secretase-dependent manner and that overexpression of ephrinB2/CTF2 also promotes the formation of these complexes. In addition, both EphB4- and ephrinB2/CTF2-induced cell sprouting require Rap1 activity. Together the above observations raise the possibility that PS1/γ-secretase affects the EphB4/ephrinB2-induced angiogenesis by regulating proteolytic processing of ephrinB2 and angiogenic complex formation. Our data raise the possibility that PS1 FAD mutations, which inhibit γ-secretase function, may decrease the production of ephrinB2/CTF2 in endothelial cells and consequently inhibit angiogenesis. This provides a potential pathogenic mechanism for the impaired angiogenesis observed in AD brains.

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Poster

791. APP Cleavage and Metabolism Processes

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Title: Presenilin 1 specifically regulates neuronal EGFR expression and signaling

Authors: *G. VOLOUDAKIS[1,2], J. BRUBAN[1], J. SHIOI[1], Y. YOON[1], Q. HUANG[1], Z. SHAO[1], M. AL RAHIM[1], M. A. GAMA SOSA[1], A. GEORGAKOPOULOS[1], N. K. ROBAKIS[1];
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Abstract: Members of tyrosine kinase growth factor receptors are regulated by presenilin1 (PS1), a protein important to Alzheimer disease (AD) as PS1 mutants are linked to familial AD (FAD). Previously, we showed that PS1 is needed for the neuroprotective activities of neurotrophin factor BDNF, a ligand for receptor tyrosine kinase TrkB. Ligands of tyrosine kinase Epidermal Growth Factor Receptor (EGFR) protect neurons from toxic insults such as excitotoxicity and ischemia. Here we report that neurons lacking PS1 show decreased ligand-induced (EGF & HB-EGF) Akt activation and reduced neuroprotection against excitotoxicity. Consistent with neuronal survival outcomes, PS1 null primary neuronal cultures contain dramatically reduced levels of this receptor, a state which can be reversed by the reintroduction of PS1. These observations are inconsistent with literature reports that PS1 negatively regulates EGFR. Acute downregulation of PS1 using siRNA decreases the expression of neuronal EGFR and brains from PS1 null mice have reduced levels of EGFR relative to WT brains. Additional experiments indicate that decreased EGFR in PS1 null neurons is due to decreased EGFR mRNA, a change independent of γ-secretase activity. Neurons that lack Presenilin 2 (PS2) exhibit normal EGFR expression levels and ligand-induced neuroprotection against excitotoxicity. Interestingly, neither primary fibroblasts nor glia cells from PS1 null mice contain increased levels of EGFR. In addition, EGFR protein levels showed no correlation with PS1 expression levels when we compared different immortalized fibroblast clones from WT, PS1 hemizygous and PS1 null mice. Together, our data suggests that in contrast to reports obtained in immortalized cells, PS1 positively regulates the expression of neuronal EGFR and the neuroprotective effects of its ligands. Furthermore, immortalized fibroblasts may not be reliable indicators for the effects of PS1 on the EGFR of primary cells and brain tissue.

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Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Else Kröner-Fresenius-Stiftung grant 2012_A304

Title: The anti-diabetic drug metformin reduces BACE protein level by interfering with the MID1-PP2A-mRNP

Authors: *S. KRAUSS, M. HETTICH, F. MATTHES, D. RYAN, N. GRIESCHE, S. SCHRÖDER, S. DORN, D. EHNINGER;
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Abstract: Alzheimer's disease (AD), the most common form of dementia in the elderly, is characterized by two neuropathological hallmarks: senile plaques, which are composed of Aβ peptides, and neurofibrillary tangles, which are composed of hyperphosphorylated tau protein. Diabetic patients with dysregulated insulin signalling have an increased risk of developing AD and in several animal models of diabetes show increased levels of expression of Aβ and hyperphosphorylated tau. As we have shown recently, the anti-diabetic drug metformin is capable of dephosphorylating tau at AD-relevant phospho-sites. Here, we investigated the effect of metformin on the main amyloidogenic enzyme BACE1 and thus on the production of Aβ peptides, the second pathological hallmark of AD in cultures of primary neurons, a human cell line model of AD and *in vivo* in mice. We show that treatment with metformin decreases BACE1 protein expression by interfering with an mRNA-protein complex that contains the ubiquitin ligase MID1, thereby reducing BACE1 activity. Together with our previous findings these results indicate that metformin may target both pathological hallmarks of AD and may be of therapeutic value for treating and/or preventing AD.

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Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 791.23/E10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG033658

Title: Snapin-mediated bace1 retrograde transport is essential for its degradation in lysosomes and regulation of app processing in neurons

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Abstract: β site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) is the major β secretase for generating β-amyloid (Aβ) peptides. The acidic environment of endosomes is optimal for β secretase activity. However, the mechanisms regulating BACE1 traffic from endosomes to lysosomes for degradation are largely unknown. Here, using snapin-deficient mice combined with gene rescue experiments, we reveal that Snapin, as a dynein motor adaptor for late endosomes, mediates BACE1 retrograde transport. hAPP mutant live neurons and mouse brains exhibited BACE1 accumulation within the altered late endocytic organelles and defective lysosomal targeting due to reduced Snapin-dynein coupling. Deleting snapin or disrupting Snapin-dynein coupling reduces BACE1 transport to lysosomes for degradation, thus enhancing APP processing. Overexpressing Snapin in hAPP neurons reduces β site cleavage of APP by enhancing BACE1 turnover. Altogether, our study provides mechanistic insights into the complex regulation of BACE1 level and activity and turnover through retrograde transport, thus controlling Aβ generation in neurons.

Disclosures: Q. Cai: None. **X. Ye:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 791.24/E11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: N-terminally truncated beta-amyloid peptides are generated by alternative beta-secretase cleavage

Authors: *H. CYNIS[1], D. SCHLENZIG[1], U. ZEITSCHEL[2], M. HARTLAGE-RUEBSAMEN[2], S. ROSSNER[2], S. SCHILLING[1], H.-U. DEMUTH[1];
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Abstract: The accumulation of beta-amyloid (Abeta) peptides is causal for the development of Alzheimer's disease (AD). Abeta is liberated by sequential cleavage of beta- and gamma-secretase and BACE I was identified as major beta-secretase mediating the N-terminal step of Abeta generation. N-terminally truncated and pyroglutamate-modified (pGlu-3) Abeta peptides are an emerging species in AD research showing an exceptionally high aggregation propensity and toxicity. Since catalysis by BACE I preferentially produces full-length Abeta peptides, the mechanism of generating N-terminally truncated Abeta peptides could lead to novel drug targets for AD treatment. Consequently, we studied pathways leading to the generation of such truncated Abeta peptides. We applied vectors containing APP (wt) or the KM595/596NL "Swedish" mutation (APP (sw)) in different cellular models including transient overexpression in different permanent cell lines and in fibroblasts generated from BACE I/II knockout mice. Abeta formation was also studied in primary neurons from APP (wt) and APP (sw)-overexpressing mouse lines. The different Abeta species were dissected by highly specific ELISAs discriminating between N-terminal Abeta variants. Results were verified by siRNA and Western Blot experiments as well as co-expression of APP (wt) with putative alternative beta-secretases. Major outcome of the study is that a significant amount of Abeta is generated by BACE I-independent cleavage in the cellular models, including primary neurons. A monitoring mutation in APP (E599Q) revealed that especially pGlu-3 Abeta is liberated by a BACE I-independent mechanism. Furthermore, classical BACE I inhibition is not sufficient to block the generation of pGlu-3 Abeta peptides. The results have implications for further drug development strategies aiming at reduction of pGlu-modified Abeta species.

Disclosures: H. Cynis: None. **D. Schlenzig:** None. **U. Zeitschel:** None. **M. Hartlage-Ruebsamen:** None. **S. Rossner:** None. **S. Schilling:** None. **H. Demuth:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG030142

NIH F32AG033445

NIH 5T32AG00026

NIH DK042394

NIH DK088227

NIH HL052173

Title: Aβ-induced BACE1 elevation in primary neurons and in 5XFAD mouse model of Alzheimer's disease is not dependent on eIF2α phosphorylation

Authors: *K. R. SADLEIR[1], W. A. EIMER[2], R. J. KAUFMAN[3], P. OSTEN[4], R. VASSAR[1]; [1]Cell and Mol. Biol., Northwestern Univ., CHICAGO, IL; [2]Dept. of Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [3]Sanford Burnham Med. Res. Inst., La Jolla, CA; [4]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The β-Secretase, β-site APP Cleaving Enzyme1 (BACE1) is the enzyme that initiates production of the β-amyloid (Aβ) peptide involved in Alzheimer's disease (AD). Reduction of BACE1 levels inhibits Aβ generation and amyloid plaque formation. Modest overexpression of BACE1 in transgenic mice increases amyloid, demonstrating that Aβ production and amyloid pathology can be modulated by BACE1 levels. BACE1 protein and activity are elevated in the brains of AD patients and mouse models of AD, accumulating specifically in dystrophic neurites around plaques, suggesting that Aβ itself causes increased BACE1. Blocking this increase in BACE1 could be therapeutically useful in slowing or preventing AD without affecting baseline levels of BACE1, which has other substrates, but first we must understand what causes the BACE1 accumulation around plaques. Previous work indicated the increased phosphorylation of the eukaryotic initiation factor subunit alpha (eIF2α) led to increased translation of BACE1 and increased Aβ generation during energy deprivation. BACE1 and eIF2α phosphorylation were elevated and correlated in brains of AD patients, and in the 5XFAD mouse model of AD. We hypothesized that increased eIF2α phosphorylation led to the Aβ-induced BACE1 increase we observe in cultured neurons and in dystrophic neurites near plaques. To test this hypothesis, we used three complementary approaches 1) dephosphorylation of eIF2α by transduction of adeno-associated virus expressing a constitutively active form of GADD34, a subunit of protein phosphatase 1c 2) a non-phosphorylatable allele, S51A, of eIF2α and 3) a BACE1-YFP transgene lacking the 5'UTR required for regulation by eIF2α. Although the constitutive active GADD34 reduced eIF2α phosphorylation by 90% in primary neurons and the brains of 5XFAD mice, there was no decrease in Aβ-induced BACE1 elevation, or BACE1 accumulation around plaques. Similarly, neurons homozygous for eIF2α S51A allele had no eIF2α phosphorylation but still showed robust BACE1 elevation upon Aβ42 exposure. 5XFAD mice heterozygous for S51A had 40% less eIF2α phosphorylation and a robust BACE1 increase. Finally we observed 125% increase in BACE1-YFP lacking the 5'UTR in 5XFAD mice compared to controls, and the BACE1-YFP protein accumulated around plaques in the same manner as endogenous BACE1. We conclude that the amyloid-associated increase in BACE1 level is not caused by translational de-repression via eIF2α phosphorylation, but appears to involve a post-translational

mechanism. These definitive genetic results resolve confusion over the role of eIF2α phosphorylation in Aβ-dependent BACE1 elevation reported in other studies.

Disclosures: **K.R. Sadleir:** None. **W.A. Eimer:** None. **R.J. Kaufman:** None. **P. Osten:** None. **R. Vassar:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 791.26/E13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS065183

Title: Par3 limits APP and BACE1 convergence by directing their trafficking to two distinct pathways

Authors: M. SUN, *H. ZHANG;
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Abstract: Alzheimer's disease (AD) is the most common form of neurodegenerative diseases affecting the elderly. It is characterized by extracellular plaques made up of β-amyloid peptides (Aβ), which is derived from the transmembrane protein APP through cleavage by β- and γ-secretases. Previous studies suggest the intracellular trafficking properties of APP and its secretases determine how APP is processed, with amyloidogenic processing occurring mostly in endocytic compartments and non-amyloidogenic processing occurring on the cell surface. However, how the trafficking properties of APP and its secretases are regulated is still not well understood. The Par polarity complex is emerging as a key regulator of vesicle trafficking. This complex consists of Par3, Par6 and atypical PKC (aPKC), which are conserved proteins that regulate cell polarization from worms to mammals. Increasing evidence suggests that the Par complex promotes polarity establishment in part by regulating the asymmetric trafficking of cellular components. Here we show that loss of the polarity protein Par3 is associated with Alzheimer's disease pathogenesis. Knockdown of Par3 promotes amyloidogenic APP processing and increases Aβ generation, while overexpression of Par3 promotes non-amyloidogenic APP processing. We further show that Par3 regulates APP trafficking by promoting its targeting to the recycling pathway, while knockdown of Par3 leads to lysosomal targeting of APP. Unexpectedly, we found that Par3 regulates the retrograde trafficking of the β-secretase BACE1.

However, loss of Par3 does not seem to cause a global disruption of vesicular trafficking. Rather, Par3 regulates APP and BACE1 trafficking through two distinct mechanisms. Taken together, our studies reveal a novel role for Par3 in AD pathogenesis through a two-pronged mechanism that involves regulation of APP and BACE1 trafficking.

Disclosures: **M. Sun:** None. **H. Zhang:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P50AG08702

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Alzheimer's Association

BrightFocus Foundation

Title: A cell-based high throughput sAPPβ screen identifies plasma membrane monoamine transporter as a regulator of BACE1

Authors: ***L. B. MCINTIRE**[1], J. C. HWANG[1], G. GANGLI[2], A. RINDERSPACHER[2], S.-X. DENG[2], S. F. LICHTENTHALER[3], K. I. SEYB[4], M. A. GLICKSMAN[4], D. W. LANDRY[2], T.-W. KIM[1];
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Abstract: The β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) is the rate-limiting enzyme mediating the first proteolytic step leading to production and deposition of amyloid-β peptide (Aβ). BACE1 is a major drug target for the treatment of Alzheimer's disease (AD) and clinical development of BACE1 inhibitors is currently being intensely pursued. While

genetic and pathological evidence support the critical role for BACE1 in pathophysiology of AD, the mechanisms regulating the cellular activity of BACE1 require further study to facilitate therapeutic development. To this end, we employed a chemical genetics approach to identify chemical probes targeting novel pathways which effect BACE1 cleavage of APP and subsequent Aβ production. We developed a cell-based assay that directly monitors BACE1-mediated cleavage of APP in intact **neuronally-derived SH-SY5Y cells** based on antibody-mediated capture of the BACE1-derived secreted APP ectodomain (sAPPβ) fused to a secreted alkaline phosphatase (SEAP) reporter. By screening small molecule libraries using this cell-based system, we identified compounds that can inhibit sAPPβ secretion through mechanisms independent of direct enzymatic inhibition of BACE1. One hit compound was a tetrahydropyrrole derivative of GBR12909, a dopamine reuptake inhibitor harboring a piperazine ring. Subsequently, analogs of GBR12909 were synthesized (C-series compounds, C2) for enhanced potency. C2 treatment led to reduced Aβ levels in primary neurons as well as in brain of a mouse model of AD. Based on homology with GBR compounds, candidate cellular targets for the Aβ-lowering activity of C2 are classical and atypical monoamine transporters. Pharmacological characterization using antagonists against monoamine transporters revealed a putative cellular target for C2 as one of the atypical monoamine transporters, plasma membrane monoamine transporter (PMAT). We found that both pharmacological inhibition and genetic suppression of PMAT expression reduced secretion of sAPPβ and Aβ in neurons. Current work is in progress to further validate PMAT as a novel cellular target in AD.

Disclosures: L.B. McIntire: None. **G. Gangli:** None. **A. Rinderspacher:** None. **S. Deng:** None. **S.F. Lichtenthaler:** None. **K.I. Seyb:** None. **M.A. Glicksman:** None. **D.W. Landry:** None. **T. Kim:** None. **J.C. Hwang:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 791.28/E15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI

Title: Endosomal recycling of BACE1 regulated by BAR domain proteins impacts on amyloid-β production

Authors: *T. TOMITA[1], T. MIYAGAWA[2], T. SASAKI[1], M. FUKUSHIMA[4], I. EBINUMA[1], R. OJIMA[1], Y. MOROHASHI[1], S. TSUJI[2], T. IWATSUBO[3];
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Abstract: Recent GWAS studies revealed that variants at BIN1 locus are associated with the onset of Alzheimer disease that is characterized by massive deposition of amyloid-β proteins (Aβ). Bin1 is a membrane trafficking-related adapter protein that contain N-terminal BAR and C-terminal SH3 domains. BAR domains recognize specific phospholipids and sculpt membranes to generate protrusions or invaginations. However, little is known how Bin1 as well as membrane dynamics contribute to the pathogenesis of the Alzheimer disease. To understand this issue, we examined the Bin1 function on the levels of secreted Aβ as well as the trafficking of secretases. Ablation of Bin1 expression in mouse primary neurons and non-neuronal cells resulted in the increased production of Aβ and sAPPβ. In addition, protein level of BACE1, which corresponds to the β-secretase, was increased without affecting its mRNA levels. Imaging analysis revealed that trafficking of BACE1 protein from early endosomes to late endosome/lysosome was impaired by loss-of-Bin1 function, suggesting that Bin1 promotes BACE1 degradation by lysosomal targeting. In addition to Bin1, we identified that the other BAR domain proteins as well as phospholipid enzymes impacted on the BACE1 activity and secreted Aβ levels. These data suggest that Aβ production is modulated by the endosomal trafficking of BACE1, which is regulated by BAR domain proteins and specific phospholipids.

Disclosures: T. Tomita: None. **T. Miyagawa:** None. **T. Sasaki:** None. **M. Fukushima:** None. **I. Ebinuma:** None. **R. Ojima:** None. **Y. Morohashi:** None. **S. Tsuji:** None. **T. Iwatsubo:** None.

Poster

791. APP Cleavage and Metabolism Processes

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG025952

NIH R01AG033016

Title: A highly novel role for the clathrin adaptor protein gga3 in anxiety and depression circuitry in the mouse brain

Authors: *K. R. WALKER[1], T. DEEB[2], J. L. ROYAL[1], S. LOMOIO[1], M. E. TOLMAN[3], S. J. MOSS[2], G. TESCO[1];
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Abstract: Golgi-localized, gamma adaptin ear-containing, ARF binding proteins (GGA's) are a family of monomeric clathrin adaptors which facilitate the trafficking of membrane bound proteins at the TGN and endosomes via clathrin coated vesicles. The neuronal family member, GGA3, plays a key role in trafficking β-secretase (BACE1) from early endosomes to lysosomes for degradation. Additionally, we have demonstrated that caspase-mediated depletion of GGA3 plays an important role in mediating BACE1 elevation and Aβ production following acute brain injury. We have generated GGA3null mice that are healthy, viable and fertile and which demonstrate BACE1 elevations of approximately 30% in the brain. To further understand the effect of GGA3 depletion in the mouse brain we performed extensive behavioral phenotyping of 8-10wk old GGA3null mice and their wild-type littermates. GGA3null mice displayed novelty-induced hyperactivity and increased exploratory behavior as evidenced by their increased exploration of an open field arena ($p<0.0001$); increased arm entries in y-maze ($p=0.0044$) and increased visits to objects in the novel object recognition task ($p=0.0020$). Additionally, GGA3null mice displayed a robust anxiolytic phenotype compared to their WT littermates in three different anxiety paradigms (Open Field Testing, Elevated Plus Maze and Light/Dark transition). GGA3null mice spent significantly more time exploring the center of the open field arena ($p= 0.0321$). While in the elevated plus maze GGA3null mice showed a robust preference for the open arm of the maze (entries: $p=0.0017$) and spent significantly more time in the open arm ($p=0.0002$). Additionally, GGA3null mice showed a preference for the light chamber entering it more often ($p=0.0029$) and spending more time in the light chamber ($p=0.0473$) than their WT littermates in the light/dark transition test. In addition to their robust anxiolytic phenotype GGA3 null mice also displayed a robust anti-depressive like phenotype when tested by the Porsolt swim test (Immobility time: $p<0.0001$). In keeping with robust anxiolytic, anti-depressive phenotype and increased motivational behaviors, GGA3null mice demonstrate elevated dopamine levels in the hippocampus. The effect of GGA3 depletion is specific for emotional and motivational behavior as no differences were observed in spatial working memory, recognition memory, spatial reference memory or fear associated memory between GGA3null and WT mice. Further work is ongoing to clarify the specific circuitry by which GGA3 depletion mediates the observed novelty induced hyperactivity, robust exploratory, anxiolytic and anti-depressive behaviors witnessed.

Disclosures: K.R. walker: None. **T. Deeb:** A. Employment/Salary (full or part-time):; Tufts - AstraZeneca Lab for Translational Research. **J.L. Royal:** None. **S. Lomoio:** None. **M.E.**

Tolman: None. **S.J. Moss:** F. Consulting Fees (e.g., advisory boards); Tufts-AstraZeneca Lab for Translational Research. **G. Tesco:** None.

Poster

791. APP Cleavage and Metabolism Processes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 791.30/F2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant P01 HD29587

NIH grant P01 ES016738

NIH grant P30 NS076411

Title: S-Nitrosylation of insulin degrading enzyme in Alzheimer's disease

Authors: *M. W. AKHTAR, S. SANZ-BLASCO, N. DOLATABADI, K. CHON, J. PARKER, R. AMBASUDHAN, W. SOUSSOU, S. R. MCKERCHER, T. NAKAMURA, S. A. LIPTON; Sanford-Burnham Ctr. for Neurosci. and Aging Res., Sanford-Burnham Med. Res. Inst., La Jolla, CA

Abstract: Alzheimer's disease (AD) is the most common form of dementia in the elderly. Cognitive dysfunction in AD is best correlated with synaptic injury, especially in the hippocampus and neocortex. AD is characterized by the presence of extracellular amyloid plaques rich in amyloid-beta (Aβ) peptides and intracellular neurofibrillary tangles of hyperphosphorylated Tau protein. Work over the last decade suggests that in most cases oligomeric Aβ is the toxic species to neurons rather than the plaques themselves. During AD onset, Aβ levels increase, apparently due to excessive production as well as decreased catabolism. Insulin degrading enzyme (IDE), a zinc-metalloproteinase, is one of the enzymes that degrades Aβ and plays an important role in maintaining the level of Aβ in the brain. Here we report that IDE can be S-nitrosylated upon exposure to the nitric oxide (NO) donor S-nitrosocysteine (SNOC) in cortical neuronal cultures, which leads to inhibition of its Aβ-degrading activity. Moreover, we found that exposure of rat cortical cultures or hippocampal slices to oligomeric Aβ, known to cause nitrosative stress via increased NO production, leads to an increase in S-nitrosylation of IDE (forming SNO-IDE). Furthermore, we observed an increase

in SNO-IDE in human AD postmortem brains compared to age-matched controls. Taken together, our results suggest that S-nitrosylation of IDE compromises its activity in AD brain.

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Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.01/F3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BMBF Grant KMU-innovativ-7

SFB944 Grant Z-project

Title: Single molecule tracking of tau reveals fast kiss-and-hop interaction with axonal microtubules in living neurons

Authors: D. JANNING, M. IGAEV, F. SÜNDERMANN, J. BRÜHMANN, O. BEUTEL, J. J. HEINISCH, L. BAKOTA, J. PIEHLER, W. JUNGE, *R. BRANDT;
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Abstract: The neuronal microtubule-associated phosphoprotein tau regulates microtubule (MT) dynamics and is involved in neurodegenerative diseases collectively called tauopathies. It is believed that tau molecules exhibit long lived interactions with axonal MTs thereby stabilizing them. It is thought that the detachment and attachment of tau are regulated by phosphorylation/dephosphorylation cycles, and that increased phosphorylation during disease leads to the detachment of tau from MTs causing their breakdown. Here we address the apparent paradox that tau regulates microtubule dynamics but does not interfere with microtubule-dependent axonal transport. By fast single molecule tracking (SMT) of HaloTag® tagged tau in living neurons we found that tau dwells on one microtubule for an unexpectedly short time of

~40 ms before it hops to the next one. The dwell time is 100-fold shorter than previously reported. We observed that even a tau construct mimicking disease-like hyperphosphorylation, pseudohyperphosphorylated (PHP) tau (Tackenberg C, and Brandt R (2009) J. Neurosci. 29 :14439-14450), still interacted with MTs although with a moderately reduced dwell time. Step-size distribution analysis revealed characteristic jump sizes that correlate well with the MT arrangement in neurites of living cells. Furthermore, we employed FDAP measurements to show that, despite tau´s rapid dynamics, it regulates the tubulin-MT balance in neurites of living cells. The rapid kiss-and-hop interaction between tau and MT-filaments explains why tau, although binding to MTs, does not interfere with the axonal transport. In addition, the data indicate that tau's dwell time on MTs is still sufficiently long to influence the life time of a tubulin subunit in a GTP cap.

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Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.02/F4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of Metformin on tau pathology in the P301S tau transgenic mouse

Authors: E. BARINI, O. ANTICO, *L. GASPARINI;
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Abstract: The accumulation of intracellular inclusions made of microtubule-associated tau protein is a defining pathological hallmark of Alzheimer disease (AD). The progression of tau pathology in the AD brain correlates with cognitive impairment and appears to be influenced by several risk factors, including brain insulin resistance and the use of antidiabetic drugs such as metformin. Indeed, there is evidence that metformin enhances protein phosphatase 2A (PP2A) activity and dephosphorylates tau in cultured mouse primary cortical neurons, suggesting a potential effect on tau pathology progression. This prompted us to investigate the potential use of metformin as a therapy for tauopathy in the P301S tau transgenic (P301S-tau) transgenic mouse model that develops widespread cerebral tau inclusions, progressive motor impairment and severe paraparesis at 5 months of age. Metformin was administered in the drinking water

(2mg/ml) to P301S-tau transgenic and C57BL6/J wild type (WT) mice starting from 4 weeks of age for 4 months and weight, glycemia, food and water intake were monitored weekly. Chronic administration of metformin does not alter weight, glycemia, water and food intake in both WT and P301S-tau mice. At the tail suspension test, the hindlimb extension reflex is impaired in P301S-tau, but not WT, mice and significantly worsens when transgenic mice are treated with metformin. Cerebral tau phosphorylation and tau inclusions were evaluated by biochemical and immunohistochemical analyses. Initial findings indicate that, in P301S-tau mice, chronic treatment with metformin significantly increases the number of β-sheet filamentous tau inclusions in the hippocampus. We hypothesize that metformin directly acts on mutant tau aggregation. In fact, in *in vitro* aggregation assays, metformin promotes the aggregation of recombinant human tau bearing the P301S mutation. We also found that, in P301S-tau mice, chronic treatment with metformin increases the levels of phosphorylated tau in the brainstem and affects the expression levels of PP2A, possibly contributing to tau phosphorylation state. These results indicate that chronic use metformin may favor the development of tau pathology and suggest that metformin should be used with caution in elderly patients with dementia.

Disclosures: E. Barini: None. **L. Gasparini:** None. **O. Antico:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.03/F5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Merit Award

Title: Mitigation of tau pathology with CD40 receptor deficiency in a mouse model of neurodegeneration

Authors: *M. TWEED[1,2,3], Z. ZAKIROVA[1,2,3], D. PARIS[1,2,3], F. CRAWFORD[1,2,3], M. MULLAN[1], G. AIT-GHEZALA[1,2,3];
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Abstract: Alzheimer’s disease (AD) is characterized by three main pathologies: ß-amyloid (Aß) deposition, tauopathy and chronic inflammation. There appears to be an important feed-forward mechanism of the inflammatory mediators on the pathology of AD, even though the primary

trigger for the inflammatory events is assumed to be accumulation of Aβ peptides or the downstream consequences thereof. The tauopathy of AD presents itself as accumulations of fibrillar microtubule associated protein tau (MAPT) that forms within neurons. The consequent neurofibrillary degeneration closely correlates with loss of cognition. The CD40-CD40L dyad is activated upon pro-inflammatory stimulation, which further enhances the inflammatory response, and we have demonstrated a significant constitutive CNS role of CD40-CD40L interaction in AD pathogenesis. For instance, in a transgenic mouse model of AD, we have shown that CD40/CD40L interaction is required to observe the full gamut of pathology. Furthermore, we have shown that CD40L or CD40 deficiency reduces Aß deposition and dystrophic neurites in Tg2576 mouse brain, as well as, cdk5 expression resulting in decreased tau phosphorylation. Overall, these data suggest that CD40-CD40L interaction has an effect on tau phosphorylation, however, we have never investigated the effect of CD40 receptor deficiency on tau pathology in a pure model of tauopathy. In our current study to investigate the effect of CD40R deficiency on tau pathology in a mouse model of tauopathy, we have crossed homozygous CD40R knockout mice with the PS19 transgenic mouse model that expresses the P301S FTD-associated mutation. The PS19 model exhibits robust tau pathology and develops neurodegeneration leading to motor neuron dysfunction and loss of locomotor coordination starting at 4 months of age. Motor function behavioral data from aged (10 month old) CD40 receptor deficient X P301S mutant tau expressing animals show improvement. Preliminary histological data demonstrates a decrease of AT8 positive tau after genetic reduction of CD40 receptor expression in the crossed animals. Ongoing pathological and biochemical analysis will be conducted to further clarify the effects of CD40 receptor deficiency on the mitigation of tau pathology in-vivo. Furthermore, a correlation in changes in the inflammatory markers to changes in tau pathology will be presented. This work demonstrates a link between the neuroinflammation associated with AD and the progression of tauopathies.

Disclosures: **M. Tweed:** None. **Z. Zakirova:** None. **D. Paris:** None. **F. Crawford:** None. **M. Mullan:** None. **G. Ait-Ghezala:** None.

Poster

792. Alzheimer's Disease: Tauopathy

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Program#/Poster#: 792.04/F6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CHIR

Alzheimer Society of Canada

Title: Increased retinal tau is neurotoxic in experimental glaucoma

Authors: ***M. CHIASSEU**, L. DESTROISMAISONS, C. VANDE VELDE, N. LECLERC, A. DI POLO;
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Abstract: Increased retinal tau is neurotoxic in experimental glaucoma Marius Chiasseu, Laurie Destroismaisons, Christine Vande Velde, Nicole Leclerc & Adriana Di Polo Retinal ganglion cell (RGC) death is the primary cause of vision loss in most optic neuropathies, including glaucoma. There is a high occurrence of glaucoma amongst Alzheimer's disease (AD) patients. Indeed, preferential loss of large diameter RGCs has been documented and may account for the impaired contrast sensitivity and motion perception experienced by individuals with AD. Moreover, AD and glaucoma share a number of pathological and clinical features such as the presence of amyloid beta plaques and tau aggregates. In this study, we examined changes in retinal tau expression and the effect of tau on RGC survival using an experimental rat glaucoma model. Ocular hypertension (OHT) was induced in rats by injection of hypertonic saline solution into an episcleral vein. Retinal tau protein expression and phosphorylation were assessed by western blot analysis using a panel of antibodies against phospho-specific tau epitopes as well as total tau. The cellular localization of tau was investigated by retinal and optic nerve immunohistochemistry using cell-specific markers. Our data demonstrate that ocular hypertension leads to rapid accumulation of retinal tau characterized by a complex phosphorylation profile. Both hyperphosphorylated and hypophosphorylated forms of tau were detected in glaucomatous retinas compared to intact controls. We show that tau accumulates within RGCs, primarily in their somato-dendritic compartment, soon after induction of ocular hypertension. Importantly, tau knockdown using short interfering RNA (siRNA) led to substantial protection of RGC soma and axons from glaucomatous damage compared to retinas treated with a control siRNA. We conclude that ocular hypertension glaucoma displays features of a tauopathy including compartmental redistribution of tau, altered phosphorylation profile and neurotoxicity.

Disclosures: M. Chiasseu: None. **L. Destroismaisons:** None. **C. Vande Velde:** None. **N. Leclerc:** None. **A. Di Polo:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.05/F7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: p62/SQSTM1 mediates tau protein degradation through proteasome system

Authors: J. IM, *S. PARK, Y.-J. KIM;
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Abstract: The accumulations of abnormal tau proteins, hyperphosphorylated, insoluble and filamentous tau proteins, lead to various neurodegenerative diseases including Alzheimer's disease. Therefore, enhancing tau degradation and decreasing its aggregation are hot research subjects for development of efficient treatments. Previously, we identified the diminished p62/SQSTM1 expression was linked to high accumulations of abnormal tau proteins in the cortex of type 2 diabetic obese rats, which suggest the critical role of p62/SQSTM1 in clearing tau proteins. To determine the molecular mechanisms of p62/SQSTM1 mediated tau degradation we started this study. We cotransfected various p62/SQSTM1 constructs with human tau constructs to cultured cell lines or primary neurons. The expression of tau proteins, both total and phosphorylated form (Thr212, Thr231, and Ser199) were decreased in dose-dependent manner with p62/SQSTM1. Also we treated cotransfected cells the proteasome inhibitor MG132, or the lysosome inhibitor leupeptin, or the autophagy inhibitor 3-Methyladenine (3-MA) to understand the clearance mechanism. p62/SQSTM1 mediated tau degradation was inhibited by treatment of MG132, but not by leupeptin or 3-MA. To know the molecular mechanism of p62/SQSTM1 mediated tau clearance various mutation constructs of p62/SQSTM1 were developed. The direct interaction between p62/SQSTM1 and Tau proteins were identified at specified domains. Our results suggest that p62/SQSTM1 regulates the degradation of tau proteins through proteasome pathway through physical interaction between two molecules at specific region.

Disclosures: J. Im: None. **S. Park:** None. **Y. Kim:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.06/F8

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Acetylcholine release in the brains of tau-transgenic mice

Authors: *C. STEIN[1], J. KLEIN[1,2], M. HOLZER[2];
[1]Pharmacol., Goethe Univ., Frankfurt Am Main, Germany; [2]Paul Flechsig Inst. of Brain Res., Univ. of Leipzig, Leipzig, Germany

Abstract: "Tauopathies" refer to a group of neurodegenerative diseases such as Alzheimer's disease (AD) or frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) which show inclusions comprised of microtubule- associated protein tau (also known as MAPT) in the brain. The occurrence and density of fibrillar tau inclusions are related to cognitive decline explaining the current interest in murine transgenic tau-models. In the present study, we applied the microdialysis technique to monitor energy metabolism and central cholinergic function in 4 and 10 month old tau-transgenic P301L and control mice. On day 1 after probe implantation, we collected basal samples and samples during behavioral stimulation in a novel environment ("open field") for 90 minutes. High performance liquid chromatography was used to measure acetylcholine (ACh) and choline. Basal levels of ACh in 4 month old control mice were 37.8 ± 3.3 fmol/5µl S.D. (N=10). Tau mice of the same age had significantly higher basal ACh concentrations in hippocampal samples (68.5 ± 12.8 fmol/5µl; N=10) ($p<0.05$). Time courses of ACh release revealed an increase to 99.1 ± 4.0 fmol/5µl in control mice (262% of basal values) and to 185 ± 15.8 fmol/5µl (270%) in tau-transgenic mice during "open field" exposure. On day 2, microdialysis probes were perfused with aCSF containing 1µM of scopolamine to stimulate acetylcholine release pharmacologically. ACh release increased to 145 ± 9.9 fmol/5µl (384% of basal values) in control mice and 345 ± 34.4 fmol/5µl (504%) in tau-transgenic mice during scopolamine stimulation. Aged, 10 months old control mice had basal ACh levels of 123 ± 11.8 fmol/5µl (N=9) which were significantly higher than the values measured in young controls. ACh levels increased to 321.2 ± 12.8 fmol/µl (261% of basal level) during behavioral stimulation on day 1. In aged P301L mice, time courses of ACh release were very similar to those measured in young P301L mice (data not shown). After sacrifice on day 3, mouse brains were used to measure acetylcholinesterase (AChE) activity which was identical in all groups. In conclusion, our data demonstrate that young tau-transgenic mice had higher ACh levels than control mice whereas ACh levels increased with age in control mice. Both groups of mice responded with a large ACh release to behavioral and pharmacologic stimulation. The reasons for this surprising finding are presently under investigation.

Disclosures: C. Stein: None. **J. Klein:** None. **M. Holzer:** None.

Poster

792. Alzheimer's Disease: Tauopathy

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Program#/Poster#: 792.07/F9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: France Alzheimer 2013

Title: Heparan sulfate sulfotransferases and pathologic phosphorylation of tau in Alzheimer's disease-related tau pathology

Authors: J. E. SEPULVEDA DIAZ[1], M. O. OUIDJA[2], S. CHANTEPIE[2], M. B. HUYNH[2], S. B. SOCIAS[1], J. VILLARES[3], *R. RAISMAN-VOZARI[1], D. PAPY-GARCIA[2]; [1]INSERM U1127/CNRS UMR 7225, Paris, France; [2]Lab. Croissance, Réparation et Régénération Tissulaires (CRRET), Univ. Paris Est-Créteil, Créteil, France; [3]Aging and Neurodegenerative Dis. Brain Bank Investigation Lab., Univ. Federal de São Paulo, São Paulo, Brazil

Abstract: The accumulation of abnormally phosphorylated tau is a central event in Alzheimer's disease. Although *in vivo* the critical pathologic phosphorylation of tau is assumed to be mediated by the same kinases that phosphorylate tau in normal brain, *in vitro*, these kinases abnormally phosphorylate tau at disease-specific sites only if the enzymatic reaction takes place in the presence of certain polyanions such as heparin, a highly sulfated analogue of the complex heparan sulfates family of glycosaminoglycans. Currently, it is unknown whether this complex family of molecules, which structural and functional diversity in brain results from the action of several heparan sulfate sulfotransferases, is involved in the cellular mechanism leading to tau pathology. Recently, we have observed that specific heparan sulfate sulfotransferases are increased in Alzheimer's disease brain and that the products of these enzymes are critically involved in the biochemical and cellular mechanisms leading to the pathologic phosphorylation of tau. Inhibiting the expression of one of these enzymes strongly attenuates the pathologic phosphorylation of tau induced in cells by oxidative stress or by expression of hTauP301L, indicating an essential role of specific heparan sulfates domains in the mechanism leading to the abnormal phosphorylation of tau at Alzheimer's disease characteristic sites. We propose a novel hypothesis for the better comprehension of the pathophysiological mechanisms leading to Alzheimer's disease related tauopathy, with groundbreaking therapeutic outcomes.

Disclosures: J.E. Sepulveda Diaz: None. **M.O. Ouidja:** None. **S. Chantepie:** None. **M.B. Huynh:** None. **S.B. Socias:** None. **J. Villares:** None. **R. Raisman-Vozari:** None. **D. Papy-Garcia:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

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Program#/Poster#: 792.08/F10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RO1 047041181103

Title: Regulation of Alzheimer's Disease tau pathology by membrane bound and soluble fractalkine

Authors: *S. M. BEMILLER[1], C. MILLER[2], G. WILSON[4], G. XU[3], S. CRISH[4], B. T. LAMB[2];
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Abstract: Hyperphosphorylation and aggregation of microtubule associated protein tau (MAPT) within neurons is an invariant feature of multiple neurodegenerative diseases collectively termed tauopathies. While recent evidence suggests that alterations in innate immune pathways within the brain directly influence the development of MAPT pathology, the exact mechanisms linking inflammatory pathways to intracellular MAPT pathology remains to be determined. One key innate immune pathway within the brain involves signaling between the neuronally-derived chemokine, CX3CL1 and the cognate microglial receptor, CX3CR1. Notably, CX3CL1 exists both as a membrane bound glycoprotein, as well as a soluble form generated by cleavage with the ADAM10/17 metalloproteases. Previous studies from the Lamb laboratory demonstrated that chemical (lipopolysaccharide/LPS) or genetic (deletion of CX3CR1) alterations in innate immunity accelerated MAPT pathology and impairment of working memory in a mouse model of tauopathy (hTau) at six months of age. Additional studies suggested that the mechanism linking micr5oglia to intraneuronal MAPT pathology occurred via the neuronal interleukin-1 receptor (IL1R) and p38 mitogen activated protein kinase (p38 MAPK) pathway. The current study examined the role of both the membrane bound as well as the soluble isoforms of CX3CL1 signaling in the development of MAPT pathology in the hTau model. Through both a CX3CL1 deficient mouse and a mouse expressing only soluble CX3CL1 (sCX3CL1), we demonstrated that; 1) CX3CL1 deficiency phenocopied the effects of CX3CR1 deficiency in promoting both microglial activation and MAPT pathology and 2) sCX3CL1 was capable of rescuing the effects on both microglial activation and MAPT pathology. Taken together these findings suggest that sCX3CL1 both ameliorates MAPT pathology and modulates microglial activation states in a mouse model of tauopathy.

Disclosures: **S.M. Bemiller:** None. **C. Miller:** None. **G. Wilson:** None. **G. Xu:** None. **S. Crish:** None. **B.T. Lamb:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AP is supported by the Roche Postdoctoral Fellowship program

Title: Phosphorylation of Tau at S422 is related to autophagic vesicle accumulation in patients with corticobasal degeneration

Authors: *A. PIRAS[1], L. COLLIN[2], F. GRUENINGER[2], C. GRAFF[1,3], A. RÖNNBÄCK[1]; [1]NVS, Ctr. for Alzheimer Research, Div. for Neurogeriatrics, Karolinska Institutet, Huddinge, Sweden; [2]F. Hoffmann-La Roche AG, NORD DTA, Pharmaceut. Res. and Early Develop., Basel, Switzerland; [3]Dept Geriatric Medicine, Genet. Unit, Karolinska Univ. Hosp., Stockholm, Sweden

Abstract: Accumulation of misfolded proteins within neurons and glia cells is a common feature of many neurodegenerative diseases. Abnormal aggregation and accumulation of the microtubule-associated protein tau underlies the neuropathology of tauopathies, such as corticobasal degeneration (CBD). It has been proposed that hyperphosphorylation of tau causes conformational changes in tau leading to an increased aggregation propensity, as well as a reducing its affinity for microtubules. Phosphorylation at the serine 422 (pS422) epitope occurs only in pathological forms of tau, and pS422 tau has been found in neurofibrillary tangles, neuropil threads and dystrophic neurites in Alzheimer's Disease (AD), as well as in Pick bodies in Pick's disease and tufted astrocytes in progressive supranuclear palsy (PSP). One mechanism that could contribute to the accumulation of aggregated proteins in the brain is dysfunction of macroautophagy (herein referred to as autophagy), a major lysosomal degradative pathway for cytoplasmic material in eukaryotic cells. Here, we hypothesized that phosphotau aggregates interfere with autophagic flux creating a vicious circle in which not only tau clearance is further impaired but also the turnover of other autophagy substrates, with toxic consequences for the cell. To explore this possibility, we analysed autophagy markers by immunohistochemistry in human post-mortem brain samples (frontal cortex) from neuropathologically confirmed CBD patients compared to control individuals (n=3/group). Interestingly, CBD patients displayed an increase in autophagic markers, such as LC3 and p62. Moreover, we assessed abnormalities in the autophagy system using *in situ* proximity ligation assay (PLA), a new technique to detect and quantify protein interactions, to study co-localization of phosphotau with autophagy vesicles (LC3). We observed clear colocalization between LC3 and phosphotau in brain tissues from

CBD patients but not in healthy controls. The increased levels of autophagic markers and co-localization between LC3 and phosphotau in human tissues from CBD patients suggested an accumulation of autophagosomes unable to degrade phosphotau, including pS422. Taken together, our results indicate a strong relationship between tau phosphorylation and autophagy vesicle accumulation in human tissues from CBD patients.

Disclosures: **A. Piras:** None. **L. Collin:** None. **F. Grueninger:** None. **C. Graff:** None. **A. Rönnbäck:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.10/F12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Appel Alzheimer's Disease Research Institute

Title: An important role for microglia in the phagocytosis and clearance of various soluble and insoluble tau species in Alzheimer’s disease

Authors: *W. LUO, W. LIU, X. HU, M. HANNA, A. CARAVACA, S. M. PAUL;
Brain and Mind Res. Institute, Helen and Robert Appel Alzheimer's disease re, Weill Cornell Med. Col., New York, NY

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by amyloid plaques and neurofibrillary tangles (NFTs). NFTs are composed of aggregated and phosphorylated forms of the microtubule-binding protein tau. Recent studies suggest that tau is “released” from neurons where it can “spread” from cell-to-cell and contribute to tau pathology in both AD and frontal temporal dementia (FTD). The exact mechanisms involved in tau metabolism and clearance in brain are still poorly understood. However, recent genome-wide association studies (GWAS) have identified several genes encoding proteins that regulate innate immunity as being strong genetic risk factors for late-onset AD (LOAD), suggesting an important role for innate immunity in AD pathogenesis. Microglia, the major component of the innate immune system in brain, play key roles in mediating the central nervous system (CNS) response to tissue injury and the clearance of cell debris. Numerous studies have shown that microglia contribute to brain Aβ/amyloid clearance. However, to our knowledge it is not known whether microglia play a similar role in the clearance of tau. To test this hypothesis, we initially

cultured primary postnatal murine microglia with sarkosyl-insoluble tau (SI-tau) isolated from AD brain. We now report that microglia from wild-type mice rapidly phagocytose/internalize and efficiently degrade SI-tau in a time-dependent manner. Confocal microscopy of AT8-positive tau aggregates revealed time-dependent phagocytosis of p-tau by microglia. Using an *ex vivo* assay, in which microglia are cultured with NFT-bearing frozen brain sections from either homozygous P301S mutant mice or from AD brain, we further demonstrated that microglia can rapidly degrade brain-derived soluble tau species (both p-tau and full length tau in the media) and eliminate NFTs (insoluble tau) from the brain sections themselves. Pre-treatment of microglia with the toll-like receptor (TLR) agonist LPS increased phagocytosis and degradation of both soluble and insoluble tau. These data identify an important role for microglia in the phagocytosis and degradation of various species of tau, including full length tau and various phosphorylated soluble and insoluble tau species. Our data suggest that impaired or senescent microglia-mediated tau "clearance" may contribute to the pathogenesis of AD and related tauopathies. Targeting microglia-mediated Aβ and tau clearance with drugs that increase clearance of both tau and Aβ may represent a novel therapeutic strategy for preventing (or reversing) both neuropathological hallmarks of AD.

Disclosures: **W. Luo:** None. **W. Liu:** None. **X. Hu:** None. **M. Hanna:** None. **A. Caravaca:** None. **S.M. Paul:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

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Program#/Poster#: 792.11/G1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2013R1A1A4A01005837

BK21+

Title: Development of novel GSK3β inhibitory peptide targeting tau hyperphosphorylation

Authors: ***H. CHUN**, E. LEE, Y. LEE, S. JI, J. KIM, K. LEE, J. LEE;
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Abstract: Tau hyperphosphorylation and accumulation in neurofibrillary tangles are closely associated with cognitive deficits in Alzheimer's disease (AD). Abnormal overexpression of

GSK3β has been implicated in tau hyperphosphorylation, thus many GSK3β inhibitors have been developed as drug candidates for AD. However, the potent GSK3β inhibitors are prone to show side effects because they can interfere with basal GSK3β function. We have recently reported that the PPPSPxS motifs in the Wnt coreceptor LRP6 are able to directly inhibit GSK3β when phosphorylated, an thus a novel GSK3β inhibitory peptide (GIP) that can be activated by Akt, was generated by combining the PPPSPxS motif and an Akt target sequence. GIP effectively blocked GSK3β-induced tau phosphorylation in hippocampal homogenate. In addition, the GIP fused with a cell permeable sequence attenuated Aβ-induced tau phosphorylation and cell death in human neuroblastoma cells. Furthermore, the designed GIP significantly reduced tau phosphorylation in the hippocampus of 3xTg-AD mice without affecting levels of Aβ plaques. In conclusion, the current findings provide a novel concept for the drug development targeting tau hyperphosphorylation in AD.

Disclosures: H. Chun: None. **E. Lee:** None. **Y. Lee:** None. **S. Ji:** None. **J. Kim:** None. **K. Lee:** None. **J. Lee:** None.

Poster

792. Alzheimer's Disease: Tauopathy

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.12/G2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Two stable SHSY-5Y cell lines over-expressing full length and truncated tau441 as screening tools for tauopathies

Authors: R. WRONSKI, N. TAUB, V. SCHIFFER, C. SCHWEINZER, *B. HUTTER-PAIER; QPS-Austria Gmbh, Grambach, Austria

Abstract: Background Tau proteins belong to the family of microtubule-associated proteins and play an important role in stabilizing the neuronal microtubules network. They are the major constituents of intraneuronal and glial fibrillar lesions described in Alzheimer's disease and numerous neurodegenerative disorders referred to as 'tauopathies', including progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, as well as the inherited frontotemporal dementia and parkinsonism linked to chromosome 17. Molecular analysis revealed that hyperphosphorylation might be the important event leading to Tau aggregation resulting in neurodegeneration and dementia. Development of new compounds capable of preventing tau hyperphosphorylation is an increasingly hot topic. Thus, reliable models are

needed that reflect tau hyperphosphorylation in human diseases. Methods For this purpose, we generated two stably transfected SHSY-5Y cell lines either over-expressing the longest human tau441 isoform comprising two disease related mutations (SH-SY5Y-TMHT441) or a truncated version of tau441 (SHSY-5Y-tTMHT441). Results Comparison of tau expression and phosphorylation levels in SHSY-5Y versus SHSY-5Y over-expressing cells confirmed relevance to human diseases. The phosphorylation pattern of tau can be reliably modulated by distinct kinase inhibitors targeting CDK, JNK or GSK3beta. Effects on tau phosphorylation (residues Thr231, Thr181, and Ser202) were determined by immunosorbent assays (Mesoscale Discovery). Conclusion These protein kinases are known to be involved in tau phosphorylation and are therefore reliable indicators for the suitability of these two cell lines as *in vitro* models for tauopathies.

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Poster

792. Alzheimer's Disease: Tauopathy

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Program#/Poster#: 792.13/G3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Appel Alzheimer's Disease Research Institute

Title: The anti-tau monoclonal antibody MC1 promotes microglia-mediated phagocytosis and degradation of pathological tau

Authors: *W. LIU[1], W. LUO[1], X. HU[1], N. POLTORATSKAIA[1], M. HANNA[1], A. CARAVACA[1], P. DAVIES[2,3], S. PAUL[1];
[1]Brain and Mind Res. Institute, Helen & Robert Appel Alzheimer's Dis. Inst., Weill Cornell Med. Coll, NEW YORK, NY; [2]Dept. of Pathology, Albert Einstein Col. of Med., New York, NY; [3]Litwin-Zucker Ctr. for Res. in Alzheimer's Dis., Feinstein Inst. for Med. Res., New York, NY

Abstract: Progressive tau pathology in brain correlates well with the age-dependent decline of cognitive function in patients with Alzheimer's disease (AD). Accumulated evidence suggests that the inter-synaptic spread and transmission of tau within and between vulnerable brain regions contributes to the progressive neurodegeneration that characterizes AD and related

tauopathies. Therefore, treatments that might reduce tau mis-folding, aggregation or spread may prevent or slow the progression of tau-dependent neurodegeneration. Recently, anti-tau immunotherapy has been shown to reduce tau pathology in various tauopathy mouse models. For example, passive immunization with the anti-tau antibody MC1 (a high affinity anti-tau antibody directed at a pathological conformational tau epitope), has been shown to significantly reduce tau pathology in several mouse tauopathy models (see Chai et al., 2011; d'Abramo et al., 2013). It has been hypothesized that these anti-tau antibodies reduce tau pathology by binding to extracellular tau and by blocking the spread and (or) seeding/aggregation of tau throughout the brain. However, it is also possible that anti-tau antibodies may promote phagocytosis of tau by microglia and clear extracellular tau via a Fc receptor-mediated microglial mechanism as has been shown previously for certain anti-Aβ antibodies which prevent the deposition and formation of brain Aβ/amyloid plaques (Bard et al., 2000). To test this possibility, we have established sensitive *in vitro* and *ex vivo* methods using sarkosyl-insoluble brain extracts and tissue sections from P301S mice and AD brain which contain abundant paired helical filaments (PHF-tau) to measure the phagocytosis and degradation of pathologic tau by primary mouse microglia. We have found that tau is rapidly degraded by mouse microglia (see companion abstract W. Luo et al) using these *in vitro* and *ex vivo* methods and several sensitive p-tau/tau ELISAs. We now report that the anti-tau monoclonal antibody MC1, but not an IgG control antibody, promotes microglia-mediated phagocytosis and degradation of various soluble and insoluble p-tau species. MC1 enhances microglia-mediated degradation of soluble and insoluble tau in a time-dependent manner. Our data suggest microglial-mediated phagocytosis may in part underlie the reduced tau pathology observed following passive immunization of tauopathy mice with certain anti-tau antibodies. We are currently testing this hypothesis *in vivo*, and also studying a variety of other anti-tau antibodies to determine whether they augment microglia-mediated tau phagocytosis and clearance.

Disclosures: **W. Liu:** None. **W. Luo:** None. **X. Hu:** None. **N. Poltoratskaia:** None. **M. Hanna:** None. **A. Caravaca:** None. **P. Davies:** None. **S. Paul:** None.

Poster

792. Alzheimer's Disease: Tauopathy

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cortice Biosciences

University of California, San Francisco

Title: Effects of TPI 287, a novel taxoid, on a transgenic mouse model of Alzheimer's disease

Authors: *E. B. DEFENSOR[1,2], G. FARMER[3], L. GAN[4,5], A. BOXER[5,6], M. SHAMLOO[1,2]; [1]Behavioral and Functional Neurosci. Lab., Stanford Univ., Palo Alto, CA; [2]Dept. of Neurosurg., Stanford Univ., Stanford, CA; [3]Cortice Biosci., New York, NY; [4]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [5]Dept. of Neurol., [6]Memory and Aging Ctr., Univ. of California, San Francisco, San Francisco, CA

Abstract: Alzheimer's disease (AD) is a common neurodegenerative disorder characterized by a progressive decline in memory along with other cognitive abilities. The pathological hallmarks of AD include elevated levels of amyloid beta peptide and abnormal tau pathology, such as hyperphosphorylation of tau, a major microtubule-associated protein in neurons. The current study tested the hypothesis that systemic administration of a microtubule-stabilizing agent reduces pathological aggregation and phosphorylation of tau and leads to cognitive improvements in a mouse model of AD and related tauopathies. Microtubule-stabilizing taxanes, including paclitaxel, have been identified as possible therapeutic agents for these indications; however, paclitaxel has poor blood-brain barrier permeability and is thus unsuitable for treatment of CNS disease. TPI 287, a novel and clinically safe taxoid, is a microtubule-stabilizing agent that readily crosses the blood-brain barrier. In a pilot proof of concept study, TPI 287 was administered intravenously, every 4 days, at 1 mg/kg or 3 mg/kg to PS19 transgenic mice overexpressing mutant human tau. Following this dosing period, behavioral tests were conducted to assess general activity, as well as contextual learning and memory. Treatment with TPI 287 improved exploratory and anxiety-like behaviors in PS19 mice. Moreover, terminal biochemical assessment of PS19 brain samples showed that TPI 287 reduced levels of hyperphosphorylated tau. We are currently confirming these preliminary findings in a follow-on study. These results suggest that modulation of tau phosphorylation and microtubule-stabilization may hold unexplored therapeutic potential with a novel mechanism of action.

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come to an institution.; Cortice Biosciences, University of California, San Francisco. F. Consulting Fees (e.g., advisory boards); Cortice Biosciences.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant AG032755

Alzheimer's Art Quilt Initiative (AAIQ)

Alzheimer's Disease Research Center at UCSD Grant AG005131

Title: Increased tau phosphorylation and aggregation in mice overexpressing corticotropin-releasing factor

Authors: *S. N. CAMPBELL[1], C. ZHENG[1], L. MONTE[1], A. D. ROE[1], K. C. RICE[2], E. MASLIAH[1], R. A. RISSMAN[1];
[1]UCSD, La Jolla, CA; [2]Natl. Inst. on Drug Abuse, Bethesda, MD

Abstract: Clinical research suggests that stress exposure can dramatically increase risk for Alzheimer's disease (AD). Although the links between stress and AD remain unsettled, data from our group and others have established that stress exposure in rodents may confer susceptibility to AD pathology by inducing tau phosphorylation (tau-P) in the hippocampus. Previous data from our lab directly implicate stress and the CRF system in neuronal vulnerability, and demonstrate that repeated stress exposure elicits cumulative effects on tau-P and its sequestration in an insoluble and potentially pathogenic form. Here, we also reported that hippocampal tau-P elicited by emotional stress was not regulated by the stress-induced rise in glucocorticoids but was dependent on corticotropin-releasing factor receptor type 1 (CRFR1). CRF over-expressing (CRF-OE) mice have marked hippocampal atrophy, learning and memory deficits by 9 months of age, and are characterized by chronically high levels of glucocorticoids. We examined how this sustained exposure to CRF and stress steroids might impact tau-P and kinase activity in the presence or absence of CRFR antagonist in the months immediately preceding cognitive impairment. In the hippocampus, CRF-OE mice had significantly elevated tau-P compared to WT mice at the AT8 (S202/T204), PHF-1 (S396/404), S262 and S422 sites.

Pretreatment with CRFR-antagonist blocked phosphorylation at the AT8 (S202/T204) and PHF-1 (S396/404) sites, but not at the S262 and S422. Although the reduction of several kinases was observed, only concomitant regulation of c-Jun N Terminal Kinase (JNK) was found with CRFR antagonist treatment. Examination of extracts from CRF-OE mice at the ultrastructural level revealed negatively stained round/globular aggregates that were positively labeled by PHF-1. These data suggest critical roles for CRF and CRFRs in tau-P and aggregation and may have implications for cognitive decline in AD.

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Poster

792. Alzheimer's Disease: Tauopathy

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARRS grant P3-0171

Title: New composite neuronal tau inclusions in two siblings with a tau mutation-associated disease

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Abstract: We present novel composite neuronal tau inclusions (CNTI) found in two siblings with a P364S tau mutation-associated tauopathy. Both sisters presented with mild cognitive decline, depression, symmetric bradykinesia, rigidity, slow and forward-bent gait. Signs of motor neuron disease were found in one sister. Bidirectional sequencing of the tau gene revealed heterozygous mutation P364S in both sisters. The patients died due to respiratory insufficiency within 2 years of initial presentation. Formalin-fixed brains and spinal cords were examined and histopathological analysis was done with selected histochemical and immunolabeling of paraffin-embedded tissue sections. Neurodegenerative changes consistent with a tauopathy were found in all regions examined. Extensive neuronal loss and reactive gliosis were found in the nucleus

basalis of Meynert, substantia nigra, locus coeruleus, transentorhinal and motor cortex, and in the anterior horn of the spinal cord. Sparse glial coiled bodies were found. Neuronal tau inclusions were globose neurofibrillary tangles (gNFT; predominant), ghost gNFT (frequent) and flame-shaped NFT (fNFT) found mostly in the pyramidal cells of CAI and CAII, the subiculum, and the transentorhinal cortex. Some granular neurons displayed Pick body (PiB)-like inclusions. A new and unique neuropathological finding in both brains were neuronal tau inclusions composed of two distinct parts. In H&E they have a pale eosinophilic core and a basophilic peripheral halo. Gallyas silver impregnation preferentially labeled the CNTI halo. AT8 immunolabeling was more pronounced at the periphery, but also labeled the core. Immunohistochemistry to 3R isoform labeled the whole CNTI, while the 4R tau epitope was found only at the periphery. Regional distribution of CNTI was similar in both brains: frequent in subiculum, subthalamic nucleus, thalamus, motor cortex, and nigra, rare in frontobasal, insular, and cingular cortex, and absent from dentate nucleus, cranial nerves nuclei, visual cortex and basal ganglia. Our cases of P364S tau mutation-associated tauopathy displayed a new type of tau neuronal inclusion composed of two distinct parts, with the periphery and core displaying characteristics of gNFT and PiB, respectively.

Disclosures: **M. Bresjanac:** None. **A. Fabjan:** None. **J. Mraz:** None. **D. Glavač:** None. **J. Magdič:** None. **A. Zupan:** None. **M. Drolec Novak:** None. **M. Popović:** None.

Poster

792. Alzheimer's Disease: Tauopathy

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 792.17/G7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Fibroblast aggregation rate converges with validated peripheral biomarkers for alzheimer's disease

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Abstract: The inaccuracy of the diagnosis for Alzheimer's disease (AD) has made the therapeutic intervention difficult, particularly early enough to prevent significant neurodegeneration and cognitive dysfunction. Here, we describe a novel, highly accurate peripheral diagnostic for AD patients based on quantitatively measured aggregation rate of human skin fibroblasts. The elevated aggregation rate with increasing cell density in AD cases is

the basis of this new bio-marker. The new biomarker, was successfully cross-validated, with two more mature assays, AD-Index, based on the imbalances of ERK1/2, and Morphology, based on network dynamics, and showed 92% overlap. A significant number of cases tested with this new bio-marker were freshly obtained, 29, and 82% of the cases are hyper-validated cases i. e. autopsy and/or genetically confirmed AD or non-Alzheimer's Demented patients(Non-ADD) and non demented age-matched controls(AC). Furthermore we show that by using a simple majority rule, i. e. two out of the three assays have the same outcome, we significantly increase the agreement with clinical AD diagnosis (100%). Based on the high accuracy of this strategy, the bio-marker profile appears to identify accurately the AD patients for therapeutic intervention.

Disclosures: F.V. Chirila: None. **T.K. Khan:** None. **D.L. Alkon:** None.

Poster

792. Alzheimer's Disease: Tauopathy

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21NS084156

R21NS077059

P50AG005134

Title: Frontotemporal lobar degeneration: Initial experience with [18f] t807 pet

Authors: *B. C. DICKERSON[1], K. DOMOTO-REILLY[2], D. HOCHBERG[2], M. BRICKHOUSE[2], N. VASDEV[2], K. JOHNSON[2];
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Abstract: BACKGROUND: A critical unmet need for FTLD research, especially therapeutic trials, is the development of biomarkers to distinguish FTLD-tau from FTLD-TDP and other non-tau FTLD pathologies. METHODS: We are using [18F] T807, a novel PET ligand, to scan a series of patients with FTLD, to date including one MAPT P301L mutation carrier with moderate severity FTD dementia, an asymptomatic carrier of the same mutation, six patients with sporadic mild primary progressive aphasia, and three patients with progressive supranuclear palsy. We analyzed SUVR (cerebellum reference) data to localize and quantify [18F] T807

signal. We also co-registered analyzed [18F] T807 images to MRI images for visualization and calculation of % atrophy relative to controls. RESULTS: [18F] T807 signal was elevated in frontal, insular, and anterior temporal cortex in the MAPT carrier with dementia, and colocalized with atrophy. In non-fluent aphasic patients, [18F] T807 signal was highest in inferior frontal and middle temporal gyri and temporal pole with marked asymmetry, most prominent in the dominant hemisphere, and localized remarkably well with atrophy. The asymptomatic carrier had mildly elevated signal in frontal, insular, and anterior temporal cortex as well as white matter. PSP patients showed elevated brainstem, basal ganglia, thalamic/subthalamic, cerebellar, and frontal signal. CONCLUSIONS: T807 is a promising new PET ligand for imaging tau pathology *in vivo* in patients with FTLD.

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Poster

792. Alzheimer's Disease: Tauopathy

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Program#/Poster#: 792.19/G9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01-AG03365

P50-AG005146

Title: Cortical regions are associated with risk of clinical symptom onset during preclinical Alzheimer’s disease

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Abstract: The pathological processes underlying Alzheimer’s disease (AD) are thought to begin during the preclinical phase, prior to the onset of clinical symptoms. Previous work suggests that thinning in specific cortical regions (known as the ‘cortical signature of AD’) is associated with increased risk of developing dementia. However, less is known about whether these regions are

also sensitive to the transition from normal cognition to Mild Cognitive Impairment (MCI), an earlier disease time point. This study examined the relationship of the AD signature regions of interest (ROIs) during preclinical AD, in order to determine whether these regions were associated with time to onset of clinical symptoms, an even earlier time point than the diagnosis of MCI. The present analyses included 212 individuals from the BIOCARD study who were cognitively normal and primarily middle aged at baseline. Participants have been prospectively followed for up to 18 years (M = 9 years) with annual clinical and cognitive assessments; to date, 50 of these participants have developed MCI or AD dementia. Baseline MRI measures of regional cortical thickness were obtained, using FreeSurfer, for the 9 'AD signature' ROIs, including two frontal regions, 3 parietal regions, and 4 temporal regions. Cox regression models (covarying baseline age and education) were used to determine whether the individual cortical ROIs, as well as the average thickness of these regions, were associated with time to onset of clinical symptoms. The mean time from baseline to the onset of clinical symptoms in this sample was 6.6 years. The average thickness of the regions was associated with time to onset of clinical symptoms (hazard ratio (HR) = 0.72, $p = .05$). However, the ROIs taken individually were not significant in these models. Follow-up analyses indicated that lower baseline cortical thickness of each ROI, as well as the combination of ROIs, was associated with increased risk of progression to clinical symptoms within 7 years after baseline (all HRs < 0.59, all $p < .05$). In contrast, these ROIs were not associated with risk of progression to clinical symptoms among individuals who progressed more than 7 years from baseline, suggesting that cortical thinning occurs more proximal to symptom onset. These results suggest that cortical thinning is detectable in cognitively normal individuals several years prior to clinical symptom onset, and that this atrophy is associated with time to onset of clinical symptoms that are a harbinger of a diagnosis of MCI. Ongoing analyses are examining the relationship between these MRI measures and other assessments in the same individuals (e.g., CSF).

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Poster

792. Alzheimer's Disease: Tauopathy

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Title: Effects of acidic oligosaccharide sugar chain on amyloid oligomer-induced impairment of synaptic plasticity in rats

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Abstract: Soluble amyloid-β protein (Aβ) oligomers have been recognized to be early and key intermediates in Alzheimer's disease-related synaptic dysfunction. In this study, using *in vitro* electrophysiology, we investigated interactions of the acidic oligosaccharide sugar chain (AOSC), a marine-derived acidic oligosaccharide, with oligomeric Aβ. We found that the inhibition of long-term potentiation (LTP) induced by Aβ oligomers can be dose dependently reversed by the application of AOSC, whereas AOSC alone did not alter normal LTP induction. Interestingly, treatment with Aβ monomers with or without AOSC did not affect LTP induction. Additionally, when fresh-made Aβ was co-incubated with AOSC before *in vitro* testing, there was no impairment of LTP induction. The results from Western blots demonstrated that AOSC prevent the aggregation of Aβ oligomers. These findings indicate that AOSC may reverse Aβ oligomer-mediated cytotoxicity by directly disrupting the amyloid oligomer aggregation, and this action is concentration dependent. Thus, we propose that AOSC might be a potential therapeutic drug for Alzheimer's disease due to its protection against oligomeric Aβ-induced dysfunction of synaptic plasticity.

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Poster

792. Alzheimer's Disease: Tauopathy

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Title: Dual use of 11C-deuterium-L-deprenyl tracer in positron emission tomography (PET) imaging of Alzheimer's disease

Authors: *E. RODRIGUEZ-VIEITEZ[1], K. BUTINA[1], S. F. CARTER[1], M. SCHÖLL[1], K. FARID[1], A. WALL[2], A. NORDBERG[1,3];
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Abstract: Background: Alzheimer's disease (AD) is the most common dementia disorder. The accumulation of amyloid plaques in AD brain has been hypothesized to play a causative role in AD. However, there is increasing evidence that additional mechanisms including neuroinflammatory and neurovascular changes may precede plaque deposition and contribute to disease development. Reactive astrocytes have been investigated in patients using the PET tracer ^{11}C-deuterium-L-deprenyl (^{11}C-DED) [1], where the presence of deuterium serves to reduce the influence of cerebral blood flow (CBF) on tracer binding, compared to a previously investigated ^{11}C-L-deprenyl compound [2]. In this study, ^{11}C-DED was applied together with ^{11}C-Pittsburgh compound B (^{11}C-PIB) and ^{18}F-FDG in a multitracer PET study in the same group of patients. The aim of this study was to further investigate the kinetic properties of ^{11}C-DED and its potential dual use to measure brain astrocytosis and cerebral blood flow (CBF). **Methods:** ^{11}C-DED, ^{11}C-PIB and ^{18}F-FDG PET scans were performed in 25 patients including sporadic AD (n=8) and mild cognitive impairment (MCI) (n=17). Healthy controls (n=14) underwent ^{11}C-DED PET imaging. PET quantification was performed in 12 bilateral regions of interest (ROIs). ^{11}C-PIB and ^{18}F-FDG uptake was quantified as target-to-pons Standardized Uptake Value ratios (SUVr). ^{11}C-DED was modeled using: (1) a modified reference Patlak model; (2) a Simplified Reference Tissue Model (SRTM) using PMOD v3.3 software with the cerebellum gray matter as a reference region. CBF was estimated by: (1) the 1-4 minute early frames of ^{11}C-DED with reference to the cerebellum (eDED); (2) the R_1 parameter (ratio of tracer delivery to a ROI with

respect to the cerebellum) obtained from the SRTM. **Results**: ^{11}C-DED binding estimated by the reference Patlak (DEDslope) and SRTM (binding potentials, BPnd) models were highly correlated in every ROI (Pearson's $r = 0.80 - 0.92$, $p < 0.001$), providing evidence that ^{11}C-DED is a reversible tracer, not strongly dependent on CBF. The eDED values were highly correlated to R_1 values in every ROI (Pearson's $r > 0.90$, $p < 0.001$), and both also strongly correlated with ^{18}F-FDG uptake. Therefore eDED and R_1 were considered measures of CBF. Neither DEDslope nor BPnd were correlated to eDED or R_1. **Conclusions**: The ^{11}C-DED tracer provided dual physiological (CBF) and pathological (astrocytosis) information from a single PET scan, and may contribute to the understanding of the time course and regional pathological brain changes at different stages in AD. Carter *et al.* (2012), JNM 53, 37-46 Fowler *et al.* (1995), JNM 36, 1255-62

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Poster

792. Alzheimer's Disease: Tauopathy

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG033036

P01 AG030128

P30 AG028383

Title: White matter integrity is associated with CSF markers of AD in normal adults

Authors: *B. T. GOLD, Z. ZHU, C. BROWN, F. SCHMITT, C. SMITH;
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Abstract: We explored whether white matter (WM) integrity in cognitively normal (CN) older adults is associated with cerebrospinal fluid (CSF) markers of Alzheimer's disease (AD) pathology. Twenty CN older adults underwent lumbar puncture and magnetic resonance imaging within a few days of each other. Analysis of diffusion tensor imaging data involved *a priori* region of interest (ROI) and voxelwise approaches. The ROI results revealed a positive correlation between CSF measures of amyloid-beta ($A\beta_{42}$ and $A\beta_{42}$/p-Tau_{181}) and WM integrity

in the fornix, a relationship which persisted after controlling for hippocampal volume and fornix volume. Lower WM integrity in the same portion of the fornix was also associated with reduced performance on the Digit Symbol test. Subsequent exploratory voxelwise analyses indicated a positive correlation between CSF $A\beta_{42}$/p-Tau_{181} and WM integrity in bilateral portions of the fornix, superior longitudinal fasciculus, inferior fronto-occipital fasciculus, and in the corpus callosum and left inferior longitudinal fasciculus. Our results link lower WM microstructural integrity in CN older adults with CSF biomarkers of AD and suggest that this association in the fornix may be independent of volumetric measures.

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Poster

792. Alzheimer's Disease: Tauopathy

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Mitchell Center for Neurodegenerative Diseases

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Title: Mixed pathology in Alzheimer could be explained by the presence of hybrid oligomers

Authors: *M. J. GUERRERO[1], D. L. CASTILLO-CARRANZA[2], A. PAULUCCI-HOLTHAUZEN[3], U. SENGUPTA[2], C. LASAGNA-REEVES[2], R. KAYED[2];
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Abstract: Introduction Alzheimer's disease (AD) is characterized by the formation of insoluble, fibrillar deposits of amyloid ß (Aß) peptide and tau protein. Recent studies in humans and in an APP mouse model demonstrate the abundance of mixed pathology proteinopathies. These results highlight the importance of pathogenic protein interactions on disease progression and possibly the disease phenotype, as well as the overlap between different neurodegenerative diseases. TDP-43, a protein highly related to Amyotrophic lateral sclerosis, has been observed to be aggregated in around 75% of brains from AD patients. Here, we show the presence of hybrid oligomers composed of TDP-43 and other amyloidogenic proteins, such as Aβ, α-synuclein, and

Prion protein in human AD brain samples, but not in age-matched controls. Methods We used a novel anti-oligomer monoclonal antibody (F11G3), anti-TDP-43 antibodies, 6E10 for Aß, 4D6 for α-synuclein, and 6D11 for PrP. We performed immunohistochemical and analyses of brain tissue from moderate to severe human AD cases, non-demented elderly controls and Tg2576 mice. Results We found hybrid oligomers composed of TDP-43 and either Aβ, PrP, or α-syn in human AD brain samples. However, the presence of hybrid oligomers in the Tg2576 AD mouse model was much lower than in human samples. Conclusions Detection of hybrid oligomers of TDP-43 and Aβ in AD human and mouse brain samples suggest that Aβ oligomers interact and seed TDP-43 aggregation which may contribute to the pathogenesis in AD. *In vitro* assay showed that TDP-43, PrP, or α-syn proteins acquire Aβ oligomer conformation.

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Poster

793. Alzheimer's Disease: Genetics and Biology II

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Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R)

National Basic Research Program of China (2013CB530900)

Title: Identification and characterization of small molecule EphA4 inhibitors as disease modifiers for Alzheimer’s disease

Authors: *W.-Y. FU[1,2,3], K.-W. HUNG[1,2,3], G. SHUO[4], J. XU[1,2,3], A. SU[1,2,3], F. IP[1,2,3], X. HUANG[4], A. FU[1,2,3], N. IP[1,2,3];
[1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., [4]Chem., Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Synaptic loss and dysfunction are associated with cognitive decline in the early stages of Alzheimer’s disease. Thus, rescuing synaptic deficits is a promising therapeutic strategy for the disease. We recently found that the receptor tyrosine kinase, EphA4, is aberrantly activated in the hippocampus of the APP/PS1 mouse model of Alzheimer’s disease and that inhibition of

EphA4 signaling alleviates synaptic dysfunctions. Using a traditional Chinese medicine database, we performed a virtual screen to identify small molecule EphA4 inhibitors that target the ligand-binding pocket of EphA4. The small molecules identified blocked both the interaction of EphA4 with its ligand, ephrin-A, as well as ephrin-A-induced tyrosine phosphorylation and growth cone collapse in cultured neurons. Furthermore, these EphA4 inhibitors attenuated the impaired synaptic plasticity induced by oligomeric Aβ, which is believed to be the major agent that contributes to the synaptotoxicity in Alzheimer's disease. Administration of these EphA4 inhibitors *in vivo* rescued synaptic impairment and Alzheimer's pathology in the APP/PS1 mouse model. Work is in progress to conduct preclinical studies for these small molecules.

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Poster

793. Alzheimer's Disease: Genetics and Biology II

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Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R)

National Basic Research Program of China (2013CB530900)

Title: Blockade of EphA4 signaling alleviates the impairment of hippocampal synaptic plasticity associated with Alzheimer's disease

Authors: *A.-Y. FU[1,2,3], K.-W. HUNG[1,2,3], H. HUANG[1,2,3], S. GU[4], Y. SHEN[1,2,3], E. CHENG[1,2,3], F. IP[1,2,3], X. HUANG[4], W.-Y. FU[1,2,3], N. IP[1,2,3];
[1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., [4]Chem., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Impairment of hippocampal synaptic plasticity is associated with learning and memory deficit in Alzheimer's disease. It is therefore important to understand the molecular mechanisms underlying synaptic dysfunctions. We found that activity of a receptor tyrosine kinase EphA4 was increased in hippocampal synaptic fractions of APP/PS1 Alzheimer's disease mouse model at 3 months, an early stage when the deposition of amyloid plaques s is observed.

Furthermore, deletion of postsynaptic EphA4 was able to alleviate the suppression of hippocampal long-term potentiation in the Schaffer collateral-CA1 pathway of APP/PS1 mice. Thus, our findings suggest that EphA4 is a potential target for disease-modifying strategies for Alzheimer's disease. Using docking analysis, we identified various small molecule inhibitors for EphA4, which were able to inhibit the binding between EphA4 and its endogenous ligands in cultured neurons. Further analysis showed that oral administration of an EphA4 inhibitor blocked the EphA4 signaling in APP/PS1 mice, and rescued the impaired synaptic plasticity observed in those mice. Taken together, blockade of the EphA4-ligand interaction is a promising approach to developing effective treatments for Alzheimer's disease.

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Poster

793. Alzheimer's Disease: Genetics and Biology II

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Support: NIH Grant R01AG037481

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Title: Histone modifications and Egr1 expression in response to prenatal Arsenic exposure and High Fat Diet

Authors: K. N. NAM[1], A. N. MOUNIER[1], D. GEORGIEV[1], N. F. FITZ[1], A. A. CRONICAN[1], R. KOLDAMOVA[1], *I. M. LEFTEROV[2];
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Abstract: Alzheimer's disease is a complex quantitative variation in disease susceptibility, controlled by multiple genes and environmental factors, as well as gene-gene and gene-environment interactions. Environmental risk factors, such as dietary patterns, exposure to environmental toxicants, intellectual and physical activity, as well as cardiovascular disease and Type 2 Diabetes, have the highest level of AD association. However, the molecular mechanisms underlying the increased susceptibility to, earlier development or aggravated course of the disease remain poorly understood. Possible molecular links between certain environmental exposures/factors and AD could be changes in regulatory and metabolic pathways due to dysregulation in transcription factor binding, or epigenetic changes known to influence synaptic transmission, memory and cognitive performance. Previously we have demonstrated that Early growth response 1 (EGR1) transcription factor regulates the constitutive expression of genes involved in vesicular transport and synaptic transmission that may be critical for memory and cognition, as well as, for the development and progression of AD. In a preliminary study we have been able to reveal changes in H3K9 acetylation profile in the proximal promoter of mouse Egr1 in response to prenatal arsenic exposure and normal diet. We also found a significantly decreased levels of Egr1 mRNAs in brains of WT offspring following in-utero exposure, as well as in 2 month old mice exposed to human relevant 100 μg/L arsenic in drinking water. The goal of this study is to identify and correlate changes in Egr1 binding to histone modifications and gene expression levels in young AD mice in response to arsenic treatment. The results and comparisons with EGR1 biding in APP mice without any treatment or exposure, will answer the question if EGR1 mediated transcriptional control is linked to histone modifications or is an independent mechanism for inducing phenotypic changes in AD mice in response to As.

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Poster

793. Alzheimer's Disease: Genetics and Biology II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIGMS U54GM104942 (Reed)

Alzheimer's Association NIRG-12-242187 (Reed)

Title: Effects of streptozotocin-induced diabetes on synaptic proteins and tau phosphorylation in mice expressing P301L human tau

Authors: *C. C. RUDY, H. C. HUNSBERGER, D. S. WEITZNER, M. N. REED;
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Abstract: The cause of sporadic Alzheimer's disease (AD) is largely believed to multifactorial, and it has been suggested that diabetes may be an important risk factor for AD. Recent work suggests diabetes-induced insulin dysfunction can induce memory deficits via its effects on glutamatergic signaling and synaptic plasticity. Though much initial work focused on the synaptic effects of beta-amyloid, emerging evidence suggests tau plays a critical role in the synaptic degeneration observed in AD. Thus, the synapse may be a point of convergence for diabetes and AD. To induce impairments in cerebral glucose utilization and energy metabolism that are assumed to play a prominent role in the pathogenesis of AD, we injected mice with low doses (50 mg/kg, i.p.) of streptozotocin (STZ) over 5 consecutive days, a protocol reported to reduce the mortality rates associated with high doses of STZ. To determine the effects of diabetes in mice with existing tau pathology, mice expressing mutant P301L human tau (tauP301L), which results in age-dependent memory deficits, neuron loss, and tangle formation, were used. We also examined the effects of STZ on mice expressing wild-type human tau (tauWT). These mice do not exhibit memory deficits or tau pathology, but by comparing the effects of STZ in tauWT to transgene negative littermates, we were able to determine whether human tau is more susceptible to the effects of diabetes than mouse tau. To determine whether diabetes increases hyperphosphorylation of tau, leading to tau accumulation in dendritic spines, tau levels and tau phosphorylation status in the hippocampal postsynaptic density (PSD) were examined. Alterations in pre- and post-synaptic proteins, such as synaptophysin, PSD95, and ionotropic glutamatergic receptors, were also examined. STZ resulted in blood glucose levels greater than 300 mg/dL, indicating hyperglycemia. We anticipate diabetes, resulting from STZ, will exacerbate tau pathology and synaptic alterations observed in tauP301L mice. We anticipate STZ will induce tau pathology and synaptic alterations in mice expressing tauWT mice above and beyond that observed in transgene negative controls. Though tauP301L mice are commonly used to model the tau pathology observed in AD, there are no known mutations in tau linked to AD. Because there are no known tau mutations resulting in AD, identifying mechanisms that alter wild-type human tau functioning is essential to understanding the etiology of sporadic AD. To date, however, there are no studies investigating the effects of diabetes in transgenic mice expressing wild-type human tau.

Disclosures: C.C. Rudy: None. **H.C. Hunsberger:** None. **D.S. Weitzner:** None. **M.N. Reed:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 793.05/H6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant MH076145

NIH Grant T32 DA07315

Title: Altered genomic mosaicism with increased DNA content and CNVs in single sporadic Alzheimer's disease neurons

Authors: *G. E. KAESER, B. SIDDOWAY, D. M. BUSHMAN, J. CHUN;
The Scripps Res. Inst., La Jolla, CA

Abstract: Somatic genomic mosaicism in the CNS is characterized by aneuploidy, neuron-specific DNA content variations (DCV), copy number variations (CNVs), and other forms of mosaically altered genomic DNA. The function(s) of these genomic changes is currently unknown. However, the identification of regional DCV as a feature of the normal human brain suggested that alterations in genomic mosaicism might play a role in brain disorders. Here we report genomic mosaicism in Alzheimer's disease (AD) characterized by increases in DNA content and amyloid precursor protein (*APP*) CNVs. Five independent methodologies were used to identify and characterize genomic mosaicism: DNA content analysis by flow cytometry, small population qPCR, dual point-paint fluorescence *in situ* hybridization (FISH), single cell qPCR, and single gene-targeted FISH. Flow cytometric analysis revealed DNA content increases in AD cortical nuclei over lymphocytes and age matched non-diseased cortical controls. Gene dosage of *APP* has been well documented in familial AD and Down syndrome yet has remained genetically unlinked to sporadic AD. By small population qPCR, half of AD samples showed mosaic increases in *APP*, reaching up to 6 copies. To assess whether these relative increases were due to trisomy 21, FISH combined with liberal counting criteria was used, which nevertheless showed no change between AD and non-diseased samples. Single cell techniques for detecting mosaic CNVs in neurons were implemented using the Biomark HD (Fluidigm) microfluidic platform, which detected increases in ~50% of AD cortical nuclei (~10% of non-diseased), ranging up to 12 copies. To visualize these CNVs directly without preamplification, we designed *APP*-specific FISH probes. Combined with super resolution microscopy, amplified loci were detected in ~50% of AD cortical nuclei and were observed as predominantly single loci of variable fluorescence intensity. Together, these data support a function for somatic changes in neuronal genomes, and

implicate genomic mosaicism in sporadic AD, with additional relevance to normal brain function.

Disclosures: **G.E. Kaeser:** None. **B. Siddoway:** None. **D.M. Bushman:** None. **J. Chun:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Program#/Poster#: 793.06/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21AG031388

Title: A common pathogenic role of peptidyl prolyl isomerase pin1 in synaptic dysfunction and protein misfolding in alzheimer's disease

Authors: ***Y. GONG**[1,2], C. LIPPA[1], F. CHOW[1], R. TSAI[1], F. RIZZOLIO[3,4], S. BOFFO[4], Z. LIAO[1];
[1]Neurol., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Dept. of Biopharmaceuticals, Nanjing Univ. of Chinese Med., Nanjing, China; [3]Sbarro Inst. for Cancer Res. and Mol. Medicine, Ctr. for Biotech., Temple Univ., Philadelphia, PA; [4]Ctr. di Riferimento Oncologico - CRO, Natl. Cancer Inst., Aviano, Italy

Abstract: Synaptic loss is the structural basis for memory impairment in Alzheimer's disease (AD). While the underlying pathological mechanism remains elusive, it is known that misfolded proteins accumulate as β-amyloid (Aβ) plaques and hyperphosphorylated Tau tangles decades before the onset of clinical disease. The loss of Pin1 facilitates the formation of these misfolded proteins in AD. Pin1 protein controls cell-cycle progression and determines the fate of proteins by the ubiquitin proteasome system. The activity of the ubiquitin proteasome system directly affects the functional and structural plasticity of the synapse. We localized Pin1 to dendritic rafts and the PSD and found the pathological distribution of Pin1 within the synapses of AD brains. The loss of Pin1 activity may alter the ubiquitin-regulated modification of PSD proteins and decrease levels of Shank protein, resulting in aberrant synaptic structure. The loss of Pin1 activity may also render neurons more susceptible to the toxicity of oligomers of Aβ and to excitation, thereby inhibiting NMDA receptor-mediated synaptic generation and exacerbating NMDA receptor-mediated synaptic degeneration. These findings provide a common pathological role of Pin1 in the development of the three pathological hallmarks of AD (Aβ plaques, Tau

tangles, and synaptic loss) and suggest that Pin1 may serve as a common target to protect synaptic function and to prevent the formation of misfolded proteins in preclinical AD.

Disclosures: Y. Gong: None. **C. Lippa:** None. **F. Chow:** None. **R. Tsai:** None. **F. Rizzolio:** None. **S. Boffo:** None. **Z. Liao:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Program#/Poster#: 793.07/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Simmons Fund for Research # 562 to DBG

Presidents Fund for Excellence to DBG

Title: The role of the extracellular domain in the regulation of transmitter release by beta amyloid, nitric oxide and cyclic guanosine monophosphate

Authors: A. K. DEGRUTTOLA[1], *D. GRAY[2];
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Abstract: Alzheimer's disease (AD), the most common neurodegenerative disorder, is strongly associated with insoluble deposits of beta amyloid (Ab) peptide in plaques within the brain. Recent work has suggested a non-pathological role of Ab as a regulator of presynaptic release of the neurotransmitter acetylcholine (ACh). In this cascade pathway which was discovered in cholinergic synapses in a chick embryo choroid layer of the eye, soluble oligomers of Ab cause the synthesis of nitric oxide (NO) which binds and activates guanyl cyclase, cyclic-guanosine monophosphate (cGMP) levels rise, protein kinase G (PKG) activates and phosphorylates exocytotic proteins, which inhibit evoked transmitter release. However the literature is split whether Ab and NO act in a neuro-protective or neuro-destructive fashion. One explanation for these discrepancies may be due to opposing actions of intracellular versus extracellular cGMP, as shown by previous research in transformed cells. Potassium-evoked radiolabeled ACh release was measured from presynaptic membrane terminals in the 14 day choroid tissues after overnight incubation with and without Ab or other pharmaceutical compounds. Here we report that membrane impermeable cGMP acts differently than membrane permeable cGMP forms (8-Bromo-cGMP) on potassium-evoked ACh release in choroid nerve terminals. Whereas 8-Bromo-

cGMP is able to mimic the inhibition of ACh release, the membrane impermeable form is not, thus suggesting that intracellular cGMP is required for the Ab-induced inhibition of evoked ACh release. However saturation of the choroid prep with soluble hemoglobin (100 uM), which strongly binds extracellular NO, reverses the Ab effect on ACh release. This suggests that NO transiently travels through the extracellular domain before interacting with intracellular cGMP during the Ab-induced pathway.

Disclosures: A.K. DeGruttola: None. **D. Gray:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

Location: Halls A-C

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Program#/Poster#: 793.08/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Scientific Prometheus Program of Ecuador

FCI-101 University of Guayaquil

Title: Determination of specific genes in neurodegenerative diseases in Ecuadorian Population

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Abstract: The increase progressive in neurodegenerative diseases (ND) prevalence in relation with the age has increased in recent years in the world population. Advances in molecular genetics have opened high development for study of neurodegenerative diseases. The aim of this research is to identify and know the gene expression and demographics data of Alzheimer's (AD), Parkinson's (PD), Multiple Sclerosis (MS) and Huntington (HD) in Ecuadorian population. Our results demonstrate significant increases in APP and HLA -DRB1 gene expression and reduction of LRRK2 gene. On the other hand we identified the IT15 gene expansion and the genetic pedigree was performed obtaining presence of autosomal dominant inheritance of Huntington's disease. When we study the relation of the (ND) with age we found that most of patients with AD, PD, MS and HD were in the age group of 71-80, 60-70, 51-60 and

41-50 years respectively. By relating the BMI with gene expression was observed the majority patients with AD were obese patients; the rest of neuropathology's present low weight. The personal medical history (PMH) in HD is present in most individuals, the other pathologies show different PMH, where the lack of PMH presence is shown in most individuals analyzed. Finally research the gender distribution, and we found the increased presence of female gender in Alzheimer's and Huntington and more individuals of male gender in Parkinson's and Multiple Sclerosis. We can conclude that has been showed the gene expression and demographic data of neurodegenerative diseases studied in this investigation in Ecuadorian population, and has been obtained similar conditions and data showed in others researcher around the world. Keywords: Neurodegenerative diseases, APP, IT15, HLA -DRB1, LRRK2, RT- PCR, pedigree.

Disclosures: R. Aviles Reyes: None. **D. Martinez:** None. **B. Cardenas:** None. **P. Palacios:** None. **S. Andrade:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Program#/Poster#: 793.09/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HI10C2020

A092004

Title: Gene-based rare allele analysis identified a risk gene of Alzheimer's disease

Authors: *J. KIM;
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Abstract: Objective: Alzheimer's disease (AD) has a strong propensity to run in families. In various complex diseases, gene studies have targeted rare alleles for unsolved heritability. Our study aims to elucidate unknown risk genes for AD by targeting rare alleles. **Methods:** We used data from five publicly available genetic studies from the ADNI and the dbGaP. The genotype information of rare alleles was imputed using 1000 genomes. We performed gene-based analysis of rare alleles (MAF $\leq$ 3%). **Results**: *ZNF628* showed a genome-wide significant association with AD. *APOE* and *TREM2* were also significantly associated with AD, although not at genome-wide significance levels. Other genes identified by targeting common alleles could not

be replicated in our study. **Conclusions:** We identified that rare variants in *ZNF628* are associated with AD. Furthermore, the significant associations of *APOE* and *TREM2* with AD imply that further deep sequencing of these genes is required in AD heritability studies.

Disclosures: J. Kim: None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P50AG025688-09

Title: Identification of differentially hydroxymethylated loci in Alzheimer's disease

Authors: *A. I. BERNSTEIN, C. STREET, L. LI, M. GEARING, A. I. LEVEY, P. JIN; Emory Univ., Atlanta, GA

Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by progressive deterioration of cognitive function. Many lines of evidence suggest a role for epigenetic regulation, in particular cytosine modifications (5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC)), in AD. Methylation of cytosine in genomic DNA is carried out by DNA methyltransferases. 5hmC, highly enriched in the nervous system, is generated from 5mC by Tet proteins. 5hmC displays temporal and spatial changes during neurodevelopment and aging. To determine the role of 5mC and 5hmC in AD, we carried out genome-wide analyses of 5mC and 5hmC of using the DNA isolated from prefrontal cortex of post-mortem AD and age-matched control brains. We also performed RNA-Seq to correlate changes in methylation status with transcriptional changes. Our analyses showed a change in the distribution of 5hmC across the genome, with 6,989 differentially hydroxymethylated loci (DhMLs) gained in AD and 15,201 DhMLs lost in AD. Annotation revealed that 30% of these DhMLs are located in intergenic regions and 70% are located within genes (intragenic). Correlation of these genes with RNA-Seq results revealed 230 genes showing significant differences between AD and age-matched controls. Three of these genes show confirmed differential splicing between the 2 conditions (ITSN2, CACNA1D, and NCOR2), while 160 genes show differential isoform expression. Gene ontology analysis of these 230 genes showed enrichment for genes located at the plasma membrane, genes involved in calcium ion transport and voltage-gated calcium

channel activity, and genes associated with clathrin-coated endocytic vesicles, suggesting a disruption of proper synaptic vesicle cycling during neurotransmission in AD. Together, these data suggest that 5hmC plays an important role in modulating gene expression in AD pathogenesis.

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Poster

793. Alzheimer's Disease: Genetics and Biology II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF Grant 2009-0070560

NRF Grant 2009-0070562

NRF Grant 2011-0030928

NRF Grant 2011-0030775

NRF Grant 2012-003338

Title: Prediction of miRNA-mRNA associations in Alzheimer's disease model mice

Authors: *H. NOH[1], C. PARK[2], S. PARK[3], Y. LEE[1], S. CHO[4,5], H. SEO[1];
[1]Dept. of Mol. and Life Sci., Hanyang Univ., Ansan, Gyeonggi, Korea, Republic of; [2]Ewha Res. Ctr. for Systems Biol., Ewha university, Seoul, Korea, Republic of; [3]Bio-Medical IT Convergence Res. Dept., Electronics and Telecommunications Res. Inst., Seoul, Korea, Republic of; [4]Mammalian Genet. Unit, Harwell Sci. and Innovation Campus, Oxfordshire, United Kingdom; [5]Program for Cancer Biol. and BIO-MAX Inst., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Transcriptome array analysis has been used to study genome-wide protein expression and its regulation by non-coding RNA. Although the functions of non-coding RNAs have been studied in many diseases, little is known about the relationships between miRNA and mRNA expression in Alzheimer's disease (AD) at early- or late-symptomatic stages. Sequence-based

target prediction algorithms and anti-correlation profiles have been applied to predict miRNA targets using omics data, but this approach often leads to false positive predictions. Here, we applied the joint profiling analysis of mRNA and miRNA expression levels to Tg6799 AD model mice at 4 and 8 months of age using a network topology-based method. We constructed gene regulatory networks and used the PageRank algorithm to predict significant interactions between miRNA and mRNA. In total, 8 cluster modules were predicted by transcriptome data for co-expression networks of AD pathology. AD networks were constructed by integrating mRNA and miRNA profiles. In total, 54 miRNAs were identified as being differentially expressed in AD. AD networks were constructed by integrating mRNA and miRNA profiles. Among those, 50 significant miRNA-mRNA interactions were predicted by integrating sequence target prediction, expression analysis, and the PageRank algorithm. We identified a set of miRNA-mRNA interactions that were changed in the hippocampus of Tg6799 mice at both early- and late-symptomatic stages compared to littermate controls. Our results demonstrate AD-specific changes in the miRNA regulatory system as well as a relationship between the levels of miRNAs and those of their targets in the hippocampus of Tg6799 mice. These data suggest the potential functions of various miRNAs and their target genes in the molecular pathology of AD.

Disclosures: **H. Noh:** None. **C. Park:** None. **S. Park:** None. **Y. Lee:** None. **S. Cho:** None. **H. Seo:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 793.12/I1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Pilot Grant from RFUMS

Title: Abnormalities in synaptic responses within dendritic spines associates with suppressed network-level CA1 plasticity in presymptomatic Alzheimer’s disease mice

Authors: S. CHAKROBORTY[1], *E. S. HILL[2], W. N. FROST[2], G. E. STUTZMANN[1]; [1]Neurosci., [2]Cell Biol. and Anat., Rosalind Franklin Univ., North Chicago, IL

Abstract: Several neurological disorders with cognitive deficits such as Alzheimer’s disease (AD) have altered hippocampal synaptic plasticity. However, the progression by which cellular synaptic function becomes impaired and manifests itself within the broader hippocampal

networks that encode memory are still unidentified and are the focus of this study. To accomplish this, we examined synaptic plasticity mechanisms at the single spine and neuron level using video-rate 2-photon calcium imaging and patch clamp electrophysiology, in parallel with observations of synaptic plasticity over the broader Schaffer collateral - CA1 hippocampal network using a fast voltage-sensitive absorbance dye and 128x128 CMOS camera sampled at 1250 Hz. Acute hippocampal brain slice preparations from 2-3 month old 3xTg-AD and control mice were used. The goal was to compare the magnitude of synaptic plasticity and calcium signals evoked in individual CA1 neurons and spines by Schaffer collateral stimulation, with synaptic plasticity responses throughout all regions of CA1. At the single neuron level, we found that calcium responses evoked by an LTP-inducing stimulus were 3-fold greater in dendrites and spines from 3xTg-AD mice compared to NonTg animals. In parallel, there was an increase in the frequency of spontaneous vesicle release in 3xTg-AD mice, and markedly reduced post-tetanic potentiation (PTP), a short-term form of calcium-dependent plasticity. Short-term potentiation (STP), a form of plasticity that likely sets the threshold for LTP, was also greatly reduced in 3xTg-AD CA1 neurons. Field potential recordings of PTP and STP from the CA1stratum radiatum subfield mirrored these findings. To map these observations from single neurons onto a larger network, we used VSD imaging to measure PTP and STP plasticity evoked by the same LTP-inducing stimulus throughout the CA1 subfield. We found a similar deficit of STP and PTP plasticity in 3xTg-AD animals vs controls in all CA1 subfields, including the stratum radiatum, stratum oriens, stratum lucidem, and stratum moleculare. The findings suggest that the increased synaptic calcium responses and reduced short term plasticity at the single cell level are reflected as synaptic plasticity deficits throughout CA1 before memory impairments emerge in AD mice.

Disclosures: S. Chakroborty: None. **E.S. Hill:** None. **G.E. Stutzmann:** None. **W.N. Frost:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Program#/Poster#: 793.13/I2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Région Rhône Alpes

ANR MAALAD

Title: A-beta oligomers induced actin cytoskeleton abnormalities and reduced synaptic plasticity in dendritic spines of cortical neurons

Authors: M. DOLLMEYER, T. J. RUSH, M. FRANDEMICHE, M. ROLLAND, E. BOREL, K. PERNET-GALLAY, F. LANTÉ, *A. BUISSON;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive deficits and memory loss due to synaptic damage. The amyloid beta peptide, deposition of which is a histo-pathological hallmark of AD, is known to be neuro- and synaptotoxic in its several forms. Soluble, low-molecular weight oligomers of amyloid beta (Aβo) are of specific interest, as they are known to occur prior to fibril formation and subsequent plaque deposition. Accumulating evidence suggests a causal role for Aβo in disease. The synaptotoxic mechanisms of Aβo are still unclear. This study aims to investigate how Aβo triggers spine dysfunction. Here we have used primary cortical neurons cultured from mouse to study the effects of Aβo on several synaptic features related to plasticity. Using video and confocal microscopy of live neurons, we exposed our culture neurons to chemical long-term potentiation (cLTP) protocol. For this purpose, we used a GABA-A receptor antagonist (bicuculine, Bic, 50 μM), and a K+-channel blocker (+4-amino pyridine, 4AP, 2.5 mM). We observed that spine volume is increased by our cLTP protocol. Pre-incubation with Aβo (at 100 nM for 15 min) reduced both the amount of intracellular free calcium and spine enlargement provoked by cLTP. The reduced calcium levels were observed both in the neuronal soma and within individual dendritic spines (using GCaMP-6 fast, a genetically encoded calcium indicator). Western blot analysis of samples subjected to a fractionation protocol to isolate post-synaptic density- (PSD) enriched and Non-PSD-enriched synaptic membrane compartments allowed several observations: exposure to Aβo disrupted two important plasticity related and stimulation-induced responses of dendritic spines: increase in the AMPA-receptor GluA1 subunit at the PSD and ERK pathway activation. Next, we performed live imaging confocal microscopy with a filamentous actin (F-actin) marker (LifeAct) to observe actin cytoskeleton in spines. We showed that short period of Aβo exposure (at 100nM) did not induce any spine loss. However, we observed an increase in spine size, characterized by the presence of F-actin enriched membrane protrusions at the spine apex. These morphological abnormalities occurred in a subset of spines characterized as mushroom spines. Understanding the mechanism(s) by which Aβo affects actin cytoskeleton and its related effectors at the spine levels would be useful to developing therapies targeting synaptic plasticity deficits in AD. Fondation Neurodis and ANR MAALAD

Disclosures: **M. Dollmeyer:** None. **A. Buisson:** None. **T.J. Rush:** None. **M. Frandemiche:** None. **M. Rolland:** None. **E. Borel:** None. **K. Pernet-Gallay:** None. **F. Lanté:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG44712

NIH AG14449

Title: Locus coeruleus projection system cellular and molecular dysfunction in amnestic mild cognitive impairment

Authors: *S. E. COUNTS[1,2], S. D. GINSBERG[3,4,5], E. J. MUFSON[6];
[1]Transl Sci. and Mol Med., [2]Family Med., Michigan State Univ., Grand Rapids, MI; [3]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY; [4]Psychiatry, [5]Physiol. and Neurosci., NYU Langone Sch. of Med., New York, NY; [6]Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: A major feature of Alzheimer's disease (AD) is the loss of noradrenergic locus coeruleus (LC) projection neurons that mediate attention, memory, and arousal. However, the extent to which the LC projection system degenerates during the initial stages of AD remains unclear. To address this question, we performed tyrosine hydroxylase (TH) immunohistochemistry and unbiased stereology of LC neurons in tissue harvested postmortem from subjects who died with a clinical diagnosis of no cognitive impairment (NCI), amnestic mild cognitive impairment (aMCI, a prodromal AD stage), or mild AD (n = 5-6/group). Stereologic estimates of total LC neuron number revealed a 30-35% decrease in aMCI vs. NCI ($p < 0.01$) and a 45% loss of cells in mild AD compared to NCI ($p < 0.01$). Furthermore, LC fiber density was selectively reduced in the hippocampus compared to the neocortex of aMCI subjects, suggesting that coeruleo-hippocampal pathway degeneration marks the transition from normal cognition to prodromal disease. To examine the molecular pathogenic processes underlying noradrenergic neurodegeneration in aMCI, we combined laser capture microdissection with custom microarray technology to quantify gene expression patterns in individual TH-immunopositive neurons accessed from LC tissue samples. These studies revealed significant reductions in select functional classes of mRNAs regulating mitochondrial metabolism (e.g., cytochrome c1, cytochrome oxidase subunit 5a, $p < 0.01$), redox homeostasis (e.g., superoxide dismutase 2, glutathione peroxidase 1, $p < 0.01$) and cytoskeletal plasticity (e.g., microtubule associated binding protein 1a, utrophin, $p < 0.01$) in both aMCI and AD subjects compared to NCI. Taken together, these observations show that noradrenergic LC projection system

degeneration is a prominent feature of aMCI and provides a rational basis for targeting LC neuroprotection as a disease modifying strategy.

Disclosures: S.E. Counts: None. **S.D. Ginsberg:** None. **E.J. Mufson:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG016573

AG00538

Title: Late onset Alzheimer's disease risk variants and duration of cognitive impairment show association with end-stage plaque load in the hippocampus

Authors: A. J. RAJIC[1], M. T. WOJNOWICZ[2], T. T. LEE[4], M. B. DICK[1], A. L. PIERCE[3], *W. W. POON[1];
[1]UCI MIND, [2]Dept. of Statistics, [3]Dept. of Neurol., UC-Irvine, IRVINE, CA; [4]Chapman Univ., Orange, CA

Abstract: Unlike Familial Alzheimer Disease (FAD), which is caused by single dominantly inherited mutations, late onset Alzheimer Disease (LOAD) is influenced by multiple genetic loci and environmental risk factors. LOAD is also characterized by greater variation in quantitative endophenotypes compared to FAD, including age of onset, rate of cognitive decline and end-stage pathology. Recent studies have attempted to elucidate the effect of single nucleotide polymorphisms (SNPs) identified by GWAS on various quantitative endophenotypes in LOAD populations. In particular, pathway analyses have been employed to identify combinatorial effects of physiologically related risk loci on these endophenotypes. Here, we tested the hypothesis that risk SNPs located within genes encoding proteins involved in APP endocytosis(PICALM, CD2AP, BIN1) and subsequent Aβ metabolism(APOE) within the CNS affect the rate of plaque deposition in AD patients. We developed a genetic risk score based on the number of risk alleles within the endocytic pathway. Our preliminary results demonstrate that the genetic risk score combined with duration of cognitive decline correlate with end-stage

hippocampal plaque burden. These results suggest that the endocytic pathway interacts with APOE to increase the rate of plaque formation in LOAD.

Disclosures: **A.J. Rajic:** None. **M.T. Wojnowicz:** None. **T.T. Lee:** None. **M.B. Dick:** None. **A.L. Pierce:** None. **W.W. Poon:** None.

Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant DA 09082

NIH Grant DA020129

Philadelphia Health Education Corporation

Title: Amyloid beta co-localizes with dopamine beta hydroxylase in axon terminals of the rat frontal cortex

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]Dept. of Biol., Drexel Univ. Col. of Arts and Sci., Philadelphia, PA

Abstract: Alzheimer's disease (AD) currently affects 5.2 million Americans and has no effective therapeutic treatment. Characterized by a progressive loss of neuronal function marked by an accumulation of amyloid beta (Aβ) peptides that aggregate to form Aβ plaques, and hyper-phosphorylated tau proteins that form neurofibrillary tangles, the mechanisms by which these features ultimately lead to neuronal death are not understood. One of the earliest sites to be affected in AD is the locus coeruleus (Zarrow et al., 2003, Arch Neurol 60:337-341), the primary site of synthesis for the neurotransmitter norepinephrine (NE) that sends projections to almost all levels of the neuraxis. While it is known that NE is produced from dopamine by the enzyme dopamine-β-hydroxylase (DBH), the precise details regarding its interaction with cellular machinery involved in Aβ processing has yet to be described. Dysregulation of NE results in complex and poorly understood interactions involving neuroinflammation that hinders Aβ degradation, therefore promoting plaque formation (Chalermpalanupap et al., 2013, Alzheimer's Research & Therapy, 5:21). *In vitro* studies utilizing neuron-like chromaffin cells demonstrate

the co-localization of Aβ with catecholamine neurotransmitters in dense core secretory vesicles (DCSV) required for synaptic transmission (Toneff et al., 2013, Peptides, 46:126-35). However, interactions between Aβ and NE have not been elucidated *in vivo*. In the present study, we examined the cellular substrates for interactions between Aβ and DBH, a marker of noradrenergic axon terminals in the rat frontal cortex (FC) using light, immunofluorescence and electron microscopy. Light microscopic analysis indicated that Aβ-immunoreactive processes are present in the FC as previously described in the mouse. Confocal immunofluorescence microscopy revealed that Aβ-immunoreactive processes are co-localized with DBH fibers and processes. Using high resolution immunoelectron microscopy Aβ is localized in both somatodendritic processes and axon terminals. When localized in axon terminals, Aβ is evident in dense core vesicles of DBH-containing axon terminals. Semi-quantitative preliminary analysis revealed that 21% of DBH-containing axon terminals (31/154) also exhibited Aβ-immunogold silver particles that are predominantly associated with dense core vesicles. These results suggest an anatomical basis for the regulated co-secretion of Aβ with NE from dense core vesicles. An on-going study aims to investigate the cellular substrate of interactions between Aβ and a noradrenergic marker in an AD transgenic mouse model.

Disclosures: J. Ross: None. **B.A.S. Reyes:** None. **A. Saunders:** None. **E.J. Van Bockstaele:** None.

Poster

793. Alzheimer's Disease: Genetics and Biology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 793.17/I6

Topic: C.05. Aging

Title: Age-related changes of the axon initial segment and subcellular localization of tau protein in the rat brain

Authors: *A. KNEYNSBERG, T. GRABINSKI, N. M. KANAAN;
Neurosci. Program, Michigan State Univ., Grand Rapids, MI

Abstract: Aging is the primary risk factor for numerous neurodegenerative diseases, including Alzheimer's disease (AD). Yet, little is known of the specific aging-related contributions that directly lead to neuron dysfunction and death in AD. AD is characterized by the pathological accumulation of the microtubule-associated protein tau and a substantial body of evidence supports the hypothesis that tau plays an important role in disease pathogenesis. The progressive

accumulation of tau pathology occurs in the brain regions responsible for cognitive functions (e.g. the hippocampus) that are compromised in AD and correlates well with progressive cognitive decline. In healthy neurons, tau is enriched in the axonal compartment, where it can stabilize microtubules and play a role in regulating axonal transport. The preferential localization of tau in axons is mediated, at least in part, by aspects of the axon initial segment (AIS), acting as a retrograde barrier. The redistribution of tau from the axonal compartment to the somatodendritic compartment is thought to be an important event in tau-mediated toxicity because tau can no longer perform its axonal functions and may actively cause problems in the soma and dendrites. Thus, disruption in the stability of the barrier properties of the AIS may allow tau to diffuse freely back into the somatodendritic compartment and potentially lead to neurotoxicity. We will examine the effects of aging on AIS integrity and tau protein localization to better understand aging-related changes in aspects of tau biology that may facilitate neuronal dysfunction. Using a combination of immunohistochemical and immunoblotting approaches we will analyze AIS proteins/structure and tau protein localization in the aging rat brain. Specifically, changes in the AIS and the distribution of tau will be analyzed in hippocampal neurons of young, middle-, and old-age (4, 14, and 24 months) Fischer 344 rats. AISs (i.e. Ankyrin G positive) will be quantified using stereological methods, while the localization of tau in the somata of neurons will be measured using confocal microscopy and Licor image analysis. Changes in the overall level of AIS and tau proteins will be measured using immunoblotting techniques. Collectively, these studies may identify whether aging is characterized by changes in the AIS or associated with a redistribution of tau from the axon to the somatodendritic compartment.

Disclosures: **A. Kneynsberg:** None. **T. Grabinski:** None. **N.M. Kanaan:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.01/I7

Topic: C.03. Parkinson’s Disease

Title: Progressive nigrostriatal neurodegeneration associated with α-synuclein spreading and pathology induced by AAV-mediated overexpression of mutant α-synuclein in mice, rats and marmosets

Authors: *S. DOVERO[1], M. BOURDENX[1], M. BASTIDE[1], S. BIDO[1], M. ENGELN[1], C. PIRON[1], F. GEORGES[2], D. SCHELLER[3], A. MICHEL[3], T. BORAUD[1], P.-O. FERNAGUT[1], E. BEZARD[1], B. DEHAY[1];
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Abstract: Animal models are an essential asset for basic pathophysiological research as well as validation of therapeutic strategies of human diseases. The absence of adequate *in vivo* experimental models has severe repercussions for therapeutic intervention success. Despite unprecedented progress in animal modelling, no mammalian model recapitulates the required age-dependent phenotypes associated with Parkinson's disease (PD). We selected an elegant series of models of ascending complexity, i.e. two different mouse strains (C57Bl/6J and senescence-accelerated mouse (SAMP8), as a model of aging), adult rats and marmoset monkeys (Callithrix jacchus) at various ages. Each model received a stereotactic injection in the substantia nigra pars compacta (SNpc) of high-titer (10E10 vg/g of body weight) bolus of adeno-associated virus (AAV) serotype 9 carrying mutant human α-synuclein (A53T) under the neuron specific synapsin promoter including a WPRE enhancer element. Sixteen weeks after injection, we systemically investigated all species specifics and age-dependent differential susceptibility of dopamine neurons regarding the extent and pattern of neuronal loss. We also assessed α-synuclein spreading, pathological state (i.e. S129 phosphorylation) and occurrence of intracellular inclusion formation. According to the species used, we observed differences in the PD-related neurodegeneration progression over time associated with α-synuclein pathology and spreading in many brain areas.

Disclosures: S. Dovero: None. **M. Bourdenx:** None. **M. Bastide:** None. **S. Bido:** None. **M. Engeln:** None. **C. Piron:** None. **F. Georges:** None. **D. Scheller:** None. **A. Michel:** None. **T. Boraud:** None. **P. Fernagut:** None. **E. Bezard:** None. **B. Dehay:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.02/I8

Topic: C.03. Parkinson's Disease

Support: 1F31NS084739-01

Title: Increased vesicular monoamine transporter 2 (VMAT2) expression opposes dopaminergic neurotoxicity in the nigrostriatal pathway

Authors: *K. M. LOHR[1], M. WANG[2], A. SALAHPOUR[3], T. S. GUILLOT[2], G. W. MILLER[2]; [1]Envrn. Hlth., [2]Emory Univ., Atlanta, GA; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: The vesicular monoamine transporter 2 (VMAT2; Slc18a2) is responsible for packaging the monoamines into vesicles for subsequent release and neurotransmission. We have previously shown that BAC-transgenic VMAT2-overexpressing mice (VMAT2-HI) have an increased vesicular capacity for dopamine and increased synaptic dopamine release. In addition to its importance in transmitter packaging, VMAT2 is also critical for sequestering both exogenous and endogenous toxicants away from their sites of action inside the neuron. Recent human data have even suggested that enhanced VMAT2 level may confer a reduced risk of Parkinson's disease. To examine the neuroprotective effects of increased VMAT2, we treated VMAT2-HI mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an established parkinsonism-inducing toxicant model. Following a 2 x 15 mg/kg MPTP dose, VMAT2-HI mice show only a 15% loss of tyrosine hydroxylase (TH) and a 55% loss of the dopamine transporter (DAT) in the striatum, compared to wildtype losses of 43% and 66%, respectively. VMAT2-HI mice also show only a 36% loss of TH+ neurons in the substantia nigra pars compacta compared to the 53% cell loss seen in wildtype littermates after a 5 x 20 mg/kg MPTP dosing regimen. Additionally, we show that elevated VMAT2 is protective against striatal terminal damage following methamphetamine treatment, another classic dopaminergic toxicant. VMAT2-HI mice show a 17.9% DAT loss, compared to the 59% DAT loss seen in wildtype littermates after a 4 x 10 mg/kg dose of methamphetamine. These data suggest that elevated VMAT2 levels may be advantageous in disease states characterized by vulnerable dopamine neurons, such as Parkinson's disease. Thus, in a hypo-dopaminergic disease state, increasing vesicle function would provide two therapeutic benefits: restoration of dopaminergic signaling and protection against further damage from neurotoxic species in the cytosol. Supported by 1F31NS084739-01

Disclosures: K.M. Lohr: None. **M. Wang:** None. **A. Salahpour:** None. **G.W. Miller:** None. **T.S. Guillot:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.03/I9

Topic: C.03. Parkinson's Disease

Support: T32 NS0007480

Title: Preservation of serotonin neurons in mice with reduced vesicular monoamine storage

Authors: *S. ALTER[1], T. N. TAYLOR[3], T. S. GUILLOT[2], G. W. MILLER[2];
[1]Envrn. Hlth., [2]Emory Univ., Atlanta, GA; [3]Univ. of Minnesota, Minneapolis, MN

Abstract: Basic and clinical research indicate that synaptic vesicle function is compromised in Parkinson's disease, while insufficient vesicular transport of catecholamines has been demonstrated to be neurotoxic. We have previously reported that mice with drastically reduced expression of the vesicular monoamine transporter 2 (VMAT2 LO) undergo progressive catecholaminergic degeneration in the substantia nigra [J. Neurosci 27, 8138 (2007)] and locus ceruleus [Neuropharmacology 76A, 97 (2014)], and exhibit motor and nonmotor symptoms of Parkinson's disease [J. Neurosci 29, 8103 (2009)]. In the present work, we examined the effects of VMAT2 deficiency on the serotonin system. We assessed the integrity of the serotonin system with immunohistochemistry and radioligand binding, and examined behaviors associated with serotonergic signaling. Notably, no differences were observed in the number of TPH2+ neurons within the dorsal raphe in young (3 months) and aged (24 months) mice. Serotonergic innervation in the forebrain was also spared, as determined by SERT immunoreactivity and paroxetine binding. VMAT2 LO mice exhibited depressive-like behaviors (increased immobility in forced swim and tail suspension tests) that were responsive to fluoxetine. Additionally, VMAT2 LO mice have alterations in 5HT receptor-mediated physiology and behavior. VMAT2 LO mice exhibited an abolished hypothermic response to the 5HT1a agonist 8-OH-DPAT, suggesting reduced autoreceptor sensitivity; in contrast VMAT2 LO mice had a ~65% increase in head twitch response following treatment with the 5HT2 receptor agonist DOI, indicating increased post-synaptic receptor sensitivity. PD consistently features degeneration of the dopaminergic and noradrenergic systems, but the serotonergic innervation is often spared, and has been postulated to compensate for the catecholaminergic loss. Collectively, these findings in VMAT2 LO mice demonstrate that loss of vesicular monoamine transport is not toxic to the integrity of serotonin system (despite neurochemical loss), but may contribute to aberrant signaling in PD in which vesicular function is compromised. Supported by T32 NS0007480

Disclosures: S. Alter: None. **T.N. Taylor:** None. **T.S. Guillot:** None. **G.W. Miller:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: KU Leuven, Industrial Research Fund (IOF)

IWT Vlaanderen

KU Leuven program financing IMIR

EC-FP7 project INMiND

Title: Development and optimization of an alpha-synuclein split-reporter complementation assay for drug discovery purposes

Authors: *A. MICHIELS[1,2], S.-A. AELVOET[1], C. VAN DEN HAUTE[1,2], Z. DEBYSER[3], R. GIJSBERS[3,2], V. BAEKELANDT[1];
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Abstract: Alpha-synuclein (α-SYN) is considered a key player in Parkinson's disease (PD). Alpha-SYN aggregates under pathological conditions into soluble oligomers and protofibrils and finally insoluble β-sheet-rich fibrils. Although the exact relationship between α-SYN aggregation and pathogenesis remains elusive, inhibition of α-SYN oligomerization is explored as a potential therapeutic strategy. We developed a bioluminescent protein-protein complementation assay based on split firefly luciferase (fLuc) and viral vector technology to obtain stable expression to quantify α-SYN oligomerization. Both in different human immortalized cell lines (i.e. HEK293T, HeLa, SH-SY5Y) and non-invasively in the mouse brain, we demonstrated that expression of a combination of two complementary split-fLuc α-SYN fusions resulted in a significantly higher bioluminescent signal than either split-fLuc α-SYN fragment alone or in combination with a non-related partner. Injection of the split-fLuc α-SYN viral vectors in the mouse substantia nigra (SN) led to extensive dopaminergic degeneration, reminiscent of what is observed in our in-house developed PD animal models based on viral vector-mediated overexpression of α-SYN in the SN. In addition, we could demonstrate that the bimolecular interaction reflected by the successful luciferase complementation results from pre-aggregate oligomeric α-SYN species. Moreover, we detected an inhibitory effect of the small molecule FK506 on the oligomerization process both in cell culture and in the mouse brain, underscoring the potential of our models to evaluate small molecules. Subsequently, we optimized and upscaled the cell-based split-fLuc α-SYN oligomerization assay towards a high throughput screening assay ($Z' > 0.5$). Together, these data indicate that α-SYN split-fLuc viral vectors can

be applied to discover small molecule compounds inhibiting α-SYN oligomerization in cell lines and to non-invasively monitor α-SYN oligomerization in live animals.

Disclosures: **A. Michiels:** None. **S. Aelvoet:** None. **C. Van den Haute:** None. **Z. Debyser:** None. **R. Gijsbers:** None. **V. Baekelandt:** None.

Poster

794. Parkinson's Disease Models III

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Program#/Poster#: 794.05/I11

Topic: C.03. Parkinson's Disease

Support: KU Leuven, Industrial Research Fund (IOF)

IWT Vlaanderen

KU Leuven program financing IMIR

EC-FP7 project INMiND

MJFF

Title: Robust and reproducible AAV-based alpha-synuclein rodent models suitable for drug discovery purposes

Authors: A. MICHIELS[1,2], A. VAN DER PERREN[1], M. OLIVERAS-SALVÁ[1], C. VAN DEN HAUTE[1,2], Z. DEBYSER[3], *V. BAEKELANDT[1];
[1]Dept. of Neurosciences, KU Leuven, Lab. for Neurobio. and Gene Therapy, Leuven, Belgium; [2]Leuven Viral Vector Core, KU Leuven, Leuven, Belgium; [3]Pharmaceut. and Pharmacol. Sci., KU Leuven, Lab. for Mol. Virology and Gene Therapy, Leuven, Belgium

Abstract: Alpha-synuclein (α-SYN) is a key protein implicated in the pathogenesis of Parkinson's disease (PD). Testing of new therapeutic strategies targeting α-synucleinopathy is currently hampered by the lack of robust and reproducible α-SYN-based animal models that display the hallmark features of PD, especially progressive dopaminergic neurodegeneration over time. We developed and optimized a rat and mouse PD model based on viral vector-mediated overexpression of α-SYN. An adeno-associated viral (rAAV) serotype 2/7 vector encoding A53T mutant or wild-type α-SYN was stereotactically injected into the substantia nigra

(SN) of rats or mice and the effects were determined by histology, non-invasive imaging and behavioral analysis. We noted a significant and dose-dependent α-synucleinopathy over time upon nigral rAAV2/7-mediated α-SYN overexpression. A strong, progressive and dose-dependent loss of approximately 80% of the dopaminergic neurons in the SN was observed at 4 to 8 weeks post-injection in rat and mouse, respectively. This effect correlated with a reduction in tyrosine hydroxylase immunoreactivity in the striatum. In rats, monitoring of dopamine transporter binding with micro-PET scan revealed the most extensive dopaminergic degeneration between day 7 and day 21 post-injection. Moreover, behavioral analysis revealed significant motor impairments in both rats and mice. In addition, we detected the presence of α-SYN-positive aggregates in the remaining surviving neurons. In conclusion, we have developed robust and reproducible rodent models for α-synucleinopathy allowing rapid and thorough evaluation of new therapeutic strategies *in vivo*.

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Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.06/I12

Topic: C.03. Parkinson’s Disease

Support: European Commission's Seventh Framework Programme CP-IP 258654

Title: Translational methods for qualitative and quantitative assessment of locomotor deficits and therapeutic improvement in pre-clinical models and patients with Parkinson’s disease

Authors: *I. VOLLENWEIDER[1], D. BORTON[1], J.-B. MIGNARDOT[1], J. LAURENS[1], L. BAUD[1], Y. LANG[2,3], L. QIN[4,3], J. BLOCH[5], J.-A. GHIKA[6], E. BEZARD[2,3,4], G. COURTINE[1,5]; [1]Ctr. for Neuroprosthetics and Brain Mind Institute, EPFL, Lausanne, Switzerland; [2]Inst. of Neurodegenerative diseases, Univ. Victor Segalen-Bordeaux 2, Ctr. Natl. de la Recherche Scientifique, Bordeaux, France; [3]Motac Neurosci. Ltd, Manchester, United Kingdom; [4]Chinese Acad. of Med. Sci. & Peking Union Med. Col., Beijing, China; [5]Univ. Hosp. of Lausanne, Lausanne, Switzerland; [6]Clinique romande de réadaptation SUVA, Sion, Switzerland

Abstract: Patients with Parkinson’s disease (PD) patients experience progressive motor impairments, including severe gait deficits. The past 2 decades have seen unprecedented

preclinical and clinical efforts to identify and validate novel antiparkinsonian therapies. Non-human primate (NHP) models of PD played a pivotal role for the safe and efficacious translation of therapeutic interventions developed in rodents to viable clinical applications for humans. Notably, MPTP-treated NHPs remain the gold standard for modelling motor and non-motor symptoms of PD and providing preclinical validation of potential therapies. However, the lack of consensus on a methodology and the almost exclusive reliance on clinical scores of PD have often hindered successful translation of interventions to clinical settings. Consequently there is a critical need to develop translational methodologies to enable robust and reliable evaluation of therapies across species. Here, we leveraged a newly established multimodal analysis platform to characterize alteration of kinematic and muscle activity during unconstrained locomotion in MPTP-treated NHPs. The connection-free platform allowed us to monitor muscle synergies and kinematics while the subjects walked on a treadmill, along a corridor, and across horizontal ladders of varying complexities. Using principal component analysis (PCA) applied on a large number of parameters, we could objectively quantify and rank task-specific deficits of gait patterns across a wide range of PD severities. This analysis also uncovered kinematic parameters that were specifically improved after the administration of L-Dopa, and others that remain resistant to dopamine replacement therapy. To assess the translational value of the developed analyses, we recorded Parkinsonian patients and rats with alpha-synuclein-mediated nigrostriatal degeneration under the same conditions as NHPs. We found striking similarities in the pattern of gait deficits across species, and on the therapeutic effects mediated by L-Dopa. The developed methodologies establish a highly-sensitive analytic framework to evaluate safety and efficacy of therapeutic interventions to alleviate locomotor deficits in animal models of PD and in Parkinsonian patients.

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Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.07/J1

Topic: C.03. Parkinson’s Disease

Support: The European Commission's Seventh Framework Programme (CP-IP 258654)

Title: Neuromodulation of spinal circuits to alleviate gait deficits in the alpha-synuclein rat model of Parkinson disease

Authors: *L. BAUD[1], N. WENGER[1], Q. BARRAUD[1], I. VOLLENWEIDER[1], E. MARTIN-MORAUD[2], M. CAPOGROSSO[2], J. GANDAR[1], P. DETEMPLE[3], P. MUSIENKO[1], S. MICERA[2], E. BEZARD[4], G. COURTINE[1];
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Abstract: Electrical neuromodulation of cervical segments increased spontaneous locomotor activity in rodent models of Parkinson's disease. However, the therapeutic effects of this paradigm have been highly variable in human patients. The use of large electrodes in animal models versus small electrodes in humans triggered a debate on the importance of electrode dimensions, electrode location, and spatial distribution of the generated voltage fields to mediate therapeutic effects. To evaluate the importance of these parameters, we leveraged a newly developed multi-electrode array that enables generation of localized or distributed voltage fields in the same neural interface. Rats were injected with AAV9 α-synuclein in the substantia nigra to induce nigrostriatal neurodegeneration. Detailed kinematic analysis during basic and skilled locomotion uncovered significant gait deficits and reduced locomotor activity that emerged around 2 months post-injection. After the occurrence of parkinsonian symptoms, we tested the therapeutic impact of electrical neuromodulation delivered through single electrodes or combinations of electrodes distributed along lumbosacral segments. We used a computational model to identify the electrical fields generated by each electrode configuration. We found that significant improvement of locomotor performance required fine-tuning of neuromodulation parameters through multiple electrodes distributed along lumbar and sacral segments. These results highlight the critical importance of mechanistic models to develop effective neuromodulation therapies that alleviate locomotor deficits after neuromotor disorders.

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Poster

794. Parkinson's Disease Models III

Location: Halls A-C

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Program#/Poster#: 794.08/J2

Topic: C.03. Parkinson's Disease

Title: Dopamine neuron produced Sonic Hedgehog (Shh) is a critical modulator of Acetylcholine neurons and important for reinforcement learning

Authors: *A. W. STUCKY[1], A. KOTTMANN[1,2];
[1]Physiology, Pharmacol. and Neurosci., Sophie Davis Sch. of Biomed. Education, CUNY, New York, NY; [2]Dept. of Pathology and Cell Biol., Columbia Univ., New York, NY

Abstract: Parkinson's Disease (PD) is recognized as a multi-systems, progressive neurodegenerative disorder with motor - and non motor - manifestations including difficulties in the expression of automatic, habitual components of motor behaviors and an increase in the display of volitional and goal directed behaviors. PD is characterized by a progressive dopaminergic and cholinergic dysfunction that spreads anteriorly and eventually involves the basal ganglia, the basal forebrain and the cortex. We find that all mesencephalic dopamine (DA) neurons express the secreted cell signaling factor sonic hedgehog (Shh) throughout life, and that DA neurons produced Shh is critical for the long term survival of cholinergic (ACh) neurons throughout the forebrain and fast spiking (FS), parvalbumin+, interneurons in the striatum [1]. We revealed that acute Shh regulates the efficacy of muscarinic autoreceptor signaling in a dose dependent manner leading to a modulation of the levels of extracellular acetylcholine. We further find that DA produced Shh regulates dendritic arborization of ACh neurons and the location and size of glutamatergic boutons on the soma of ACh neurons in the striatum, which likely impacts cortico-striatal neuro-transmission. Importantly, Shh signaling is graded across functionally distinct domains in the striatum, with the greatest impact in the dorsal lateral striatum, an area crucial for reinforcement learning and motor habit formation. Consistent with this finding we reveal that animals without Shh expression in mature DA neurons manifest hypercholinergic sensitivity, habituation deficits and impaired reinforcement learning before developing motor signs. Our data indicate that altered Shh expression in DA neurons of PD patients could contribute to the manifestation of cognitive dysfunction in PD. We will present recent data on the dynamic regulation of Shh expression in adult DA neurons and on the involvement of Shh signaling from DA neurons in the devaluation of reward and in motor habit formation.

Disclosures: A.W. stucky: None. **A. Kottmann:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

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Topic: C.03. Parkinson's Disease

Support: NIH Grant MH65561

NIH Grant MH73057

Title: Impulsivity in GDNF heterozygous mice receiving chronic unpredictable stress

Authors: *B. Z. YANG, M. BUHUSI, C. V. BUHUSI;
Psychology, Utah State Univ., Logan, UT

Abstract: Administration of dopamine (DA) agonists is a common therapy used to help people suffering from Parkinson's disease (PD). However, DA agonists have been found to be a double-edged sword: they alleviate the motor symptoms of PD, but increase the levels of impulsivity in these patients. Partial glial cell line-derived neurotropic factor deficient (GDNF+/-) mice have been found to have a marked reduction in striatal DA levels, hinting at a dysfunctional substantia nigra DA neurons, as well as an accelerated age-related deterioration of motor functions. These neuroanatomical and behavioral symptoms match those observed in patients suffering from PD, supporting the GDNF+/- mice model as a mouse model of PD. At the same time, chronic unpredictable stress has been found to aggravate the onset of experimentally induced PD in rats, suggesting the possibility of a double hit model for the onset of PD. Therefore, in our study we evaluated impulsivity in GDNF+/- mice and wildtype littermates(WT) via a temporal discounting paradigm before and after chronic unpredictable stress. In our paradigm, mice chose between a smaller-sooner (SS) option, and a larger-later (LL) option delivered at varying delays of 0s, 4s, 16s, and 64s for 7 days. Afterwards, mice were subjected to 18 days of daily unpredictable chronic stress. The type of stressor and the time of stress activity was pseudo-randomized throughout the course of the 18 days. Finally, mice were tested again with the same temporal discounting paradigm. Results indicate that prior to stress, GDNF+/- mice were reliably less impulsive than their WT counterparts, possibly due to lower levels of DA. Post-stress, GDNF+/- mice showed a reliable increase in impulsivity while WT mice exhibited a less steep (not reliable) increase in impulsivity. Post-stress, the levels of impulsivity in GDNF+/- mice were not reliably different from those of WT mice, suggesting that GDNF+/- mice may be more susceptible to stress than WT mice. Taken together, our findings support the GDNF+/- mice as a dual-hit mouse model of PD.

Disclosures: B.Z. Yang: None. **M. Buhusi:** None. **C.V. Buhusi:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.10/J4

Topic: C.03. Parkinson's Disease

Title: Aggregated α synuclein intoxication of primary dopaminergic neurons as an *in vitro* model of Parkinson disease

Authors: D. BUTTIGIEG, E. GRAS LAVIGNE, M. OUAMER, R. STEINSCHNEIDER, *B. DOROTHEE;
Neuron Experts, Marseille, France

Abstract: Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder leading to severe motor symptoms. Major pathological features of PD are the loss of dopaminergic neurons in the substantia nigra (SN) and the presence of intraneuronal proteinacious cytoplasmic inclusions (termed Lewy bodies, LB) majority composed by alpha-synuclein (a-syn), a pre-synaptic protein implicated in dopamine trafficking but also in neuronal protection. A large body of evidence suggests that aggregation of a-syn plays a crucial role in the pathogenesis because of its neurotoxic activity and its ability to trigger inflammatory process. To study the mechanisms by which aggregated a-syn acts, we developed a relevant *in vitro* cellular model where dopaminergic neurons are reproductively intoxicated by a-syn. For this, we first assessed a-syn toxicity on dopaminergic neuron survival according to its form (linear or prefibrillar) and preparation. After having confirmed the predominantly toxic effect of a-syn prefibrillar oligomers, its assumed most toxic form; we develop a new method to produce a-syn oligomers in a more predictable manner. We investigated its toxicity according to its concentration and time of incubation. Finally, we validated our cellular model by using several reference compounds and demonstrated protective and restorative effects of two neurotrophic factors belonging to the Glial Cell Line-Derived Neurotrophic Factors (GDNF) Family of Ligands (GFLs). This improved and reproducible cellular model displays a prerequisite to further experiments on a-syn involvement in Parkinson disease and drug discovery through *in vitro* high content screening.

Disclosures: D. Buttigieg: None. **E. Gras Lavigne:** None. **M. Ouamer:** None. **R. Steinschneider:** None. **B. Dorothee:** None.

Poster

794. Parkinson's Disease Models III

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.11/J5

Topic: C.03. Parkinson's Disease

Support: MJFF grant: Synuclein Therapeutic Acceleration Program 2013

Title: Correlation between different alpha-synuclein pathological species and onset and progression of behavioral and electrophysiological deficits in a viral mediated rat model of human wt alpha-synuclein overexpression

Authors: L. B. VESTERAGER[1], K. J. ANDERSEN[1], F. SOTTY[1], N. MAJBOUR[2], N. N. VAIKATH[2], S. MENON[2], *P. KALLUNKI[1], O. M. EL-AGNAF[2], K. FOG[1];
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Abstract: α-Synuclein has long been recognized as a key player and a potential therapeutic target for Parkinson's disease, as well as other synucleinopathies. Supporting this, mutations/multiplication in the gene encoding α-synuclein increases the risk for developing PD. Several observations underline the pathophysiological role of α-synuclein, including its presence in cellular inclusions in a range of neurodegenerative disorders in different parts of the brain, the association between increased α-synuclein expression and cell death or susceptibility to stress-induced toxicity in cells, as well as the causative link between α-synuclein overexpression and pathology in various species. While α-synuclein exists in different forms depending on the pathophysiological state, detection of aggregated and/ or phosphorylated α-synuclein in post-mortem brains has been widely accepted as a pathological marker of PD. To further elucidate the connection between α-synuclein and the progressive pathology of PD, the presence of total, phosphorylated as well as aggregated α-synuclein was analyzed in a rat model with virally-mediated overexpression of human wt α-synuclein targeted to the nigro-striatal dopamine system. A progressive loss of dopaminergic neurons in the substantia nigra leading to loss of terminals in the striatum was observed between 3 and 17 weeks post-virus injection, and was associated to progressive motor deficits. In addition, an abnormal firing pattern of neuronal populations in the basal ganglia circuits, including the subthalamic nucleus (STN) and the Substantia Nigra reticulata (SNr), was observed, recapitulating some of the neurophysiological alterations reported in PD patients. The progression of α-synuclein pathology in this animal model was evaluated from 3 to 17 weeks post virus injection, using three different methods: 1) ELISA detection using antibodies with a preferential binding to aggregated α-synuclein, 2)

Western Blotting of enriched fractions of higher molecular weight species of α-synuclein and 3) Immunohistochemistry. Analysis of the correlations between the levels of various pathological forms of α-synuclein and progression of behavioral and electophysiological deficits in our animals models is necessary in order to design studies aimed at investigating the effects of drugs targeting α-synuclein.

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Poster

794. Parkinson's Disease Models III

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Topic: C.03. Parkinson’s Disease

Support: Longevity Sciences (24-15) from NCGG, Japan

Title: The downregulation of longevity-related gene, FOXO3 induces the neurodegeneration of the Lewy body disease model cells

Authors: N. LONG[1], Y. K. NOSE[1], *M. MINAMIYAMA[1], N. MOTOYAMA[1], M. S. NAGAI[1], K. IBARAKI[1], T. HAYAKAWA[1], H. YAMADA[1], K. KANAMORI[1], A. YAMAOKA[2], M. NAOI[3], W. MARUYAMA[1];
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[3]Psychological and Physical Sci., Aichi Gakuin Univ., Nisshin, Japan

Abstract: Lewy body diseases (LBDs) are a series of neurodegenerative illness represented Parkinson’s disease (PD), and pathologically characterized by the neuronal inclusion (Lewy Body). Genetical and biochemical studies discovered the disease-related genes especially from the familial cases, but the pathogenesis has not been elucidated yet. On the other hand, the therapy for PD has become available with the modification of neurotransmitters. However, it cannot stop the progressing neuronal cell death. An appropriate model to understand the pathogenesis and to develop the cell therapy is needed. So far, mainly two types of LBDs model have been developed. One is the pharmacological model with neurotoxin like MPTP or rotenone, and another is the genetical model representatively with α-synuclein (α-syn), the major ingredient of Lewy body. The pharmacological model was originally found to cause

Parkinsonism by neurotoxicity, but it cannot explain all LBDs pathogenesis. On the other hand, the genetical model with the wild type of α-syn cannot induce the neuronal death. And so, we newly focused on the point that these diseases develop in elderliness, and are progressing with aging, and started to develop the new LBDs model with aging factor added. Here, we report the functional analysis of the longevity-related gene with the LBDs model cells, and the new model creation trial from the cell level to the mouse level in progress. FOXO3 (Forkhead box O3), which is one of the longevity-related genes, is a multifunctional transcription factor. It functions in stress response, apoptosis, tumor suppression, and etc. Also, it is reported to be colocalized in Lewy body of the patients' autopsied brain, which implicates that it may play a role for the LBD pathology. Therefore, we analyzed the influence of LBD model cells which were made by α-syn overexpression, with the downregulation of FOXO3 by RNA interferance. FOXO3 downregulated cells were more fragile compared with the control. And the α-syn overexpressed cells upregulated FOXO3, and this gene was suggested to have an association with stress response caused byα-syn. We searched the influence of the FOXO3 downregulation biochemically and pathologically with westernblotting, RT-PCR, and etc, and the autophagy system was found to be impaired in the FOXO3 downregulated cells. And α-syn monomer was found to be decreased by the knockdown of the FOXO3 gene. We are now studying more about the impairment of the autophagy, and about the influence for the oxidative stress. If possible, we also want to report about the creation trial of the new LBDs mouse model.

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Poster

794. Parkinson's Disease Models III

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Topic: C.03. Parkinson's Disease

Support: NIH Grant RO1 ES010975

Title: Retinal structural injury in animal models of parkinson's disease and chronic manganese exposure detected by optical coherence tomography

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Abstract: Optical coherence tomography (OCT) is a non-invasive procedure for analysis of retinal morphology. OCT has been effectively used to assess disease progression in multiple sclerosis (MS) patients and OCT and retinal imaging has been suggested as a surrogate measure for brain damage in MS patients. OCT may also be a valuable tool for longitudinally following other neurodegenerative conditions such as Parkinson's disease (PD) and potentially for monitoring disease progression as well as assessing efficacy of neuroprotective treatments. Recent studies using OCT to assess retinal histology *in vivo* in PD patients have reported significant inverse correlations between foveal or peripapillary retinal nerve fiber layer (RNFL) thickness and Unified Parkinson's Disease Rating Scale scores, and have even suggested the possibility of differentiating between rigid/akinetic and tremor dominant PD patients by differences in retinal morphology between these subgroups. The present study was conducted to investigate the extent to which changes in retinal morphology could be detected *in vivo* using OCT in an established animal model of PD, the MPTP-treated nonhuman primate. We also examined the extent to which OCT could detect retinal changes in a nonhuman primate model of chronic to exposure to manganese (Mn) at levels in the ranges of those reported for human environmental or occupational exposures. Eyes from 10 normal, neurologically intact adult male cynomolgus monkeys, 5 adult male cynomolgus monkeys previously made parkinsonian by chronic administration of MPTP, and 7 adult male cynomolgus monkeys with Mn exposures ranging from were examined from approximately 20 - 72 weeks. MPTP-treated animals were all moderately parkinsonian and exhibited stable parkinsonian signs of at least 4 years duration. Statistically significant reductions in RNFL thickness were found in MPTP-treated animals vs. controls particularly in nasal and inferior RNFL quadrants. Significant foveal thinning and decreases in macula volume were also detected. RNFL thinning was also observed in Mn-exposed animals with changes related to duration of Mn exposure. Foveal thickness and macula volume were not different from controls. These data show that in a nonhuman primate PD model, OCT imaging detects changes in the retina similar to those detected in PD patients. These data suggest that OCT may be a useful measure to employ in preclinical studies of PD and other neurodegenerative conditions and that retinal measurements may have sufficient specificity to be used as biomarkers for different neurodegenerative conditions.

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Poster

794. Parkinson's Disease Models III

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Topic: C.03. Parkinson's Disease

Support: EXPL/DTP-DES/0104/2013

PEst-C/SAU/UI3282/2011

Title: Quiescent RAGE in dysfunctional striata from MPTP-treated mice

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Abstract: Parkinson′s disease (PD) is a motor system disease characterized by a progressive dopaminergic nigrostriatal degeneration underlying a severe striatal dysfunction, which can be partially recapitulated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Although etiology of PD is not fully understood, recent data suggest the receptor for advanced glycation endproducts (RAGE), which plays a major role in oxidative stress and gliosis, seems to contribute to the pathogenesis. We aimed to characterize RAGE density and its cellular localization in striatum after a chronic MPTP paradigm (20mg/Kg i.p., 2 i.d-12h apart, 5 days/week for 2 weeks). C57BL/6 mice were euthanized 7 days following this toxic insult after probing their locomotor activity for striatal neurochemical analysis. This new chronic MPTP model triggered striatal dopaminergic nerve terminals toxicity gauged by a mild loss of tyrosine hydroxylase levels, severe dopamine (DA) depletion and by increased DA turnover. This monoamine disruption underlined motor impairments as assessed by the pole test and rota-rod. We provide novel evidence that RAGE-positive immunoreactivity was exclusively observed in striatal neurons of MPTP-treated mice in spite of an intense astrogliosis (GFAP and GS upregulation) and increased oxidative stress (augmented HNE adducts levels). Additionally, this MPTP paradigm failed to change striatal density of RAGE and its astrocytic ligand S100β. These findings demonstrate for the first time that striatal RAGE is quiescent in an astrogliotic and oxidative environment underlying striatal dysfunction in a PD experimental model. This research was supported by PEst-C/SAU/UI3282/2011 and by EXPL/DTP-DES/0104/2013 under the frame of "Programa Operacional Temático Fatores de Competitividade (COMPETE/QREN)" and "Fundo Comunitário Europeu (FEDER)". SDV is a recipient of a PhD grant from Fundação para a Ciência e a Tecnologia (FCT, Portugal, SFRH/BD/78166/2011).

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Poster

794. Parkinson's Disease Models III

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Topic: C.03. Parkinson's Disease

Support: NSF of Korea 2012-008656

Title: Molecular and neural correlates of olfactory dysfunction by paraquat-induced oxidative stress and modeling for Parkinson disease in *Drosophila* melanogaster

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Abstract: An environmental toxicant like paraquat is known to induce the increase of oxidative stress, which is likely to lead to various neuropathological symptoms. One of the ultimate questions is what are the molecular and neural target eliciting various neural diseases as time proceeds. Here in this study, using a fruit fly, *Drosophila melanogaster*, as a model animal, we have examined the effect of paraquat on olfactory sensation and behaviors as well as molecular targets based on this phenomenon. Dietary feeding of paraquat with sucrose solution demonstrates that olfactory sensitivity in the periphery was decreased and olfactory behaviors such as food choice and olfactory learning and memory were affected. Present study also demonstrates that the dietary feeding of phytochemicals prior to paraquat exposure has anti-oxidative and neural protective effects, which leads to the recovery of behaviors and lifespan in fruit flies. We will present the more details on molecular and neural correlates associated with this environmental toxicant.

Disclosures: **H. Kwon:** 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.; NRF of Korea. **J. Jung:** None. **H. Lee:** None.

Poster

794. Parkinson's Disease Models III

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Taiwan Chang Gung Memorial Hospital CMRPG8A0151

Title: Effects of 6-hydroxydopamine exposure on motor activity and biochemical expression in zebrafish (Danio rerio) larvae

Authors: *C. W. FENG[1,2], H.-C. HUNG[2], C.-H. CHEN[2], Z.-H. WEN[2,3], S.-Y. HUANG[3], W.-F. CHEN[4,5];
[1]Natl. Sun Yat-Sen Univ., Kaohsiung, Taiwan; [2]Doctoral Degree Program in Marine Biotech., Natl. Sun Yat-Sen University/Academia Sinica, Kaohsiung, Taiwan; [3]Dept. of Marine Biotech. and Resources, Natl. Sun Yat-sen Univ., Kaohsiung, Taiwan; [4]Dept. of Neurosurg., [5]Ctr. for Parkinson's Dis., Kaohsiung Chang Gung Mem. Hosp. and Chang Gung Univ. Col. of Med., Kaohsiung, Taiwan

Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra. However, current treatments for PD are mainly palliative. Recently, researchers discovered that neurotoxins can induce Parkinsonian-like symptoms in zebrafish. No study to date has investigated the characteristics of PD, such as neuroinflammation factors, oxidative stress, or ubiquitin dysfunction, in this model. Therefore, the current study was aimed at utilizing commonly used clinical drugs: minocycline, vitamin E, and Sinemet, to test the usefulness of this model. Previous studies had indicated that dopaminergic cell loss was greater with 6-hydroxydopamine (6-OHDA) than with other neurotoxins. Thus, we first challenged zebrafish with 6-OHDA immersion and found a significant reduction in zebrafish locomotor activity, we then reversed the locomotor disruptions by treatment with vitamin E, Sinemet, or minocycline. The present study also analyzed the mRNA expression of *parkin*, *pink1*, and *cd-11b*, because expression of these molecular targets has been shown to lead to attenuation in mammalian models of PD. Vitamin E, Sinemet, and minocycline significantly reversed 6-OHDA-induced changes of *parkin*, *pink1*, and *cd-11b* mRNA expression in zebrafish. Moreover, we assessed tyrosine hydroxylase (TH) expression to confirm the therapeutic effects of vitamin E tested on this PD model and established that vitamin E reversed the 6-OHDA-induced damage on TH expression. Our results provide some support for the validity of this *in vivo* Parkinson's model, and we hope that this model will be more widely used in the future.

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Poster

794. Parkinson's Disease Models III

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Harvard Stem Cell Institute

Title: Axon guidance molecules in Parkinson's disease

Authors: ***J. A. KORECKA**, J. R. MCLEAN, J. L. JANSSON, J. A. BEAGAN, Z. SCHNEIDER-LYNCH, D. AHMADI, T. M. OSBORN, O. ISACSON;
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Abstract: Recently important evidence came to light implicating axonal degeneration as an early hallmark of developing pathology in Parkinson disease (PD). More importantly, gene expression studies, genome wide association studies (GWAS) of single-nucleotide polymorphism variations and pathway analysis studies identified axon guidance signaling pathways to be associated with PD development (Edwards et al., 2011, Plos One;6(2):e16917, Bossers et al, 2009, Brain Pathol;19(1):91-107, Lin et al., 2009, Trends Neurosci;32(3):142-9, Srinivasan et al., 2009, Hum. Mutat;30(2):228-38, Lesnick et al., 2007, Plos Genet;3(6):e98, Maraganore et al., 2005, Am J Hum Genet;77(5):685-93). Based on GWAS and pathway analysis Lin et al. (2009, Trends Neurosci;32(3):142-9) identified five axon guidance genes associated and predictive of PD outcome: DCC, EPHB1, NTNG1, SEMA5A and SLIT3. Axon guidance cues persist to be expressed in the adult CNS maintaining the structural plasticity of the neuronal circuits (Mironova and Giger, 2013, Trends Neurosci;36(6):363-73). It is thought that through this maintained expression of guidance cues in the adult host brain neuronal transplants reconnect their dopaminergic (DA) axons to their specific target- the dorsal lateral striatum (Isacson & Deacon 1996, Neuroscience;75(3):827-37). Mouse ventral midbrain (VM) primary cultures and DA neurons derived form embryonic stem cells are sensitive to Slit and Netrin

signaling (Lin et al., 2005, Mol Cell Neurosci;28(3):547-55, 2006, Stem Cells;24(11):2504-13). For the first time we show that human iPS cell-derived DA neurons may also be sensitive to known DA axon guidance regulators by expressing Ephrin and DDC receptors. Preliminary data from human iPS cell-derived DA neurons indicate that LRRK2 may play a role in neurite outgrowth regulation. IPS cell-derived DA neurons carrying LRRK2 mutations showed increased neurite collapse and lack of recovery after thapsigargin and alcohol stress *in vitro*. These data indicate that axon growth and maintenance is potentially aberrant in PD. We hypothesize that these changes can either take place in the years preceding the clinical diagnosis, or are already present during the development of CNS.

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Poster

794. Parkinson's Disease Models III

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Michael J. Fox foundation

Title: Characterization of G2019S-LRRK2 BAC transgenic rats

Authors: *H. ABDELMOTILIB, X. HU, J. DAHER, T. GROEN, A. B. WEST;
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Abstract: Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene can cause late-onset Parkinson’s disease (PD). Recently, transgenic rats that express LRRK2 from the human promoter with the pathological mutation G2019S became available. Localization of LRRK2 expression in these transgenic rats revealed high levels of LRRK2 in numerous brain nuclei and neuronal subtypes, strongly divergent from endogenous rat LRRK2 distribution. In initial characterizations, normal neuronal counts of neurons in the substantia nigra were observed, yet deficits in motor co-ordination compared to non-transgenic littermates suggested deficits in motor pathways. Characterization of these rats with a battery of motor and behavioral assays will help reveal the extent to which G2019S-LRRK2 expression alters normal physiology. LRRK2

kinase-activity dependent phenotypes will be defined through exposures to potent and selective LRRK2 inhibitors to begin to understand reversible deficits from neural developmental alterations. These studies may help provide valuable baseline data for a new pre-clinical model useful for the characterization of novel LRRK2-targeting therapeutics.

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Poster

794. Parkinson's Disease Models III

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Title: Overexpression of fragments of the kinase domain of LRRK2 in the rat substantia nigra produces cell disturbances but does not lead to dopaminergic cell loss or motor symptoms

Authors: *E. P. BROUILLET, N. CRESTO, M.-C. GAILLARD, E. DIGUET, D. BELLET, L. LEGROUX, L. FRANCELLE, J. MITJA, G. AURÉGAN, M. GUILLERMIER, D. HOUITTE, G. BONVENTO, C. JAN, F. PETIT, P. HANTRAYE, N. DÉGLON, K. CAMBON, A.-P. BEMELMANS;
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Abstract: Mutations in the LRRK2 gene are the most common genetic causes of autosomal dominant Parkinson’s disease (PD). Genetic models of LRRK2 mutations have been developed in animals and cell cultures. They provided new insights in the possible mechanisms underlying mutant LRRK2 toxicity, pointing to a key role of its kinase domain. However, transgenic models in rodents show limited neurodegeneration of dopaminergic neurones in the substantia nigra pars compacta (SNc). An alternative to transgenic models is the use of gene transfer technology with viral vectors. Adenoviral vectors and Herpes Simplex virus amplicons coding for mutant LRRK2 have been successfully used to induce SNc neurodegeneration in rodents. The aim of the present study was to evaluate the potential toxicity of lentiviral vectors (LV) and adeno-associated viral

(AAV) vectors expressing three different fragments of the C-terminal part of wild type (WT) or G2019S mutant LRRK2: the kinase domain (K), the ROC-COR-kinase (RCK) and the RCK-WD40 domain. *In vitro* autophosphorylation assays indicated that only the mutant RCK-WD40 domain had increased kinase activity as compared to the other fragments. Transcriptomic analysis of the rat striatum infected with LV-RCK-WD40 showed that the G2019S mutation produced cellular disturbances as compared to the WT fragment and a G2019S/D1994A double mutant fragment encoding a "dead-kinase". LVs coding the different constructs were stereotaxically injected in the rat SNc. Histological evaluation was carried out 10 and 25 weeks later. Results showed that 20-60% of the tyrosine-hydroxylase-(TH)-positive dopaminergic neurones were transduced with LV coding the WT or G2019S fragments. However, no loss of TH-positive cells could be evidenced at these time points. We also infected the SNc with AAV9 coding wild type RCK-WD40 domain or the RCK-WD40-G2019S mutant. Evaluation of motor performance (Catwalk and the elevated board test) at 3 and 6 months after the AAV injections showed no significant motor alterations in these animals as compared to sham-operated / age matched controls. Histological evaluation indicated no loss of TH positive cells despite high levels of expression of LRRK2 RCK-WD40 fragments. Our data show that sustained overexpression of the active kinase domain of mutant LRRK2-G2019S can produce cellular disturbances in neurons *in vivo*, but is not sufficient to rapidly trigger loss of TH-positive cells or motor changes in adult rats.

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Poster

794. Parkinson's Disease Models III

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MDSCRF 2009-MSCRFII-0125-00

MDSCRF 2013-MSCRFII- 0105-00

Title: Generation of Parkinson's disease-linked LRRK2 mutation models with genetically engineered human embryonic stem cells

Authors: *J. W. KIM[1,2,3], T. M. DAWSON[1,2,4,5,6], V. L. DAWSON[1,2,3,4,5];
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Abstract: Leucine-rich repeat kinase 2 (LRRK2) is a large multi-domain protein with known GTPase and kinase domains. Mutations in LRRK2 have been identified as risk factors for Parkinson's disease (PD). Gain-of-function mutations in LRRK2 are the most common genetic cause of familial PD and the mutations are also found in sporadic PD patients, highlighting the importance of LRRK2 in PD pathology. Various animal models including rodent models have been generated to investigate the molecular etiology of PD, and the genetic animal models have provided insights into the neuropathology of LRRK2 mutations. In addition, recent advances in genetic engineering tools and in differentiation techniques for human pluripotent stem cells enable us to generate new PD models with highly enriched human dopamine neurons in culture. To generate a human dopamine neuron PD model with controlled LRRK2 mutant expression, we have generated genetically engineered human embryonic stem cells with an inducible G2019S-LRRK2 overexpression cassette. Expression of the LRRK2 transgene is under the control of the Tet-Off system, which consists of the tetracycline/doxycycline responsive tet-promoter and the tTA transcriptional activator. To avoid transgene silencing and to increase integration efficiency, transcription activator-like effector nucleases (TALENs) were employed to introduce the inducible LRRK2 construct into the AAVS1 safe locus. We expect that this new PD model with inducible LRRK2 expression will allow for a more comprehensive understanding of the molecular mechanisms underlying DA neuron degeneration.

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Poster

794. Parkinson's Disease Models III

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NIGMS (PRAT) Postdoctoral Research Associate Fellowship

NIH Intramural Programs

Title: The loss of endogenous parkin causes dopaminergic neurodegeneration in a mouse model of mitochondrial aging

Authors: *A. M. PICKRELL, C.-H. HUANG, R. J. YOULE;
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Abstract: Parkinson's disease is the most common motor deteriorating neurodegenerative disease caused by the loss of a subpopulation of dopaminergic (DA) neurons in the substantia nigra (SN). Early-onset familial cases have identified recessive PARK2 gene mutations encoding the E3 ubiquitin ligase Parkin. Parkin selectively translocates to dysfunctional mitochondria promoting their removal by autophagy (mitophagy) by targeting mitochondria with low mitochondrial membrane potential and/or pathogenic mitochondrial DNA (mtDNA) mutations. Patients with parkin mutations often confer a loss of mitochondrial function, postulated as a consequence due to a defect in the surveillance of mitochondrial quality. To study the role of Parkin *in vivo* in relation to its role in mitochondrial quality control, we utilized an aging mouse model that accumulates dysfunctional mitochondria caused by an accelerated generation of mtDNA mutations (Mutator mice) crossed to a Parkin knockout (KO) mouse. We hypothesized that the loss of endogenous Parkin would exacerbate phenotypes of a mouse model harboring deleterious mtDNA mutations. Mutator Parkin KO mice performed behavioral testing revealing the appearance of L-DOPA (a pharamcological drug used to alleivate PD symptoms) reversible motor coordination defects at 52 weeks-of-age. At this time point, we found 40% of DA neurons in the SN degenerated and a significant reduction in the amount of DA axons projecting into the striatum of Mutator Parkin KO mice. This specific neuronal loss caused a depletion of DA in the striatum, which led to the motor deficits displayed in these mice. Age-matched wildtype, Parkin KO, and Mutator mice exhibited none of these abnormal behaviors or had any DA neurodegeneration present. The gross neuroanatomy and brain weight appeared normal in Mutator Parkin KO mice suggeting that DA neurons are particularly sensitive to mitochondrial defects. Our study provides the first evidence that endogenous Parkin protects DA neurons haboring high levels of deleterious mtDNA mutations implying an important role for Parkin in mitochondrial quality control *in vivo*.

Disclosures: A.M. Pickrell: None. **C. Huang:** None. **R.J. Youle:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.22/K4

Topic: C.03. Parkinson's Disease

Support: Investissements d'avenir" ANR-10-IAIHU-06

Title: Role of pedunculopontine cholinergic neurons in the vulnerability of nigral dopaminergic neurons in Parkinson's disease

Authors: *M. BENSAID, P. P. MICHEL, E. C. HIRSCH, C. FRANCOIS;
ICM, Paris, France

Abstract: Pedunculopontine nucleus (PPN) cholinergic neurons, which exert excitatory nicotinic control over substantia nigra dopaminergic (DA) neurons, are largely affected in Parkinson's disease and their loss correlates with the level of DA denervation (Karachi et al, J Clin Invest, 2010). This finding and other studies showing that nicotine, the preferential agonist of nicotinic acetylcholine receptors, is neuroprotective in experimental models of Parkinson's disease (Quik et al, Mov Disord, 2013), suggest that PPN excitatory cholinergic inputs might contribute to the survival of substantia nigra DA neurons (Toulorge et al, Faseb J, 2011). To explore this possibility, we used lesion paradigms of DA and/or cholinergic systems in rats and in monkeys. Four groups of rats were used: 1) a group with a bilateral stereotaxic cholinergic lesion of the PPN with diphtheria toxin coupled to urotensin II, 2) a DA lesioned group with unilateral injection of 6-hydroxydopamine (6-OHDA) in the striatum in order to obtain a progressive and significant DA loss in the substantia nigra 3) a doubled lesioned group with unilateral 6-OHDA injection in the striatum followed by a bilateral injection of diphtheria toxin in the PPN, 4) a SHAM group with saline injections in the striatum and in the PPN. Consistent with our hypothesis, we observed that bilateral stereotaxic lesioning of PPN cholinergic neurons in rats resulted in a significant loss of DA neurons in the substantia nigra (27%). Unexpectedly, however, a loss of substantia nigra DA neurons, induced by unilateral striatal injection of 6-OHDA in rats, also produced an important and statistically significant decrease in PPN cholinergic neurons (32%). MPTP intoxication in macaques produced a small (7.2%) but statistically significant reduction in PPN cholinergic neurons compared to control animals. Lastly, when the PPN cholinergic lesion was performed in rats having initially received 6-OHDA in the striatum, i.e., when the process of DA degeneration had therefore already started, losses of DA and cholinergic neurons were more drastic than when each neurotransmitter system was lesioned separately. Thus, our results further highlight the key role of PPN cholinergic neurons in the pathophysiology of Parkinson's disease and suggest strong reciprocal interactions with nigral DA neurons.

Disclosures: **M. Bensaid:** None. **P.P. Michel:** None. **E.C. Hirsch:** None. **C. Francois:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.23/K5

Topic: C.03. Parkinson's Disease

Title: Behavioral pharmacological effects of dopaminergic drugs in common marmosets pretreated with subcutaneous MPTP and intra-cerebral 6-OHDA

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Central Inst. for Exptl. Animals, Kawasaki, Japan

Abstract: The purpose of the present study was to investigate behavioral characterization of MPTP (1-1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) and 6-hydroxydopamine (6-OHDA) in the common marmoset, a small primate. Behavioral pharmacological effects of dopaminergic drugs were also investigated in the marmosets pretreated with above toxins. MPTP-treated marmosets: MPTP at 2, 2 and 1 mg/kg on day 1, 2 and 3, respectively, was subcutaneously administered to marmosets (MPTP-marmosets). These with marked degeneration of dopaminergic neurons continued to exhibit moving tremor, immobility and other Parkinson's disease (PD)-like signs. In behavioral pharmacological test of apomorphine for observing circling behavior in cylinder-type equipment, four marmosets out of six exhibited either ipsilateral or contralateral circling after apomorphine at 0.125 mg/kg, s.c. Two other MPTP-marmosets and all six intact marmosets exhibited no circling behavior. Hemi-lateral brain infusion of 6-OHDA: 6-OHDA at 3 or 6 μg was infused into the right striatum. These marmosets did not exhibit clear PD-like signs after the infusion. Nevertheless, clear contralateral circling behavior was observed after apomorphine at 0.5 mg/kg, s.c. (n=12). However, marked degeneration of dopaminergic neurons in the striatum was not detected by tyrosine hydroxylase (TH) antibody histology in these marmosets. Bilateral brain infusion of 6-OHDA: The neural degeneration in the striatum was detected by the histology when 6-OHDA was infused into the medial forebrain bundle (MFB). Therefore, 6-OHDA at 30 μg was infused into each side of MFB (n=3). Some marmosets exhibited slight moving tremor after the infusion but no other clear PD-like signs were observed. Spontaneous motor activity of these marmosets in their individual living cages were measured and compared with intact marmosets (n=3) after administration of each dopaminergic drug. Increased levels of motor activity were observed after apomorphine at 0.5 mg/kg, s.c. during 30 - 60 min period and after L-DOPA at 10 mg/kg, p.o. during 60 - 120 min period in comparison with intact marmosets administered the same drug doses. Decreased

level was observed after methamphetamine at 1 mg/kg, s.c. during 0 - 180 min period. Discussion: Unbalanced neural degeneration in both sides of the brain was suggested by apomorphine-induced circling behavior in the MPTP-marmosets received peripheral administration of this toxin. In the preclinical study of PD, 6-OHDA-marmosets can provide bases for understanding actions of the new compounds in comparing with the dopaminergic agonist, dopamine releaser and drug for dopamine synthesis.

Disclosures: K. Ando: None. **C. Nishime:** None. **R. Inoue:** None. **E. Nishinaka:** None. **N. Okahara:** None. **T. Inoue:** None. **T. Itoh:** None. **H. Tsutsumi:** None. **K. Kawai:** None.

Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.24/K6

Topic: C.03. Parkinson's Disease

Title: Pharmacological validation of a new invertebrate model of Parkinson's disease: The moon jellyfish

Authors: *P. F. ARAVICH[1], V. CHEN[2], A. QUIDORT[2], Z. M. ARAVICH[1], J. H. PAVELA[2], F. A. LATTANZIO[3], D. B. SPANGENBERG[1];
[1]Path/Anat & Med., [3]Physiological Sci., [2]Eastern Virginia Med. Sch., NORFOLK, VA

Abstract: Parkinson's disease (PD) is a complex neurodegenerative disorder that needs simpler animal models. We explored the validity of a new invertebrate model using the ephyra stage of the widely available moon jellyfish (*Aurelia aurita*). It can be seen without a microscope, is inexpensive and easy to maintain, and flew on two space shuttle missions. It also has a full complement of physiological systems in a transparent body that is easily penetrated by toxins/drugs; a simple nervous system with transmitters like dopamine and glutamate; and a known mitochondrial genome. We previously found face validity for this model since the mitochondrial complex-1 inhibitor, rotenone, caused a dose-dependent poverty of movement. However, the validity of a new PD model requires responsiveness to L-DOPA, the classic treatment for PD. L-DOPA efficacy was tested in jellyfish matched for baseline motor activity (swimming pulses/min) and exposed to rotenone (final concentration 1 micromolar in 0.01% DMSO in artificial sea water) according to a 3x4 factorial design: zero, 0.5 & 5.0 nM L-DOPA concentrations (n=15 animals/group); and repeated measures over 1, 12, 24 & 48 hrs). It was found that L-DOPA reliably mitigated rotenone-induced motor deficits in a time and

concentration-dependent fashion: compared to controls, the high concentration significantly increased motor pulses at both 24 & 48 hrs while the low concentration increased pulses at 48 hrs. It was also shown that motor activity improved in all groups when removed from the rotenone, demonstrating a specific and reversible effect of the toxin rather than general malaise. It is concluded that these data show the predictive validity of a new model of PD using one of the evolutionarily oldest nervous systems on earth.

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Poster

794. Parkinson's Disease Models III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 794.25/K7

Topic: C.03. Parkinson's Disease

Title: A comprehensive tissue based biomarker approach to explore the roles of oxidative stress, mitochondrial dysfunction, and DNA damage in the pathogenesis of parkinson's disease by using a leucine-rich repeat kinase 2 (LRRK2) knockout rat

Authors: *W. A. SPECKMANN[1], Z. FENG[2], R. BROCKETT[4], F. AHMADI[5], K. LONG[5], J. NUCKOLLS[6], A. MCCOY[6], J. BODE[3];
[1]Millipore, TEMECULA, CA; [2]R&D, [3]Eurofins Pharma Bioanalytics Services US Inc, St. Charles, MO; [4]R&D, [5]Marketing, EMD Millipore, Temecula, CA; [6]SAGE Labs, Saint Louis, MO

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disease, affecting ~2% of the population that is 60 years of age or older. Autosomal-dominant mutations in the leucine-rich repeat kinase 2 (LRRK2) gene is associated with both familial and late-onset PD which compose ~13% of PD cases. Oxidative damage, mitochondrial dysfunction, and abnormal protein aggregation have been identified as key players in the pathogenesis of PD and LRRK2 has been implicated in each of these diverse effects in addition to its known functionality in normal cellular activities including neurite outgrowth, vesicular trafficking, phosphorylation, protein translation, and autophagy. Yet, LRRK2's exact etiology in disease progression remains unclear. To further elucidate the role of LRRK2 with PD, we have initiated a comprehensive tissue based biomarker screening analysis of LRRK2 knockout rats. Kidney and brain samples were collected from 11 week old male homozygous LRRK2 knockout rats and

from age matched wild type controls (SAGE Labs). Tissue sections were prepared and stained for histopathology and immunohistochemistry (IHC) and whole slide images were subsequently captured. Histopathological abnormalities in kidneys of the knockout rat were characterized by a loss of tubular architecture, damage to proximal /distal tubule and cast formation. Nissl staining of the brains indicated that there were no observable neuropathological changes. For IHC, a range of targets were analyzed focusing on oxidative stress, mitochondrial function, DNA damage, and kidney function screening both brain and kidney samples. The cellular levels for many of the targets in the panels were essentially unchanged for both wild type and knockout animals, but certain markers showed significant fluctuations in their expression suggesting that the knock out was affecting the normal physiology of the animals. In conjunction with the published data, our results indicate that the kidney abnormality observed in the young LRRK2 knockout rats could be further developed as a potential biomarker for assessing safety liabilities of putative LRRK2 inhibitors as PD therapeutics. Understanding integration of LRRK2 signaling processes and elucidating interaction with other pathways could provide insights for target identification, mechanism study, and ultimately lead to novel therapeutic development for this multiple etiological factors disease.

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Poster

794. Parkinson's Disease Models III

Location: Halls A-C

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Topic: C.03. Parkinson’s Disease

Support: CAPES

FINEP

INCEMAQ

FAPERN

SWISS BRAZILIAN Scientific & Technology Cooperation Fund

AASDAP

Title: Underlying mechanisms from motor symptoms of the Alpha-synuclein Parkinson Disease rat model

Authors: *I. BRYS[1,2], R. FUENTES[1], J. NUNES[1,3], B. SCHNEIDER[4], P. AEBISCHER[4], M. A. L. NICOLELIS[1,5];
[1]Edmond and Lily Safra Intl. Inst. of Neurosci. of Natal, Natal, Brazil; [2]Psychobiology, Federal Univ. of Rio Grande do Norte, Natal, Brazil; [3]Univ. Potiguar, Natal, Brazil; [4]École Polytechnique Federale de Lausanne, Lausanne, Switzerland; [5]Duke Univ., Durham, NC

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder associated with motor symptoms, progressive loss of dopaminergic neurons in the nigrostriatal pathway and aberrant low-frequency synchronous corticostriatal oscillations. Alpha-synuclein is the major component of Lewy Bodies, the cytoplasmic protein aggregates present in the neurons of PD patients. Mutations in the gene encoding alpha-synuclein have been associated with familial cases of PD. In recent years the injection of viral vectors to overexpress alpha-synuclein in the substantia nigra has been used to reproduce the features of PD in animals. However, the underlying mechanisms of the motor symptoms from this model are still not totally understood. The aim of this work was to investigate the behavioral, histological and electrophysiological features of an AAV6 alpha-synuclein rat model of PD. Sprague-Dawley male rats were injected with empty viral vector suspension AAV6 (Control) or the vector carrying the gene for wild type human alpha-synuclein (Synuclein) in the substantia nigra. Animals were tested for locomotor activity in the Open Field test and asymmetry in the Cylinder test 1 week before viral injections and weekly from weeks 4 - 10 following injection. A separate group of animals was injected with empty viral vector AAV6 in the substantia nigra of one hemisphere and the vector carrying the gene for alpha-synuclein in the other hemisphere. These animals were implanted in both hemispheres with 64 recording electrodes in the motor cortex and striatum. Electrophysiological recordings were performed in freely moving rats for 10 weeks following the viral injection. Animals were perfused at the end of the survival time, the brains were collected and the tissue was used for Tyrosine Hydroxylase (TH), microglial activation (ED1) and alpha-synuclein immunostaining. Animals from the Synuclein group showed a significant decrease in the use of the forepaw contralateral to the lesion after the viral injection [$F(3.8,58.1)=3.4$, $p<0.05$] in the Cylinder test. Alpha-synuclein resulted in ED1 expression in the injection region. TH staining revealed a reduction of 32.9% in the striatal innervation and 43.4% of neuronal loss in the substantia nigra of the synuclein expressing side. Changes in the spectral power components of the local field potential of motor cortex and striatum were assessed. Motor symptoms present in the alpha-synuclein PD model are associated with dopaminergic neuronal loss and inflammatory

response resulting from alpha-synuclein overexpression. The model reproduces the major features of PD and might be useful to study the underlying mechanisms of PD and for preclinical testing.

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Poster

794. Parkinson's Disease Models III

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Topic: C.03. Parkinson's Disease

Support: NIH Grant 3R01NS070190-03S1

NIH Grant 5R01NS070190-03

Title: N-α-synuclein specific CD4+ effector T cell line exacerbates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced neurodegeneration in a mouse model of Parkinson's disease

Authors: R. A. WILSHUSEN, K. M. ANDERSON, A. M. SZLACHETKA, *R. MOSLEY; Dept Pharmacol. and Exp Neurosci., Univ. Nebraska Med. Ctr., OMAHA, NE

Abstract: Inflammation and oxidative stress play critical roles in Parkinson's disease (PD). We have previously shown in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model that adoptive transfer of effector T cells (Teffs) that recognize nitrated α-synuclein (N-α-syn) as non-self exacerbates microglial-mediated neuroinflammation with amplified dopaminergic neurodegeneration as well as accelerated and prolonged MPTP-induced neuropathology. Moreover, Teffs that secrete IL-17 (Th17 phenotype) exacerbate neurodegeneration to greater levels than those Teffs that secret IFNɣ (Th1 phenotype). The specific cellular and molecular mechanisms by which these Teffs modulate neurodegeneration remain enigmatic. However, increasing evidence suggests that neurotoxic inflammatory activities have a profound effect on the pathogenesis and progression of PD. Herein, we report on the generation and long-term culture of an N-α-syn specific CD4+ Teff line that induces increased production of reactive nitrogen species (RNS) from myeloid lineage cells and also intensifies the loss of tyrosine hydroxylase (TH) positive neurons within the SN of MPTP-intoxicated mice. Flow cytometric analysis showed the T cell line exhibited very distinct

expression of CD3 and CD4 cell surface markers and intracellular IL-17a cytokine, moderate staining for surface CD146, and little expression of CD8 and FoxP3. Analysis of secreted cytokines revealed the N-α-syn-specific CD4+ Teff line produced high levels of IL-17, TNF-α and IFN-γ, intermediate levels of IL-10, but low levels of IL-2, IL-6, and IL-4 suggesting characteristics consistent with a Th17 cell line, but also exhibiting attributes of Th1-type T cells. Co-culture of the T cell line with N-α-syn and BV-2 cells increased RNS production by BV-2 cells by 150% compared to BV-2 controls cultured in the absence of T cells. Lastly, adoptive transfer of the cells from the Teff line to MPTP-intoxicated mice increased the loss of TH+ dopaminergic neurons by 63% compared to controls. Together, these results support the notion that effector T cells specific for N-α-syn increase oxidative stress as measured by RNS release from reactive myeloid microglia, increase the loss of TH+ neurons, and thus may play a pivotal role in the tempo and progression of dopaminergic neurodegeneration in PD.

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Poster

795. Huntington's Disease Mechanisms II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 795.01/L1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JPB Foundation

Title: Dysregulation of polycomb repressive complex 2 (PRC2) activity in Huntington's disease

Authors: *R. J. FENSTER[1,2,3], A. M. HEILBUT[1,4], R. KULICKE[1,2], L. J. HACHIGIAN[1,6,2], E. D. KOLACZYK[5,4], J. P. MESIROV[1,4], M. HEIMAN[6,1,2];
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Abstract: Huntington's disease (HD) is an autosomal dominant, fatally progressive neurodegenerative disorder caused by the expansion of CAG tri-nucleotide repeats in the *huntingtin* (*HTT*) gene. Although the gene is expressed nearly ubiquitously throughout the brain, some cell types display enhanced vulnerability in HD. The reason for this enhanced vulnerability

is unknown, but offers the potential to identify a therapeutic opportunity. Transcriptional dysregulation is known to be a characteristic of the pathophysiology seen in HD, but whether this dysregulation contributes to the enhanced vulnerability seen for certain cell types in the disease remains unclear. We have used the cell-type specific translating ribosome affinity purification (TRAP) methodology to profile gene expression changes occurring in the most vulnerable cell types in various HD mouse models, at different time points of disease progression. Using these TRAP data, in conjunction with published human HD transcriptional studies, we searched for over-representation of regulatory motifs among the genes whose expression was altered in the most HD-vulnerable cell types: deep-layer cortical neurons and striatal medium spiny neurons. Our analyses revealed an enrichment of genes regulated by the polycomb repressive complex 2 (PRC2) in these most vulnerable cell types, and that a large fraction of those genes with altered expression in HD disease models also contained PRC2 regulatory motifs in their promoters. Given that mutant HTT has been previously shown to activate PRC2 in a CAG-repeat-length-dependent manner, we have further investigated PRC2 function in HD mouse models. By immunostaining, we identified changes to PRC2-dependent histone methylation marks in several mouse models of HD versus control littermates. Of note, the largest of these PRC2-dependent methylation changes were found in the striatum, a region of highest vulnerability in HD. These data together demonstrate that aberrant PRC2 activity is involved in the transcriptional dysregulation that is observed in HD, and that there are brain region-specific differences to changes in PRC2 activity in HD.

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Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: United States Public Health Service Grant MH18501

CHDI Foundation

Title: Dysregulated cysteine homeostasis mediates neurodegeneration in Huntington’s disease

Authors: *B. D. PAUL, J. I. SBODIO, R. XU, M. S. VANDIVER, J. Y. CHA, A. M. SNOWMAN, S. H. SNYDER;
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Abstract: Cysteine is a versatile thiol, which is a building block for proteins and the antioxidant glutathione. Cysteine by itself has substantial antioxidant potential and is the substrate for generation of the gaseous signaling molecule, hydrogen sulfide. One of the modes by which hydrogen sulfide acts is by the process of sulfhydration, wherein the -SH group of cysteine is converted to an -SSH. Sulfhydration mediates various cytoprotective functions in the brain. Cystathionine gamma lyase (CSE) is the sole enzyme which synthesizes cysteine via the transsulfuration pathway in mammals. CSE is induced in response to oxidative stress, which confers cytoprotection via cysteine and hydrogen sulfide production. Depletion of CSE causes elevated oxidative stress and susceptibility to stress induced by 3-nitropropionic acid (3-NP) and excitotoxins such as kainate and glutamate leading to a reduction in neuronal glutathione content and hydrogen sulfide production. We show that CSE is depleted in Huntington's disease (HD) which contributes to neurotoxicity. The decrease in CSE levels seems to stem from the inhibitory effect of mutant huntingtin on specificity factor 1 (Sp1), the transcription factor for CSE. Treatment of striatal cells derived from transgenic models of HD or transgenic R6/2 mice with cysteine donors alleviates this stress and improves associated behavioral abnormalities in mice. We propose that stimulation of the transsulfuration pathway could be a therapeutic measure for treatment of neurodegenerative diseases involving oxidative stress such as HD. Taken together, our results unravel a hitherto unrecognized role for CSE in redox homeostasis in the brain.
References 1. Paul BD and Snyder SH (2012). H_2S signalling through protein sulfhydration and beyond. Nat. Rev. Mol. Cell Biol. 13, 499-507. 2. Paul BD, Sbodio JI, Xu R, Vandiver MS, Cha JY, Snowman AM and Snyder SH (2014). Cystathionine γ -lyase deficiency mediates neurodegeneration in Huntington's disease. Nature 509, 96-100.

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Poster

795. Huntington's Disease Mechanisms II

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Program#/Poster#: 795.03/L3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS085880

EMBO post doctoral fellowship

Title: *In vivo* synthetic lethal screening in the mammalian central nervous system

Authors: *R. SHEMA[1,2,3], R. KULICKE[1,2,3], M. HEIMAN[1,2,3];
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Abstract: Understanding the molecular basis of neurodegenerative diseases (NDDs), and how they interact with the aging process, is one of the greatest challenges in neuroscience. As the most common NDDs, including Alzheimer's, Parkinson's, and Huntington's diseases remain essentially without a cure, the search for therapeutic targets becomes imperative. We have developed a novel platform for the study of NDDs, utilizing the disease-relevant cellular populations in their natural environment. For these screens, which we term SLIC (Synthetic Lethal In the Central nervous system), pooled libraries of lentivirus for knock-down, knock-out, or over-expression of all known genes in the genome are injected into the relevant disease regions in the mouse brain, with one barcoded virus infecting one cell. Comparison, by genomic sequencing, of lentiviruses that are retrieved from wild-type animals, but not from disease model littermates, after various times of incubation in the mouse brain, reveals target genes that function as enhancers of toxicity specific to the disease-associated mutation. We have implemented SLIC for the study of Huntington's disease, the most common inherited neurodegenerative disorder, which is caused by abnormal expansion of a cytosine-adenine-guanine (CAG) tri-nucleotide repeats in the coding region of the huntingtin gene (Htt). Employing SLIC screening across several Huntington's disease mice models, we demonstrate that not only is SLIC capable of identifying enhancers of lethality *in vivo* in the mammalian brain, but that it is able to generate novel hypotheses regarding disease mechanisms and potential therapeutic interventions.

Disclosures: R. Shema: None. **R. Kulicke:** None. **M. Heiman:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CiberNed

MICINN/MINECO

Comunidad Autonoma de Madrid

Fundación Ramón Areces

European Commission

CSIC JAE-pre

Title: Study of tau expression in Huntington´s disease

Authors: M. FERNANDEZ-NOGALES[1], J. CABRERA[1], M. SANTOS-GALINDO[1], J. HOOZEMANS[2], I. FERRER[3], A. ROZEMULLER[2], F. HERNANDEZ[1], J. AVILA[1], *J. J. LUCAS[1];
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Abstract: Tauopathies are a group of neurodegenerative diseases such as Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), or frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) that are characterized by altered metabolism and deposition of the microtubule associated protein tau. Alternative splicing of *tau* exon 10 results in tau isoforms containing either three or four microtubule-binding repeats (3R-tau and 4R-tau). Discovery of silence and intronic mutations leading to increased 4R/3R ratio in FTDP-17 affected families revealed that a disbalance in 4R-tau and 3R-tau in favor of the 4R isoform is sufficient to cause neurodegeneration with personality disturbances, dementia and motor dysfunction. Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by involuntary movements, psychiatric symptoms and dementia that is caused by an expanded CAG repeat in exon 1 of the *Huntingtin* (*HTT*) gene. HD thus belongs to the group of dominant trinucleotide repeat diseases that include many other CAG repeat disorders such as the spinocerebellar ataxias SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, dentatorubropallidoluysian atrophy (DRPLA) and spinobulbar muscular atrophy (SBMA) as well as the CUG repeat disorders SCA8 and myotonic dystrophy 1 (DM1). A key element in the pathogenesis of the latter is the binding of splicing factors by the mutant CUG transcript, thus leading to alternative splicing aberrations in multiple genes. Intriguingly, CAG repeats have recently been shown to mimic CUG repeats in the misregulation of alternative splicing. Since it has also been very recently reported that aberrant splicing contributes to the generation of the highly toxic short N-terminal species of HTT, it is conceivable that splicing alterations significantly contribute to HD pathogenesis. Here we aim to explore whether the neurodegeneration-causing increase in 4R/3R-tau mRNA ratio occurs in HD

by performing quantitative RT-PCR from RNA extracted from striatum and cortex of HD and control subjects.

Disclosures: **M. Fernandez-Nogales:** None. **J. Cabrera:** None. **M. Santos-Galindo:** None. **J. Hoozemans:** None. **I. Ferrer:** None. **A. Rozemuller:** None. **F. Hernandez:** None. **J. Avila:** None. **J.J. Lucas:** None.

Poster

795. Huntington's Disease Mechanisms II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 795.05/L5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Inhibition of excessive monoamine oxidase A/B activity protects against increased stress-induced neuronal death in Huntington disease

Authors: ***M. A. POULADI**[1], J. OOI[2], M. R. HAYDEN[3];
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Abstract: Monoamine oxidases (MAO) are an important component of the homeostatic machinery that maintains the levels of monoamine neurotransmitters, including dopamine, in balance. Given the imbalance in dopamine levels observed in HD, the aim of this study was to examine MAO activity in cellular models of HD and in patient dermal fibroblasts. We show that cells expressing mutant HTT exhibit increased MAO expression and activity. Using cellular stress paradigms, we further demonstrate that the increase in MAO activity in mutant cells is accompanied by enhanced susceptibility to oxidative stress and cell death. Treatment of mutant cells with MAO inhibitors ameliorated oxidative stress and reduced cell death. Consistent with the findings in striatal HD cells, we observe increased MAO expression and activity in patient dermal fibroblasts. Altogether, this study demonstrates abnormal MAO expression and activity and suggests a potential use for MAO inhibitors in HD.

Disclosures: **M.A. Pouladi:** None. **J. Ooi:** None. **M.R. Hayden:** None.

Poster

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Program#/Poster#: 795.06/L6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Marie Curie IIF (PIIF-GA-2011-300197)

Title: Sphingosine-1-phosphate metabolism/axis as potential therapeutic target in Huntington disease

Authors: *V. MAGLIONE, A. DI PARDO, E. AMICO, M. FAVELLATO, F. SQUITIERI; Ctr. for Neurogenetics and Rare Dis., IRCCS Neuromed, Pozzilli, Italy

Abstract: Background. Sphingosine-1-phosphate (S1P) is a potent signaling lipid that regulates a number of processes essential for cellular homeostasis, differentiation, motility and cell viability. S1P is normally found both in the intracellular and extracellular compartments. When S1P is exported outside of the cell, it acts as a high affinity agonist at five known G protein-coupled receptors, S1PR1-S1PR5, normally expressed in neurons, astrocytes and oligodendrocytes. S1P metabolism is quite complex and involves the action of different enzymes. S1P is synthesized by sphingosine kinase-1 and -2 (SPHK1 and 2 and degraded either by S1P-phosphate (SPP) or by S1P-lyase (SGPL1). Thus, the overall cellular S1P level is determined by a balance between its synthesis and degradation and any alteration in its metabolism is likely to have profound impact on both brain homeostasis and function. Emerging evidence indicates that pharmacological interference with S1P metabolizing enzymes may represent a promising therapeutic approach for multiple disorders including neurological diseases. Aim. Recently, we have demonstrated that chronic administration of FTY720, a synthetic analog of S1P, is neuroprotective and neurorestorative in Huntington disease (HD) R6/2 mouse model. In this study, our aim is to explore whether S1P metabolism/signaling pathway may represent a molecular target for developing new therapeutic strategies for the treatment of HD. Results. Our findings highlight an altered expression of some of the S1P metabolizing enzymes in both cellular and animal models of HD. In particular, we found that immunoblotting analysis on lysate from HD mouse brain tissues revealed increased expression of SGPL1 which might likely result in its increased activity and subsequent alteration of S1P bio-availability. We also demonstrate that the stimulation of specific S1PR subtypes activates pro-survival pathways in HD cells. Conclusion. These results let us suppose an aberrant metabolism of S1P in HD and indicate that modulation of its signaling pathway may be beneficial in HD. We believe S1P axis has the potential of providing the possibility to develop new therapeutic

interventions for treating HD, therefore further studies are warranted. This research is supported by Marie Curie International Incoming Fellowship (PIIF-GA-2011-300197) granted to V.M. within the 7th European Community Framework Programme.

Disclosures: **V. Maglione:** None. **A. Di Pardo:** None. **E. Amico:** None. **M. Favellato:** None. **F. Squitieri:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Dendritic loss is an early phenotype in rat cortico-striatal co-cultures expressing mutant huntingtin

Authors: S. DIJKSTRA[1], R. VAN DE BOSPOORT[1], S. LACHIZE[1], S. FRATANTONI[1], J. VEENMAN[1], N. VAN DEN BERG[1], G. MCALLISTER[2], *D. F. FISCHER[1], S.-W. JANG[3]; [1]BioFocus, Leiden, Netherlands; [2]BioFocus, Saffron Walden, United Kingdom; [3]CHDI Mgmt. / CHDI Fndn., New York, NY

Abstract: Phenotypic assays in primary neurons can be used to discover and validate potential novel therapeutic strategies for Huntington's disease (HD). We are using rat primary neuronal cortico-striatal co-cultures based upon Kaltenbach et al 2010, J Biomol. Screen., but with inducible expression of expanded repeat huntingtin (mutHTT) exon 1 fragments. Cortical and striatal neurons are isolated from E18 rat embryos and transfected with a HTT exon 1 fragment containing 73 CAG repeats (Q73), together with a plasmid encoding a fluorescent reporter (different for striatal and cortical neurons), before being plated on a glial cell feeder layer. Striatal and cortical cell populations are identified within the co-cultures by their respective fluorescent reporter signals, using automated high content imaging and analysis. The inducible expression system allows for a period of neuronal maturation *in vitro* before mutHTT expression to explore polyQ dependent phenotypic changes that may be relevant to the neuronal dysfunction found in HD. A unique feature of this inducible system is that it allows candidate compound testing both as a preventive treatment and as a therapeutic treatment. We have previously characterized the time course of mutHTT expression, aggregation and neuronal death in this

system, showing that when mutHTT was induced at 4 days *in vitro* (DIV), cell loss was observed between 11-14 DIV. Here, we describe mutHTT-induced changes in the striatal dendritic tree. To measure the striatal dendritic tree, a MAP2 tag was fused to the striatal reporter to localize the fluorescent signal to dendrites. Using this reporter we observed a gradual reduction in total striatal dendrite area in Q73- compared to non-induced controls, reaching ~50% at DIV12. No dendrite loss was observed in control cultures transfected with a non-expanded HTT fragment (Q8), indicating that the effect was specific for mutHTT. Striatal dendrite loss was evident within days after induction and appeared to precede loss of striatal or cortical cells. The MLK inhibitor CEP-1347, but not the neurotrophin BDNF, was able to significantly ameliorate the Q73-induced dendrite loss at DIV10. We are currently profiling additional compounds targeting pathways implicated in dendritic remodeling.

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Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NINDS1RO1-NS065867

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NIHM 5P50-NS071669

Title: Age dependent changes in M4-mediated control of striatal glutamate and dopamine signaling in the YAC128 mouse model of Huntington's disease

Authors: *T. PANCANI[1], T. BICHELL[2], D. FOSTER[2], E. BRADLEY[2], M. C. FERGUSON[2], T. BRIDGES[2], S. DANIELS[2], A. BOWMAN[2], C. W. LINDSLEY[2], P. J. CONN[2], Z. XIANG[2]; [1]Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ. Medcenter, Nashville, TN; [2]Vanderbilt Univ., Nashville, TN

Abstract: Huntington disease (HD) is a neurodegenerative disease characterized by severe motor and behavioral alterations. The appearance of a hyperkinetic phenotype (chorea) marks the beginning of the motor symptomatic stage, and is followed by akinesia and progressive loss of striatal medium spiny neurons (MSNs). Changes in signaling of glutamate and other neurotransmitters including dopamine are demonstrated to be responsible for behavioral and neuropathological changes. Interestingly, these changes seem to largely precede the onset of the disease's symptoms. At early age, the YAC128 mouse model of HD shows motor hyperactivity associated with increased striatal glutamate transmission and likely dopamine signaling as well, while late akinesia is accompanied by striatal neuronal loss and decreased glutamatergic/dopaminergic transmission. Striatal cholinergic activity and muscarinic receptor (mAChR) functions seem to be also compromised in HD. A handful of clinical studies show that cholinesterase inhibitors might provide symptomatic relief in HD patients. The M4 mAChR, one of the functionally predominant mAChR subtypes in the striatum, is involved in the modulation of striatal glutamate and dopamine signaling (Pancani et al. 2014, Byun et al. 2014) and is also known to play a pivotal role in locomotor activity and control. Using slice electrophysiology and fast cyclic voltammetry in conjunction with behavioral assay, we found that eEPSC as well as sEPSC amplitude in MSNs and evoked dopamine (DA) release in the striatum are increased in YAC128 compared to WT at a pre-symptomatic stage (2 months). Interestingly, the increased release of neurotransmitters is accompanied by increases in M4 brain expression as well as M4-

mediated inhibition of cortico-striatal glutamate transmission and striatal DA release, suggesting that striatal M4 function is up regulated in HD. Deletion of M4 in YAC128 mice dramatically exacerbates the increase in glutamate release in the striatum. Finally, alterations in Rotarod and open field performance were detected at as early as 5 months of age and were significantly correlated with a decrease in glutamatergic signaling in YAC128 compared to WT mice. Our data suggest that the early increase in striatal glutamate and dopamine release in YAC128 might contribute to the increased neurotoxicity, cortico-striatal "disconnection" and behavioral impairment at later age (5 months). The transient early increase in M4 activity could represent a neuroprotective, compensatory mechanism aimed to decrease glutamate and dopamine tone in the YAC128 mouse.

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Poster

795. Huntington's Disease Mechanisms II

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CHDI Foundation Inc

Title: RTP801 mediates mutant huntingtin-induced cell death

Authors: N. MARTÍN-FLORES[1], J. ROMANÍ-AUMEDES[1], M. CANAL[1], P. SANDERS[2], M. STRACCIA[2], L. RUÉ[2], C. SVENDSEN[3], N. D. ALLEN[4], J. ALBERCH[2], J. CANALS[2], *E. PEREZ-NAVARRO[2], C. MALAGELADA[1];
[1]Dept. of Pathological Anatomy, Pharmacol. and Microbiology, Barcelona, Spain; [2]Dept. Cell Biology, Immunol. and Neurosciences, Univ. of Barcelona, Barcelona, Spain; [3]Regenerative Med. Institute, Cedars-Sinai Med. Ctr., Los Angeles, CA; [4]Divisions of Pathophysiology & Repair and Neuroscience, Sch. of Biosci., Cardiff, United Kingdom

Abstract: Selective neuronal death is a hallmark in the pathogenesis of Huntington's disease (HD) although the mechanisms underlying the toxicity of mutant huntingtin (htt) are still unclear. RTP801 is a stress responsive protein that has a pro-apoptotic role in non-proliferating differentiated cells such as neurons. The mechanism by which RTP801 triggers neuronal cell death is by a sequential inhibition of mTOR and Akt survival kinases. RTP801 is induced in cellular and animal models of Parkinson's Disease and its expression is elevated in nigral neurons from PD human brains. Thus here we have investigated whether RTP801 is involved in mutant htt-induced neuronal death. Overexpression of exon-1 mutant htt in NGF-differentiated PC12 cells elevated RTP801 protein levels by induction of gene expression, and by lengthening RTP801 protein half-life. RTP801 induction was confirmed in rat cortical neurons in culture. During the differentiation to medium spiny neurons of human induced pluripotent stem cell (iPSC) lines containing either a wild type or mutant htt with 60 CAG repeats, elevation of RTP801 protein levels was observed in the mutant iPSC line in comparison to the wild type control. Consistently in NGF-differentiated PC12 cells, silencing RTP801 expression with shRNAs blocked mutant htt-induced cell death. In HD animal models that display motor deficits but no neuronal death such as the knock-in HdhQ7/Q111 and the exon-1 R6/1 mice, RTP801 levels in the striatum did not differ from their wild type littermates at several stages of the disease. Importantly, RTP801 protein levels were elevated in human HD brains, meaning that the relationship between mutant htt and RTP801 may be relevant to the human disease. Taken together these results indicate that RTP801 is a novel downstream effector of mutant htt toxicity.

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Poster

795. Huntington's Disease Mechanisms II

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Program#/Poster#: 795.10/L10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIA/NIH AG031153

NIA/NIH AG019206

Title: Expression of mutant huntingtin in astrocytes suppresses BDNF secretion

Authors: *Y. HONG, T. ZHAO, X.-J. LI, S.-H. LI;
Human Genet., Emory Univ., Atlanta, GA

Abstract: Huntington's disease (HD) is a fatal, inherited, neurodegenerative disease that affects one in every 10,000 Americans. There is no effective treatment for HD to date because of the unknown pathological mechanism. Previous studies mainly focus on neurons, because neuronal loss occurs preferentially in the striatum and then extends to other brain regions as HD progresses. However, astrocyte dysfunction has been found in several neurological disorders, including Epilepsy, ALS and Stroke. In the central nervous system, astrocytes are the largest cell population and play multiple roles in regulating neuronal functions. Among their multiple functions, astrocytes can synthesize and release some important neurotrophic factors including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell derived neurotrophic factor (GDNF). All of these factors are involved in neuronal survival, development, and function. A large number of studies in HD mouse models indicate that BDNF deficit in the striatum and cerebral cortex occurs before brain pathology, which has led to the idea that reduced endogenous neurotrophic support may contribute to HD onset and/or progression. Mutant huntingtin (mhtt) is found in glial cells in the brains of HD mice and patients; however, little is known about the pathological roles of mhtt in astrocytes. Whether mhtt expressed in astrocytes interferes with BDNF synthesis and release to contribute to selective neuronal degeneration in HD remains unknown. In this study, we used a transgenic HD mouse model (GFAP-160Q) that specifically expresses N-terminal mhtt in astrocytes to study the effect of mhtt on BDNF secretion. Western blot and real-time PCR results show no significant difference of BDNF level in astrocytes between WT and GFAP-160Q mice. However, ELISA results demonstrate that the secretion level of mature BDNF is decreased in the culture medium of astrocytes from GFAP-160Q mice compared with WT. These results indicate that mhtt may impair secretion of BDNF from astrocytes, which might contribute to the neuronal dysfunction and degeneration in HD. The mechanism of decreased BDNF secretion is under investigation

Disclosures: Y. Hong: None. **T. Zhao:** None. **X. Li:** None. **S. Li:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NS041669

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NIH NS045016

Title: Studying turnover of mutant huntingtin in neuronal and glial cells at subcellular level

Authors: *T. ZHAO, Y. HONG, S.-H. LI, X.-J. LI;
Human Genet., Emory Univ., Atlanta, GA

Abstract: Huntington's disease (HD) is an autosomal dominant, neurodegenerative disease that affects one in every 10,000 Americans. About 200,000 Americans are at risk of inheriting the disease from affected parents. HD patients are characterized by motor, cognitive and neuropsychiatric abnormalities. HD is caused by the expansion of the trinucleotide CAG (>37 units) encoding an expanded stretch of polyglutamine (PolyQ) in the N terminal region of mutant huntingtin (mhtt). Mhtt is neurotoxic and induces neuronal death by disturbing gene expression, axonal transport, and mitochondrial function. Mhtt is prone to forming insoluble aggregates. Appearance of mhtt aggregates is indicative of the accumulation of mhtt. In HD, progressive emergence of mhtt aggregates in neurons is observed. Compared to neurons, fewer and smaller mhtt aggregates are found in astrocytes. Furthermore, the mhtt aggregates preferentially form in neuronal neurites and nuclei, and few aggregates form in the cytosol of soma. This implicates that degradation rates of mhtt in different subcellular compartments are uneven. In order to study degradation rates of mhtt in subcellular compartments, we conjugate dendra2, a photoconvertible fluorescent protein, to the N-terminal fragmented mhtt (N230-130Q) and wild-type htt (N230-23Q) that is used as the control. Dendra2 is irreversibly photoconverted from a green to a red fluorescent state with 405nm light in the neuronal compartments. After photoconversion, decline of red signal over time is used to measure the degradation rates of N230(130/23Q)-dendra2 in the subcellular compartments. In the present study we used brain slice and primary culture models. We found that mhtt is degraded faster than wild-type htt in the cytosol of soma in neurons. However, expanded polyQ stabilizes mhtt in neurites. By contrast, mhtt is cleared faster than wild-type htt in both cytosol of soma and processes of astrocytes. Our data demonstrates

uneven clearance rates of mhtt in distinct subcellular compartments and indicates that astrocytes and neurons cope with mhtt in different ways.

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Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Hereditary Disease Foundation

NICHD R37-HD028341

R01 GM089903

Title: CalDAG-GEFI and CalDAG-GEFII are down-regulated in the R6/2 Huntington's disease mouse model and show reduced H3K4 trimethylation in a pattern typical of dysregulated neuronal genes

Authors: *J. R. CRITTENDEN[1], F. YILDIRIM[2], H. A. BOWDEN[1], T. A. GIPSON[2], C. W. NG[2], E. FRAENKEL[2], D. E. HOUSMAN[2], A. M. GRAYBIEL[1];
[1]McGovern Inst. for Brain Research, Brain & Cognitive Sci., [2]MIT, Cambridge, MA

Abstract: Huntington's disease is caused by the expansion of a glutamine tract in the Huntingtin protein, resulting in neurodegeneration in the striatum and cerebral cortex and progressively severe psychiatric and motor symptoms. In rodent models that express full-length or pathogenic fragments of mutant Huntingtin, there is notably less cell death than in the human disease, but there is nevertheless a strong overlap in the sets of dysregulated genes observed. This overlap in gene dysregulation appears to be, at least in part, related to shared changes in the function of transcription factors and epigenetic regulators. By genome-wide analyses of an epigenetic mark, histone H3K4 trimethylation, and gene transcription in the R6/2 mouse model of Huntington's disease, we identified the striatum-enriched genes CalDAG-GEFI and CalDAG-GEFII (aka RasGRP2 and RasGRP1) to have lower expression and reduced histone H3K4 trimethylation levels. CalDAG-GEFI is predominantly expressed in the matrix compartment of

the striatum, which receives input from sensorimotor cortices and sends projections to the primary output nuclei of the basal ganglia. By contrast, CalDAG-GEFII is differentially expressed in the striosome compartment, which receives inputs from limbic cortical regions and sends projections to dopamine-containing neurons in the substantia nigra pars compacta, placing striosomes in a circuit involved in mood and motivation. In Huntington's disease, differential degeneration in striosomes, relative to degeneration in the matrix, has been linked to disease onset with prominent mood symptoms. At the protein level, we previously showed that CalDAG-GEFI is reduced in Huntington's disease. We now report that mRNA transcript and protein for CalDAG-GEFII are severely reduced in striatum from aged R6/2 mice. Furthermore, we show that the genes encoding CalDAG-GEFI and CalDAG-GEFII show reduced H3K4 methylation and display a distinct H3K4 trimethylation profile around their transcription start sites that is typical of Class I neuronal genes that are down-regulated in expression in Huntington's disease (Vashishtha et al., 2013). The functional consequences of CalDAG-GEFI and CalDAG-GEFII down-regulation are under evaluation by comparing the phenotypes in CalDAG-GEFI and CalDAG-GEFII knockout mice to those in Huntington's disease models. Vashishtha M, Ng CW, Yildirim F, Gipson TA, Kratter IH, Bodai L, Song W, Lau A, Labadorf A, Vogel-Ciernia A, Troncosco J, Ross CA, Bates GP, Krainc D, Sadri-Vakili G, Finkbeiner S, Marsh JL, Housman DE, Fraenkel E, Thompson LM. Targeting H3K4 trimethylation in Huntington disease. Proc Natl Acad Sci U S A. 2013;110:E3027-E3036.

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Poster

795. Huntington's Disease Mechanisms II

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Program#/Poster#: 795.13/M1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Mutant huntingtin causes loss of mechanisms underlying early cognitive deficits in Huntington's disease

Authors: *R. P. MURMU[1,2], W. LI[2], J.-Y. LI[2];
[1]Panum Inst., Copenhagen, Denmark; [2]Dept. of Exptl. Med. Sci., Lund Univ., Lund, Sweden

Abstract: In Huntington's disease (HD), cognitive symptoms and cellular dysfunction precede the onset of classical motor symptoms and neuronal death in the striatum and cortex by a decade.

This suggests that the early cognitive deficits may be due to a cellular dysfunction rather than being a consequence of neuronal loss. Abnormalities in dendritic spines and abnormal synaptic plasticity, which are observed in HD patients and in HD animal models are thought to underlie the early cognitive symptoms in HD. However, the exact kinetics of spine alterations and plasticity in HD remain unknown. Experience-dependent synaptic plasticity caused by mechanisms such as LTP or novel sensory experience potentiates synaptic strength, enhances new dendritic spine formation and stabilization and may contribute to normal cognitive processes, such as learning and memory. Although, HD is a autosomal dominant disorder studies show that the disease phenotype is modulated by environmental factors. In this study, we used long-term, two-photon imaging through a cranial window, to track individual dendritic spines in a mouse model of HD (R6/2) as the disease progressed. Additionally, we investigated whether pathological processes of HD interfered with the normal experience-dependent plasticity of dendritic spines in the R6/2 model. Six weeks of two-photon *in vivo* imaging revealed that under baseline condition (without any sensory manipulation) neuronal circuitry in HD mouse model is highly unstable which led to a progressive loss of persistent-type, mature spines in these mice. Although, more new spines formed in the R6/2 mice, the probability that newly formed spines transformed into persistent spines was greatly reduced in these mice. Further, we could show that mutant huntingtin was directly involved in the loss of persistent-type, mature spines. In addition, six-weeks of two-photon imaging before and after whisker trimming revealed that sensory deprivation exacerbates loss of persistent-type, stable spines in R6/2 mice and led to impaired transformation of newly generated spines into persistent spines in these mice thereby reducing synaptic density is barrel cortical neurons of R6/2 mice. Interestingly, dendritic spine alterations in the R6/2 mice were evident before the onset of motor symptoms suggesting that decreased stability of cortical synaptic circuits could underlie the early cognitive deficits in HD. This data also suggests that mutant huntingtin is implicated in maladaptive synaptic plasticity which could be one of the plausible mechanisms underlying early cognitive deficits observed in HD.

Disclosures: R.P. Murmu: None. **W. Li:** None. **J. Li:** None.

Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG039818

CHDI

Title: Processing deficits in the behavioral modulation of striatal local field potential activity in the YAC128 mouse model of Huntington's disease

Authors: E. S. ZHANG, S. J. BARTON, *G. V. REBEC;
Program Neurosci & Dept. Psychological & Brain Sci.,, Indiana Univ., Bloomington, IN

Abstract: Growing evidence from transgenic mouse models suggests that dysfunctional neural processing in dorsal striatum plays a critical role in the behavioral phenotype of Huntington's disease (HD), a dominantly inherited condition. For example, in freely behaving symptomatic R6/2 mice, which express truncated human mutant huntingtin (mHTT) and develop an early behavioral phenotype, striatal local field potentials (LFPs) are characterized by high frequency oscillations not normally expressed in wild-type controls (Hong, S.L. et al., 2012, PLoS ONE, 7(10): e470246). Here, we recorded striatal LFPs in YAC128 mice, a full-length mHTT mouse model with adult-onset symptoms, and age-matched controls (FvB/N), while each mouse freely explored an open-field arena. We focused on LFP activity when the animals engaged in the following behavioral categories: quiet rest (no overt movement), repetitive grooming (face washing and fore- or hind limb scratching), and active exploration (locomotion, rearing, or climbing). All mice were between 33 and 65 weeks of age, a range in which some neurological signs are evident in the YAC128 model (van Raamsdonk, J.M. et al., 2005, J. Neurosci., 25:4169). We calculated an average power spectrum for each animal for each behavior. Both groups spent similar amounts of time in each behavioral category, but episodes of quiet rest were significantly longer in YAC128s than wild-types. Analysis of resting LFP activity indicated significant group power differences in the theta (4-7 Hz), beta (13-24 Hz), and low gamma range (25-40 Hz), consistent with our R6/2 data. Moreover, active exploration in wild-type and YAC128 mice was accompanied by differential changes in delta (1-4 Hz) and alpha (8-12 Hz) power. Collectively, our results confirm behavior-related striatal processing deficits in HD mice. In the YAC128 model, these deficits occur during quiet rest and active exploration at frequency bands associated with cognitive and motor processing. Thus, our results provide fundamental information on striatal activity that may be central to the HD behavioral phenotype.

Disclosures: E.S. Zhang: None. **G.V. Rebec:** None. **S.J. Barton:** None.

Poster

795. Huntington's Disease Mechanisms II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 795.15/M3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation, Inc.

Title: *In vivo* characterization of the basal ganglia direct, indirect, and hyperdirect pathways in the zQ175 heterozygous knock-in mouse model of Huntington's disease

Authors: S. ZHONG[1], H. LIN[1], N. KEATING[1], A. GHAVAMI[1], R. CACHOPE[2], *R. F. ROGERS[3], V. BEAUMONT[2];
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Abstract: Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disorder caused by expansion of CAG repeats in the *Huntingtin (Htt)* gene. Post-mortem evaluation of HD patient brains reveal a devastation of both the caudate and putamen, in addition to other progressive grey matter, white matter, whole brain and regional atrophy[1]. Cortico-striatal-thalamo-cortical circuits are particularly affected in HD[2], and choreic symptoms especially have been strongly correlated with the preferential susceptibility of indirect pathway originating striatal medium spiny neurons (MSNs) to mutant Htt insult[3-4]. Impairments in corticostriatal transmission, and dysfunction of medium spiny neuron (MSN) activity has been extensively studied *in vitro* in HD models, but there has been very little functional investigation of the consequences of this on the immediate indirect pathway downstream nuclei, namely the external Globus Pallidus (GPe), and the subthalamic nucleus (STN). The purpose of the current study was to characterize the properties of cortical to basal ganglia transmission in microcircuit-defined neurons in early symptomatic zQ175 heterozygous knock-in mice (6 month old zQ175 HET), a model of HD that carries one allele of expanded CAG repeats contained within the native murine *huntingtin* gene. Under urethane anesthesia, the responses of striatal MSNs were recorded following stimulation of primary motor cortex (M1). MSNs were identified as belonging to either the (putative) indirect pathway (IP) or to the direct pathway (DP) by their (lack of) antidromic activation from electrical stimulation of the Substantia Nigra, respectively. Preliminary data obtained from these recordings indicate that zQ175KI HET mice show a reduced probability of MSN firing in response to M1 stimulation. Furthermore, a higher percentage of spontaneously active MSNs were recorded in zQ175KI HET compared to WT mice. Studies are underway to determine whether the stimulation-response curves are affected in both IP and DP neurons, or whether one pathway is preferentially affected. We have also started to investigate the “hyperdirect” cortico-STN pathway (HP) by recording STN neurons in response to M1 stimulation. Preliminary data indicates a modest reduction in STN responses to M1 stimulation in zQ175 Het mice. Our data together suggests that zQ175KI HET mice exhibit basal ganglia circuitry dysfunction relevant to the human HD condition. These studies may

produce physiological assays for assessing compounds that could rescue these circuitry disturbances.

Disclosures: **S. Zhong:** None. **H. Lin:** None. **N. Keating:** None. **A. Ghavami:** None. **R. Cachope:** None. **R.F. Rogers:** None. **V. Beaumont:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR grant MOP-84438

NIH K99 MH102244

Title: Ultra-sensitive detection of huntingtin protein in biofluids from Huntington disease patients and model mice using immunoprecipitation followed by flow cytometry

Authors: S. E. P. SMITH[1], A. L. SOUTHWELL[2], T. R. DAVIS[1], E. B. VILLANUEVA[2], *B. LEAVITT[2], A. G. SCHRUM[1], M. R. HAYDEN[2];
[1]Immunol., Mayo Clin. Col. of Med., Rochester, MN; [2]Dept. of Med. Genet., Ctr. For Mol. Med. & Therapeut., Vancouver, BC, Canada

Abstract: Huntington disease (HD) is a progressive neurodegenerative disorder caused by a CAG trinucleotide repeat mutation in the gene encoding for the huntingtin protein (HTT) resulting in the expansion of a polyglutamine tract. Although age of onset in HD can be predicted based on CAG repeat length, this variable accounts for only 50-70% of the variation, while multiple known and unknown genetic and environmental factors account for the remainder. Thus, there is a need for biomarkers that could more accurately predict disease conversion or progression. Levels of mutant HTT protein or of specific species of mutant HTT protein in the brain could be such biomarkers. Additionally, there are multiple HTT lowering therapeutics in clinical development, for which quantification of HTT levels in the brain would be useful to validate and quantify target engagement. Unfortunately, it is not currently possible to directly quantify HTT levels in the living brain of patients. HTT protein is present in cerebrospinal fluid and plasma, though at much lower concentrations than in brain. We propose that cerebrospinal fluid and/or plasma HTT levels could be used as a proxy for brain HTT levels,

but traditional protein quantitation methods have failed to accurately detect and measure HTT protein in biofluids. To overcome this limitation, we have adapted the technique of immunoprecipitation followed by flow cytometry (IP-FCM). IP-FCM is a highly sensitive method of protein detection that has previously been used to quantitatively measure both absolute protein abundance and stable or transient protein-protein interactions. We have screened available anti-HTT antibodies and identified capture-probe pairs that accurately detect HTT protein with enough sensitivity to allow detection of mutant huntingtin protein in the cerebrospinal fluid of HD patients and mice. By using different antibody pairs, specific species of HTT protein relevant to HD pathogenesis and progression, such as total HTT, mutant HTT, full-length HTT, and caspase 6 cleaved HTT, can be rapidly and accurately measured. This technique has potential applications both as a clinical diagnostic and as a research tool to further our understanding of levels and localization of specific HTT protein species over time.

Disclosures: S.E.P. Smith: None. **A.L. Southwell:** None. **T.R. Davis:** None. **E.B. Villanueva:** None. **B. Leavitt:** None. **A.G. Schrum:** None. **M.R. Hayden:** A. Employment/Salary (full or part-time):; Teva Pharmaceuticals.

Poster

795. Huntington's Disease Mechanisms II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 795.17/M5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R01NS078008

Title: Respiratory activity of isolated mitochondria, cultured neurons, and whole YAC128 mice, a model of Huntington’s disease

Authors: *J. HAMILTON[1], T. BRUSTOVETSKY[1], N. BRUSTOVETSKY[1,2];
[1]Pharmacol. and Toxicology, [2]Stark Neurosciences Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Huntington’s disease (HD) is a hereditary neurodegenerative disorder characterized by severe motor and cognitive abnormalities. It is believed that HD is caused by a mutation in the huntingtin protein. Huntingtin is a ubiquitous 350 kDa protein that is involved in developmental processes and anti-apoptotic defense. In healthy individuals, the N-terminus of huntingtin possesses a polyglutamine stretch containing <35 glutamines. Increased number of

glutamines correlates with development of HD. In recent years, different hypotheses were proposed to explain the mechanism by which mHtt expression leads to neuronal dysfunction and loss. It has been shown that mHtt may affect autophagy, mitochondrial dynamics, and trafficking. In addition, there were reports of mHtt-mediated impairment of mitochondrial respiratory activity, although some investigators failed to find any difference in respiration of mitochondria and neurons derived from HD mice and wild-type (WT) animals. In the present study, we used YAC128 mice and their WT littermates. Each animal was genotyped and tissue samples were analyzed for mHtt using Western blotting. We evaluated respiratory activity and mitochondrial membrane potential in isolated, purified synaptic and non-synaptic brain mitochondria as well as in liver and heart mitochondria from WT mice and 4-5 month old YAC128, which displayed clasping behavior typical for HD mice. We used high-precision respirometry and evaluated mitochondrial membrane potential following tetraphenyl phosphonium (TPP^+) distribution across the inner membrane with a TPP^+-sensitive electrode. In addition, we evaluated respiration in cultured striatal and cortical neurons derived from postnatal day 1 YAC128 mice and their WT littermates. The neurons were grown for 9 days *in vitro*, then their respiration was analyzed in a bath solution containing either 10 mM glucose plus 15 mM pyruvate or 2.5 mM glucose alone, using Seahorse XF24 flux analyzer. Finally, we analyzed respiratory activity of whole YAC128 mice compared to WT littermates, using LabMaster Animal Monitoring System (TSE Systems, Midland, MI). In experiments with isolated mitochondria, we found no difference in respiratory activity and membrane potential between mitochondria from YAC128 mice and WT animals. Similarly, in experiments with cultured neurons no difference in respiratory activity was observed. Lastly, there was no difference in respiratory activity of whole YAC128 mice and WT animals. Thus, our data strongly argue against mHtt-mediated impairment of mitochondrial respiratory activity assessed either with isolated mitochondria, cultured neurons, or whole YAC128 mice.

Disclosures: **J. Hamilton:** None. **T. Brustovetsky:** None. **N. Brustovetsky:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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CHDI grants

Title: Activation of axonal JNKs represents an early pathogenic event in Huntington's disease that correlates with axonal degeneration

Authors: *R. G. GATTO[1], E. TAVASSOLI[1], Y. CHU[2], J. KORDOWER[2], G. A. MORFINI[1]; [1]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL; [2]Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Huntington disease (HD) involves degeneration of specific populations of projection neurons within selected areas of the basal ganglia circuit. Nonetheless, the mechanisms underlying differential neuronal vulnerability of selected neuronal populations in HD remain unknown. Results from our previous studies showed that m Htt inhibits axonal transport through a mechanism involving activation of the c-Jun amino-terminal kinase (JNK) pathway and phosphorylation of the motor protein kinesin. Because axonal connectivity critically depends upon appropriate axonal transport, we analyzed the distribution and activation of several JNK pathway components in brains of transgenic R6/2 mice. Using a novel R6/2-YFP reporter mouse model, we present direct evidence that axonal degeneration represents a very early pathogenic event in mutant Huntingtin (mHtt)-affected neurons. Results from quantitative immunohistochemical and immunoblotting studies further revealed heterogeneous cellular distribution of specific JNK isoforms. Additionally, these studies revealed presymptomatic activation of axonal JNK in selected neuronal populations of R6/2 mice, compared to wild type littermates. Immunoblot analysis of control and HD patients confirmed these observations. Taken together, results from our work suggest that axonal degeneration represents a very early pathogenic event in HD. Further, our data suggest that the tissue pattern of JNK activation might contribute to the unique cellular topography of HD.

Disclosures: R.G. Gatto: None. **E. Tavassoli:** None. **Y. Chu:** None. **J. Kordower:** None. **G.A. Morfini:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: M-channel activators rescue homeostatic regulation of striatal excitability and ameliorate locomotor deficits in the R6/2 Huntington's mouse

Authors: Y. CAO, D. BARTOLOME-MARTIN, N. ROTEM, C. ROZAS, S. S. DELLAL, M. A. CHACON, B. KADRIU, M. GULINELLO, K. KHODAKHAH, *D. S. FABER;
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Abstract: We describe a fast activity-dependent homeostatic (fADH) regulation of the intrinsic excitability of medium spiny neurons in mouse dorsal striatum. It can be induced *in vitro* by repeated brief bursts of evoked impulse activity, for example, by applying 300ms suprathreshold depolarizing pulses at a rate of $1s^{-1}$. With this paradigm, fADH is manifest as a progressive reduction in the number or frequency of stimulus evoked impulses and increased variability in interspike interval. That is, it appears as enhanced accommodation or adaptation. fADH develops and decays with time constants of 10 and 20s, respectively, and thus we hypothesize that it limits repetitive firing and can convert tonic neurons to phasic ones. fADH is not due to a rise in intracellular Ca^{2+}, as it is not affected by chelating this cation with BAPTA in the recording pipette. The properties of fADH are consistent with the hypothesis that it is due to a progressive recruitment or facilitation of the M-current that is mediated by KCNQ2/3 channels and is regulated by the level of phosphatidylinositol 4,5-bisphosphate (PIP2) in the plasma membrane . Three lines of evidence support this notion: 1) fADH is blocked by perfusion with the M-current antagonist XE 991 (6uM), 2) whole cell voltage clamp recordings indicate that fADH induction increases the magnitude of an outward XE 991 sensitive current, and 3) blocking PIP2 synthesis with 10uM Wortmannin reduces fADH. This homeostatic mechanism is significantly depressed in medium spiny neurons of the R6/2 and BAC HD transgenic mouse models of Huntington's Disease, at ages when the affected mice begin to exhibit overt locomotor impairments. This deficit in fADH can be at least partially reversed *in vitro* by superfusion with KCNQ channel activators, such as 10uM retigabine. Finally, to test whether M-current activation could ameliorate the locomotor deficits in the transgenic models, R6/2 mice were treated with daily i.p. injections of retigabine (10mg/kg), for four weeks, starting at 4 weeks of age, and locomotor activity was assessed with the open field test in weeks 5-7. Activity levels in the treated HD mice were significantly improved, in comparison with the untreated transgenic controls. Thus, we suggest that a functional down regulation of the M-current contributes to a neuronal hyperactivity and network dysregulation that may lead to the neurodegeneration observed in these model systems, and that KCNQ2/3 channel regulation may be a target for therapeutic intervention.

Disclosures: Y. Cao: None. **D. Bartolome-Martin:** None. **D.S. Faber:** None. **N. Rotem:** None. **C. Rozas:** None. **S.S. Dellal:** None. **B. Kadriu:** None. **M. Gulinello:** None. **K. Khodakhah:** None. **M.A. Chacon:** None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Canadian Institutes of Health Research

Cure Huntington Disease Initiative

Michael Smith Foundation for Health Research

Title: Characterization of signature changes mediated by mutant huntingtin expression in cortico-striatal co-culture: Towards a drug screening platform

Authors: *C. BUREN, M. P. PARSONS, L. A. RAYMOND;
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Abstract: Huntington disease (HD) is caused by a polyglutamine (polyQ) expansion in the N-terminal region of the huntingtin protein (Htt). This mutation results in synaptic dysfunction and eventual neurodegeneration that particularly affects the spiny projection neurons (SPNs) of the striatum. Several animal models of Huntington's disease (HD) show altered cortical-striatal (C-S) presynaptic glutamate release, glutamate uptake and trafficking/signaling of postsynaptic glutamate receptors. Synaptic changes precede the HD-like motor phenotype, and synaptic proteins form interaction hubs with Htt. Therapy to ameliorate functional deficits in neuronal circuitry may therefore delay HD onset and provide neuroprotection. Here, we aimed to characterize the "signature" changes in C-S synaptic transmission associated with mutant Htt expression in a reduced model system, the C-S co-culture, with the goal of using the model as a platform for testing therapeutics. Our results demonstrate progressive synaptic development on SPNs between day *in vitro* (DIV) 7 to DIV 21 in co-cultures from wild-type mice. Co-cultures from the YAC128 HD mouse model show similar development as wild-type up to DIV 14, after which there is a decline in excitatory, but not inhibitory, synaptic transmission. The reduction in frequency of miniature excitatory synaptic currents (mEPSCs) in YAC128 SPNs is not a result of reduced density of dendritic synapses, since the density of spines and co-localized puncta of postsynaptic (PSD-95) and presynaptic (VGLUT1) proteins were similar for wild-type and YAC128 SPNs at DIV 21. Surprisingly, YAC128 SPNs show significantly shorter total dendritic length by DIV21. In order to determine whether these changes are driven by aberrant cortical activity or secretion of growth factors, versus a cell autonomous effect of mHtt expression in SPNs, we employ "chimeric" co-cultures, comparing the four different combinations of cortical

and striatal neurons from wild-type and YAC128 mice. Potential treatments for recovery of excitatory synaptic transmission in YAC128 SPNs are under investigation.This co-culture system can facilitate mechanistic studies and serve as a drug screening platform for early HD synaptopathy.

Disclosures: C. Buren: None. **M.P. Parsons:** None. **L.A. Raymond:** None.

Poster

795. Huntington's Disease Mechanisms II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS078008

Title: Calcium uptake capacity and induction of the permeability transition pore in brain mitochondria from YAC 128 mice

Authors: *J. J. PELLMAN[1], T. BRUSTOVETSKY[1], N. BRUSTOVETSKY[1,2];
[1]Pharmacol. and Toxicology, [2]Stark Neurosci. Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Aberrant mitochondrial calcium handling has been proposed to contribute to mitochondrial damage and neuronal injury in Huntington's disease (HD), a rare hereditary neurodegenerative disorder characterized by motor and cognitive dysfunction. HD pathogenesis is linked to a mutation in the huntingtin protein, a ubiquitous 350 kDa protein involved in developmental processes. The mutant huntingtin (mHtt) protein has an elongated polyglutamine stretch in the N-terminus that is believed to result in HD neuropathology. Despite numerous studies, the mechanisms of deleterious mHtt action are not completely understood. It has been previously reported that mHtt causes bioenergetic deficits, increases likelihood of induction of the mitochondrial permeability transition pore (PTP), and, therefore, decreases mitochondrial Ca^{2+} uptake capacity. However, some investigators found neither a decrease in Ca^{2+} uptake capacity, nor an increase in propensity to PTP induction in mitochondria isolated from brains of HD mouse models. In the present study, we investigated the effect of mHtt on Ca^{2+} uptake capacity and PTP induction in synaptic and non-synaptic brain mitochondria isolated from YAC128 mice and wild-type (WT) littermates. We confirmed that mHtt binds to isolated mitochondria and can be removed by alkali treatment in a medium with pH 11.5. In both

synaptic and non-synaptic mitochondria, mHtt failed to diminish Ca^{2+} uptake capacity. Moreover, addition of mHtt to brain mitochondria isolated from WT mice failed to decrease Ca^{2+} accumulation. In experiments with cultured striatal neurons derived from YAC128 mice and their WT littermates, a short application of glutamate produced similar transient elevations in cytosolic Ca^{2+}. After removal of glutamate and recovery of resting cytosolic Ca^{2+}, FCCP triggered a release of Ca^{2+} from mitochondria and produced similar Ca^{2+} elevations in neurons from both YAC128 and WT mice. This suggests similar amounts of Ca^{2+} accumulated in mitochondria and, consequently, the lack of detectable alterations in Ca^{2+} uptake by neuronal mitochondria exposed to mHtt. The level of cyclophilin D expression, the rate of ROS generation, and an induction of the PTP by Ca^{2+} in mitochondria isolated from both YAC128 and WT mice appeared to be similar. Thus, our experiments do not provide evidence for the decreased Ca^{2+} uptake capacity and argue against increased likelihood of PTP induction in brain mitochondria from YAC128 mice.

Disclosures: J.J. Pellman: None. **T. Brustovetsky:** None. **N. Brustovetsky:** None.

Poster

795. Huntington's Disease Mechanisms II

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Title: Huntingtin is required for normal excitatory synapse development in cortical and striatal circuits

Authors: *S. U. MCKINSTRY[1], Y. B. KARADENIZ[1], A. K. WORTHINGTON[1], V. HARAPETYAN[1], M. I. OZLU[1], K. SERAFIN-MOLINA[1], W. C. RISHER[1], T. USTUNKAYA[1], I. DRAGATSIS[2], S. ZEILTIN[3], H. H. YIN[1], C. EROGLU[1];
[1]Duke Univ., Durham, NC; [2]The Univ. of Tennessee, Memphis, TN; [3]Univ. of Virginia, Charlottesville, VA

Abstract: Huntington's disease (HD) is a neurodegenerative disease caused by the expansion of a poly-glutamine (poly-Q) stretch in the huntingtin (Htt) protein. Gain-of-function effects of mutant Htt have been extensively investigated as the major driver of neurodegeneration in HD. However, loss-of-function effects of poly-Q mutations recently emerged as potential drivers of disease pathophysiology. Early synaptic problems in the excitatory cortical and striatal connections have been reported in HD, but the role of Htt protein in synaptic connectivity was unknown. Therefore, we investigated the role of Htt in synaptic connectivity *in vivo* by conditionally silencing Htt in the developing mouse cortex. When cortical Htt expression was silenced, cortical and striatal excitatory synapses formed and matured faster through postnatal day 21 (P21), but this exuberant synaptic connectivity was lost by 5 weeks. Synaptic decline in the cortex was accompanied with layer- and region-specific reactive gliosis. To determine whether the disease-causing poly-Q mutation in Htt affects synapse development, we next investigated the synaptic connectivity in a full-length knock-in mouse model of HD, the KIQ175 mouse. Similar to the cortical conditional knockouts we found abnormal excitatory synapse formation and maturation in the cortices of P21 KIQ175, which was lost by 5 weeks. Taken together, our findings reveal that cortical Htt is required for the correct establishment of cortical and striatal excitatory circuits, and this function of Htt is lost when the mutant Htt is present.

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Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Canadian Institutes of Health Research

Michael Smith Foundation for Health Research

Title: Imaging the spatiotemporal dynamics of evoked glutamate release in a mouse model of Huntington disease using iGluSnFr

Authors: *M. P. PARSONS, M. P. VANNI, T. H. MURPHY, L. A. RAYMOND;
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The cell death that occurs in certain neurodegenerative diseases can be attributed in part to glutamate spillover outside of the synaptic cleft where it can activate extrasynaptic NMDA receptors (eNMDARs). In Huntington disease (HD), there is an early increase in the quantity of eNMDARs on vulnerable neurons in the striatum which contributes to their subsequent death. Moreover, the reported decrease in the expression and function of glutamate transporters in the HD striatum provides a potential means whereby otherwise normal synaptic transmission may activate eNMDARs. Here, we employed a novel fluorescent glutamate sensor, iGluSnFr, to assess its usefulness as a tool to visualize and quantify glutamate spill-over in the acute slice preparation. We then used this sensor to compare the spatiotemporal dynamics of glutamate rise (release) and decay (uptake) in the striatum of wild-type (WT) and YAC128 mice, a well-characterized mouse model of HD. Glutamate indicator expression was obtained by injecting AAV-syn-iGluSnFR virus into the striatum of 1-month old male mice. Acute brain slices were obtained 3 weeks later. Wide field fluorescence recording was performed with a CCD camera. iGluSnFR was excited with a 470nm LED and emission fluorescence was collected using a 530nm bandpass filter. Striatal glutamate release was induced by local electrical stimulation with a tungsten electrode. iGluSnFr responses could not be detected following single pulses (100μA, 0.1 ms), despite the presence of excitatory postsynaptic potentials by whole-cell patch clamp recording. Interestingly, clear fluorescent responses to single pulses were observed at higher stimulation intensities, which may indicate the requirement of glutamate spillover to detect a response under our experimental conditions. Furthermore, at 100 μA, paired pulses or high frequency trains were required to observe a response, and response magnitude was inversely correlated with the inter-pulse interval. Pharmacological inhibition of glutamate transporters dramatically lengthened the response decay, confirming the glutamatergic nature of the signal. Despite previous reports of a glutamate uptake deficiency in HD, our preliminary data show no clear differences in the iGluSnFr decay in the striatum of WT and YAC128 mice. We are currently aging a cohort of animals to test glutamate dynamics in symptomatic HD. Our study demonstrates the usefulness of iGluSnFr to explore glutamate dynamics following electrically evoked release in the acute slice preparation. By using this technique, we expect to gain a better understanding of the contribution of glutamate spill-over to HD pathogenesis.

Disclosures: **M.P. Parsons:** None. **M.P. Vanni:** None. **T.H. Murphy:** None. **L.A. Raymond:** None.

Poster

795. Huntington's Disease Mechanisms II

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Program#/Poster#: 795.24/M12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Vaccinia-related kinase 2 mediates accumulation of polyglutamine aggregates via negative regulation of the chaperonin tric

Authors: *J. LEE;
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Abstract: Misfolding of proteins containing abnormal expansions of polyglutamine (polyQ) repeats is associated with cytotoxicity in several neurodegenerative disorders, including Huntington's disease. Recently, the eukaryotic chaperonin TRiC hetero-oligomeric complex has been shown to play an important role in protecting cells against the accumulation of misfolded polyQ protein aggregates. It is essential to elucidate how TRiC function is regulated to better understand the pathological mechanism of polyQ aggregation. Here, we propose that vaccinia-related kinase 2 (VRK2) is a critical enzyme that negatively regulates TRiC. In mammalian cells, overexpression of wild-type VRK2 decreased endogenous TRiC protein levels by promoting TRiC ubiquitination, but a VRK2 kinase-dead mutant did not. Interestingly, VRK2-mediated downregulation of TRiC increased aggregate formation of a polyQ-expanded huntingtin fragment. This effect was ameliorated by rescue of TRiC protein levels. Notably, small interference RNA-mediated knockdown of VRK2 enhanced TRiC protein stability and decreased polyQ aggregation. The VRK2-mediated reduction of TRiC protein levels was subsequent to the recruitment of COP1 E3 ligase. Among the members of the COP1 E3 ligase complex, VRK2 interacted with RBX1 and increased E3 ligase activity on TRiC *in vitro*. Taken together, these results demonstrate that VRK2 is crucial to regulate the ubiquitination-proteosomal degradation of TRiC, which controls folding of polyglutamine proteins involved in Huntington's disease.

Disclosures: J. Lee: None.

Poster

795. Huntington's Disease Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: University of Luxembourg- Institute for Systems Biology Strategic Partnership

CHDI

National Science Foundation GRFP

Title: Integrated systems biology approach reveals relationships between age, Htt genotype, and transcriptional dysregulation in knock-in mouse models of Huntington's disease

Authors: *J. R. PEARL[1], S. A. AMENT[2], J. C. EARLS[2], C. PLAISIER[2], N. GOODMAN[2], G. CARLSON[3], A. GRINDELAND[3], V. WHEELER[4], J. CARROLL[5], N. D. PRICE[2], L. E. HOOD[2];
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Abstract: Transcriptional dysregulation is an early and progressive phenotype in Huntington's disease (HD), a fatal neurodegenerative disease caused by expanded poly-glutamine (CAG) repeats in the Htt gene. Longer CAG repeat lengths are associated with earlier age of onset in HD patients and mouse models, but the relationships between Htt genotype, transcriptional networks, and pathogenesis remain poorly understood. We generated a comprehensive microarray dataset profiling the effects of Htt CAG repeat length across age and four background strains in a knock-in mouse model of HD. We profiled gene expression in striatal tissue from 827 mice with nominal Htt CAG allele lengths Q7 (wild-type), Q50, Q92, and Q111 harvested between 4 and 20 weeks of age. All of these timepoints precede the onset of overt behavioral deficits. One of the primary aims of the analysis was to identify the earliest perturbations in Htt-related transcriptional networks. By looking at these early changes, we hope to infer the most proximal mechanisms of the disease. Linear modeling revealed a total of 809 genes that were differentially expressed between Q111 and wild-type mice across four genetic backgrounds, with the largest effect size in the C57BL/6J strain. In addition, we found that an increase in the expansion of the CAG repeat tract led to a cascade of transcriptional relationships that occurs earliest in the longer repeat lengths. We used transcriptional network inference approaches and Differential Rank Conservation (DiRAC) to explore specific transcriptional regulatory mechanisms underlying these age- and CAG-length dependent changes in gene expression. Our analysis indicates that progressive reorganization of gene modules related to synaptic dysfunction and chromatin remodeling begins after just 10-12 weeks in the Q111 mice. We identified putative transcriptional regulators involved in these processes. Based on these results,

we are optimistic that mapping premanifest phenotypes of HD will lead to better clinical markers and novel therapeutic targets.

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Poster

796. Motor Neuron Disease Mechanisms

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Title: Promoter methylation is an epigenetic disease modifier of mutant C9orf72

Authors: J. RUSS[1], K. WU[1], D. NEAL[2], E. SUH[2], D. J. IRWIN[2,3], C. T. MCMILLAN[3], E. M. WOOD[2], S. X. XIE[4], L. ELMAN[3], L. MCCLUSKEY[3], M. GROSSMAN[3], V. M. VAN DEERLIN[2], *E. B. LEE[5,6,7];
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Abstract: *C9orf72* promoter hypermethylation inhibits the accumulation of pathologies which have been postulated to be neurotoxic. We tested here whether *C9orf72* methylation is associated with prolonged disease in *C9orf72* mutation carriers. *C9orf72* methylation was quantified from brain, blood or saliva using methylation-sensitive restriction enzyme digest-qPCR in a cross-

sectional cohort of 133 *C9orf72* repeat expansion carriers and 37 non-carrier family members. Multivariate regression models were used to determine whether *C9orf72* methylation was associated with age at onset, disease duration, or age at death. Permutation analysis was performed to determine whether *C9orf72* methylation is heritable. While there were differences in *C9orf72* methylation between blood and saliva, there was a high correlation between *C9orf72* methylation in brain versus blood (r_S=0.74, p=0.0012). *C9orf72* methylation was not significantly different between ALS and FTD, and did not predict age at onset. However, brain and blood *C9orf72* methylation was associated with later age at death in FTD (brain: β=0.18, p=0.006; blood: β=0.15, p<0.001). Furthermore, blood *C9orf72* methylation was associated with longer disease duration in FTD (β=0.03, p=0.007). Finally, analysis of pedigrees with multiple mutation carriers demonstrated a significant association between *C9orf72* methylation and family relatedness (p<0.0001). *C9orf72* methylation is associated with prolonged disease in *C9orf72* repeat expansion carriers with FTD. The attenuated clinical phenotype associated with *C9orf72* hypermethylation suggests that slower clinical progression in FTD is associated with reduced expression of mutant *C9orf72*. These results support the hypothesis that expression of the hexanucleotide repeat expansion is associated with a toxic gain of function.

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Poster

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Title: C9orf72 hypermethylation protects against repeat expansion associated pathology in ALS/FTD

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Abstract: Hexanucleotide repeat expansions of C9orf72 are the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal degeneration. The mutation is associated with reduced C9orf72 expression and the accumulation of potentially toxic RNA and protein aggregates. CpG methylation is known to protect the genome against unstable DNA elements and to stably silence inappropriate gene expression. Using bisulfite cloning and restriction enzyme based methylation assays on DNA from human brain and peripheral blood, we observed CpG hypermethylation involving the C9orf72 promoter in cis to the repeat expansion mutation in approximately one third of C9orf72 repeat expansion mutation carriers. Promoter hypermethylation of mutant C9orf72 was associated with transcriptional silencing of C9orf72 in patient-derived lymphoblast cell lines, resulting in reduced accumulation of intronic C9orf72 RNA and reduced numbers of RNA foci. Furthermore, demethylation of mutant C9orf72 with 5-aza-deoxycytidine resulted in increased vulnerability of mutant cells to oxidative and autophagic stress. Promoter hypermethylation of repeat expansion carriers was also associated with reduced accumulation of RNA foci and dipeptide repeat protein aggregates in human brains. These results indicate that C9orf72 promoter hypermethylation prevents downstream molecular aberrations associated with the hexanucleotide repeat expansion, suggesting that epigenetic silencing of the mutant C9orf72 allele may represent a protective counter-regulatory response to hexanucleotide repeat expansion.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Title: Angiogenin is a secreted ribonuclease that cleaves RNAs in paracrine

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Abstract: Angiogenin is a secreted member of the pancreatic ribonuclease A superfamily. Angiogenin is expressed in motorneurons and secreted under serum deprivation conditions, whereupon it is endocytosed by neighbouring astroglia. Numerous ALS-associated loss-of-function point mutations have been identified in both familial and sporadic forms of the disease. ALS-associated angiogenin mutations either lead to loss of ribonuclease activity or modulate subcellular localisation. We have previously shown that systemic angiogenin delivery increases lifespan and ameliorates symptoms in the SOD1 G93A ALS mouse model. We have established a paracrine cell culture system to further investigate the function of angiogenin using SH-SY5Y neuroblastoma cells overexpressing wild type or mutant angiogenin. In our system the K40I angiogenin mutant is produced at significantly lower levels than wild type angiogenin. Under serum-starvation conditions angiogenin is secreted into the media. The angiogenin conditioned media is then applied to astrocytoma cell line MZ-294 and angiogenin is internalised. We demonstrate that angiogenin expression and treatment generates RNA fragments by PAGE analysis and SYBR Gold staining of small RNAs. Cleavage of tRNAs within the anticodon loop was detected in both neuroblastoma and astrocytoma cell lines using custom taqman assays and northern blotting. However angiogenin uptake into astrocytes induces the cleavage of distinct RNA molecules, in addition to tRNA fragments, suggesting angiogenin has broader substrate specificity than tRNA alone.

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Poster

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5 R01 NS061867 06

Title: The role of the rna binding protein rbm45 and nuclear stress bodies in als and ftld

Authors: *M. A. COLLINS[1], Y. LI[2], R. BOWSER[2];
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Abstract: The aggregation of RNA binding proteins into inclusion bodies is a pathological hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Evidence suggests that the aggregation process can be initiated by the incorporation of these proteins into protein-RNA complexes, such as stress granules. We recently demonstrated that the RNA binding protein RBM45 forms intracellular inclusions in neurons and glia in ALS, FTLD, and Alzheimer's disease. The mechanisms by which RBM45 forms inclusions are unclear, as it does not incorporate into cytoplasmic stress granules. In our post-mortem disease tissues, RBM45 staining exhibited a speckled nuclear staining pattern, suggestive of the protein's incorporation into a subnuclear structure. Despite this, RBM45 does not associate with a variety of known subnuclear structures, including nuclear speckles, Cajal bodies, and nuclear gems. During conditions of cellular stress, including oxidative stress, genotoxic stress, and UV light, the distribution of the protein changes to a punctate staining pattern. Using a combined immunocytochemistry/digital deconvolution approach, we show that the RBM45-positive puncta in stressed nuclei correspond to the protein's incorporation into nuclear stress bodies (NSBs). Under conditions of cellular stress, RBM45 exhibits significant colocalization with the NSB marker HSF1 ($p < .05$), but does not do so under basal conditions ($p > .05$). Likewise, the number of RBM45-positive puncta in the nucleus of cells increases in a stress-dependent manner. We also show that the number of these puncta is significantly increased in neurons from ALS and FTLD post-mortem tissue when compared to control ($p < .05$). Overexpression of the protein in cells is, itself, sufficient to cause the formation of these structures. Using a domain mapping approach, we show RBM45's ability to associate with NSBs depends critically upon specific structural elements of the protein, including a putative nuclear localization sequence. Collectively, these results demonstrate that RBM45 is a component of NSBs and NSBs represent a novel disease-associated protein-RNA complex that is a potential therapeutic target for disorders such as ALS and FTLD.

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Poster

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NSF GRFP Fellowship

Target ALS

Title: Examining the role of necroptosis signaling in motor neuron dysfunction and death in amyotrophic lateral sclerosis

Authors: K. POLITI[1], D. RE[2], V. LE VERCHE[2], S. PHANI[2], R. PRADHAN[2], *S. E. PRZEDBORSKI[3];
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Abstract: Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset paralytic disorder. ALS can be familial (fALS) through inheritance of genetic mutations such as superoxide dismutase-1 (mSOD1) or sporadic (sALS) with no familial history. Transgenic mice expressing mSOD1 develop a phenotype which emulates the clinical and pathological hallmarks of ALS, including motor neuron (MN) degeneration and nerve terminal retraction from the neuromuscular junction (NMJ). Importantly, the molecular mechanisms leading to these two pathogenic events remain unknown and studies suggest they may result from distinct mechanisms. Previously, we reported that wild-type primary or embryonic stem cell-derived spinal MNs show significant death and axonal shortening *in vitro* upon exposure to primary astrocytes from mice expressing fALS-linked mSOD1 or from human sALS patients. We also demonstrated that mSOD1 MNs cell autonomously exhibit a similar axonal length defect. Recently, we reported the death of MN in response to ALS astrocytes is driven by necroptosis, a non-classical form of programmed cell death. Indeed, necrostatin 1 (Nec1), an inhibitor of the kinase activity of the necroptosis initiator, receptor interacting protein kinase (RIP1), prevented MN death in both *in vitro* models. Now, we propose to dissect further the first necroptotic cascade described in non-neoplastic neurons and to determine whether necroptosis also drives the axonal defects we previously observed. A canonical step in necroptosis initiation is the formation of a complex containing RIP1, RIP3 and mixed lineage kinase domain like protein (MLKL). Here, we provide evidence that RIP3 also drives MN death, as RIP3 silencing by shRNA renders MNs resistant to mSOD1 astrocytes. We are currently optimizing the silencing of MLKL in MNs to confirm its involvement. Additionally, our preliminary data suggest that Nec1 may prevent the axonal defects non-cell autonomously mediated by mSOD1 astrocytes. Analysis is ongoing to determine the effect of Nec1 on the cell-autonomous axonal defect in mSOD1 MNs. Our next step will be to genetically confirm the Nec1 results by silencing RIP1 in these two experimental conditions. The validation in our models of RIP3 and MLKL is critical, as in contrast with RIP1, which has several known functions independent of necroptosis, RIP3 and MLKL have not yet been shown to have non-redundant roles independent of necroptosis. Accordingly, they may prove more selective targets to be pursued *in vivo* for the development of

neuroprotective therapies for ALS. Finally, our study will help to predict whether anti-necroptosis strategy can also target muscle denervation in ALS.

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Poster

796. Motor Neuron Disease Mechanisms

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Title: The role of HIF-1α and SREBP1 in models of ALS

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Abstract: In Amyotrophic Lateral Sclerosis (ALS), degeneration of motor neurons is accompanied with mitochondrial dysfunction, leading to disrupted ATP production and reactive oxygen species (ROS) accumulation. We asked if degenerating neurons could employ alternate pathways for producing ATP and fighting ROS build-up, and if these could be beneficial to disease progression. The two arms of glucose metabolism – glycolysis and the pentose phosphate pathway (PPP) – lead to ATP production and NADPH production to combat ROS accumulation respectively. The mammalian target of rapamycin 1 (mTORC1) is poised to control flux through both arms of glucose breakdown via two transcription factors – hypoxia inducible factor 1α (HIF-1α) and sterol regulatory element binding protein 1 (SREBP1). HIF-1α regulates transcription of key genes in glycolysis (phosphofructokinase, pyruvate dehydrogenase kinase etc.) and SREBP1 regulates transcription of key genes in the PPP (glucose-6-phosphate dehydrogenase) and lipid biosynthesis (fatty acid synthase). We hypothesize that mTORC1, HIF-1α and SREBP1 are downregulated in ALS, preventing motor neurons from combating dysfunctional mitochondria. Additionally, we hypothesize that increasing the activity of these factors will be protective in models of ALS. Preliminary experiments show that constitutive

activation of HIF-1α in the mutant SOD1 background in *C. elegans* is protective against mutant SOD1-induced deficits in worm locomotion. Ongoing experiments will investigate the mRNA and protein levels of HIF-1α and SREBP1 targets *in vitro* and *in vivo* in mammalian models of the disease (cultured rat neurons and mice). Preliminary experiments have also established a pharmacological agent (FG-4497, from Fibrogen, Inc) upregulates HIF-1α function. Ongoing experiments will use this compound to test the role of this compound in protecting against mutant SOD1-induced toxicity in cultured neurons. We have also designed a microRNA against TSC2, a negative regulator of mTORC1 function, and will test the role of this microRNA in protecting against mutant SOD1-induced toxicity. Mitochondrial dysfunction is a key contributor to changes in cellular metabolism observed in ALS. By exploring the role of specific transcription factors aimed at addressing these changes, we hope to gain insight into pathogenesis as well as identify targets for therapeutic intervention in this devastating disease.

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Poster

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Title: Quantitative assessment of endogenous calcium homeostasis in adult hypoglossal motoneurons of wildtype and SOD1-G93A ALS mice

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Abstract: In amyotrophic lateral sclerosis (ALS) hypoglossal (hMN) and spinal motoneurons but not occulomotor neurons (oMN) degenerate, leading to paralysis of skeletal muscles in the whole body, while even terminal patients can still move their eyes. Previous work suggested that differences in calcium homeostasis in spinal and hypoglossal motoneurons compared to oMNs might partially account for this differential vulnerability. Until now, quantitative parameters of calcium homeostasis were determined only in early postnatal mice (up to postnatal day P6) because of methodological limitations. However, as death of hMNs starts not before P60 and disease endstage is reached around P130 in a mouse model of ALS (SOD1-G93A), calcium homeostasis should be studied in this relevant adult age range to assess its direct pathophysiological involvement. Recently, we developed a mouse brainstem slice preparation that allowed patch-clamp recordings of adult hMNs up to disease endstage and filling of the cells with a calcium indicator dye (Fuchs et al. J Physiol 2013). Visualization of the neurons enabled the combination of electrophysiological recordings with fluorometric calcium measurements. Endogenous calcium homeostasis of adult (P130) hMNs in wildtype (wt) and SOD1-G93A mice was quantified by using the "added buffer" approach. In this case, the gradual disruption of endogenous calcium homeostasis by the calcium indicator dye is utilized to quantify endogenous calcium parameters in the cell. Interestingly, we found an endogenous buffering capacity $\kappa s < 70$ in adult wt hMNs. This is small compared to capacities in other central neurons (e.g. hippocampal CA1 pyramidal cells $\kappa s \approx 200$) and provides direct support for the idea that low calcium buffering capacities represent a significant risk factor for selectively vulnerable cells. κs was even lower in SOD1-G93A hMNs ($\kappa s < 50$) compared to wt, indicating that the mutated SOD1-driven disease process further impaired the already constitutively low calcium buffering capacity of vulnerable motoneurons. The extrapolated decay time of calcium transients τ in the absence of indicator dye was 0.29 ± 0.07 s ($n = 52$) for wt and 0.12 ± 0.09 s ($n = 42$) for SOD1-G93A, reflecting rapid somatic calcium dynamics and efficient cytoplasmic clearing by extrusion and calcium uptake into intracellular compartments under physiological conditions. For the first time, our data quantitatively describe the calcium homeostasis in adult motoneurons in health and disease and might help to explain the differential vulnerability of different motor nuclei and even single neurons in ALS.

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Poster

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Title: The involvement of the notch signaling pathway in amyotrophic lateral sclerosis

Authors: A. H. NONNEMAN[1,2], T. PHILIPS[3], *P. DUPONT[4], R. LEMMENS[1,2,5], P. VAN DAMME[1,2,5], L. VAN DEN BOSCH[1,2], W. ROBBERECHT[1,2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a late-onset progressive neurodegenerative disease affecting motor neurons. This results into fasciculation, muscle atrophy and ultimately paralysis. Denervation of respiratory muscles and diaphragm is often the fatal event and typically occurs between 1 and 5 years after symptom onset. Studies using mutant SOD1 mice have shown that ALS is a non-cell autonomous disease, meaning that non-neuronal cells contribute to motor neuron degeneration. Typically for ALS is the presence of very reactive microglia and astrocytes, so-called microgliosis and astrocytosis, determining disease progression. Recently, also oligodendrocytes were found to actively contribute to ALS pathogenesis, as they degenerate extensively. Although degenerating oligodendrocytes are replaced by newly differentiated oligodendrocytes, differentiation seems to be incomplete. These new oligodendrocytes appear to be immature, unable to myelinate properly and unable to supply neurons with energy substrates. The Notch signaling pathway is important in regulating cell-cell communication, axonal retraction, microgliosis, astrocytosis, proliferation and differentiation of oligodendrocyte precursor cells into mature oligodendrocytes, and even cell death. Notch signaling is also involved in several neurodegenerative diseases, such as Alzheimer's disease and ischemic stroke. This knowledge suggests that the study of Notch signaling is relevant for ALS pathogenesis. We hypothesize that Notch signaling may be disturbed in ALS, thereby contributing to disease progression. More particularly, we hypothesize the involvement of Notch in the non-cell autonomous glial cell contribution to motor neuron degeneration. First, we investigated whether

the expression of components of Notch signaling, such as ligands, receptors and targets, is altered in ALS mice (SOD1G93A), compared to control mice (SOD1WT and non-transgenic), and whether the expression changes with disease progression. We used qRT-PCR, western blot and immunofluorescent staining, and found remarkable changes in the expression of several Notch ligands, receptors and targets, with a clear disease progression dependency. In addition, we studied to what extend modulation of the Notch signaling pathway in the SOD1G93A mice has beneficial effects on ALS pathological hallmarks. A tamoxifen-induced Cre-Lox mediated recombination approach was used for modulating Notch signaling. In conclusion, our data suggest that Notch signaling is severely altered in ALS mice and may play an important role in the non-cell autonomous component of ALS pathogenesis.

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Poster

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Title: Loss of TIA1 impairs development of male reproductive organs in a mouse model of spinal muscular atrophy

Authors: *M. D. HOWELL[1], N. N. SINGH[1], J. SEO[1], E. M. WHITLEY[2], R. N. SINGH[1]; [1]Biomed. Sci., [2]Vet. Pathology, Iowa State Univ., Ames, IA

Abstract: Spinal muscular atrophy (SMA) results from low levels of survival motor neuron (SMN) protein in the central nervous system and peripheral tissues. While death of spinal cord motor neurons and deficits in neuromuscular junctions have been characterized in SMA patients and mouse models of the disease, the consequences of low SMN on many peripheral tissues,

including reproductive organs, is unknown. Low levels of SMN in SMA results from the loss of the SMN1 gene coupled with predominant SMN2 exon 7 skipping that leads to the synthesis of the truncated protein SMNΔ7. We previously reported that depletion and overexpression of TIA1, a RNA-binding protein, promotes SMN2 exon 7 skipping and inclusion, respectively. To assess the impact of the loss of TIA1 on severity of SMA, we generated TIA1 knockout mice on the background of the recently reported allele C mouse model, a mouse that exhibits mild SMA-like symptoms. We examined the gross appearance and histology of testes at six weeks of age in wild type (WT; Smn+/+, Tia1+/+), TIA1-KO (Smn+/+, Tia1-/-), allele C (SmnC/C, Tia1+/+) and allele C-TIA1-KO (SmnC/C, Tia1-/-). Compared to WT mice, we found strikingly smaller testes in allele C mice. Histologically, allele C testes exhibited poorly organized seminiferous tubules and apparent disrupted spermatogenesis. As further evidence of impaired spermatogenesis, sperm count in allele C epididymides was significantly reduced compared to WT males. TIA1-KO testes were slightly larger than WT testes, but there was no difference in histology or sperm count compared to WT mice. Allele C-TIA1-KO mice, however, had significantly reduced testes and further reduced epididymal sperm count compared to allele C mice. Interestingly, SMN protein level in testis remained unchanged in allele C and allele C-TIA1-KO compared to WT and TIA1-KO mice. These results suggest that low levels of SMN in other tissues, particularly the autonomic nervous system that controls testicular blood flow, is responsible for the impaired development of testes. TIA1 is a glutamine-rich protein that regulates stress-granule formation, a critical cellular process involving several factors including SMN. Earlier studies reported that mice lacking Tia1 are hypersensitive to toxic effects of lipopolysaccharides and exhibit dysregulated expression of lipid storage and membrane dynamics factors in nervous tissue. Further, mutations in TIA1 have been associated with Welander distal myopathy, an adult onset autosomal dominant disorder characterized by distal limb weakness. Our results uncover a novel role for TIA1 in modulating testicular development in SMA, a leading genetic disease of children.

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Poster

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Title: Investigation of the timing and extent of sensory nervous system degeneration in ALS

Authors: *A. LAGRIMAS[1], B. GENC[1], H. WILSON[2], R. HESS[9], M. YASVOINA[1], M. TU[1], D. M. MENICHELLA[1], R. J. MILLER[3,4], A. PALLER[2,5,6,7], P. OZDINLER[1,5,8]; [1]Neurol., [2]Dermatol., [3]Mol. Pharmacol. and Biol. Chem., [4]Robert H. Lurie Cancer Ctr., [5]Robert H. Lurie Comprehensive Cancer Ctr., [6]Ann & Robert H. Lurie Children's Hosp. of Chicago Res. Ctr., [7]Ctr. for Genet. Med., [8]Cognitive Neurol. and Alzheimer's Dis. Ctr., Northwestern Univ., Chicago, IL; [9]Univ. of Notre Dame, Notre Dame, IN

Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of the motor neuron circuitry. However the spatial and temporal degeneration of sensory nervous system with respect to disease progression has not been studied in detail. We recently generated a novel ALS mouse model reporter line, in which corticospinal and spinal motor neurons, together with sensory neurons, are genetically labeled with enhanced green fluorescent protein (eGFP) expression under the UCHL1 promoter. This mouse model offers, for the first time, a comparative study of peripheral neurons together with motor neurons and allows investigation of the sensory nervous system involvement in motor neuron disease pathology. In this study we investigated the timing and extent of potential sensory nervous system degeneration, using one of the most characterized mouse models of ALS. Qualitative and quantitative analysis of epidermal nerve density of $eGFP^{+}$ distal peripheral nerves in the footpads of the $hSOD1^{G93A}$-UeGFP mice at postnatal days (P) 30, 60, 90, and 120 revealed a significant decrease in epidermal nerve density only by P120, end-stage of the disease. Our preliminary results are in line with previously reported skin biopsy results obtained from ALS patients, and suggest that peripheral nervous system degeneration is most apparent toward the end of the disease.

Disclosures: **A. Lagrimas:** None. **B. Genc:** None. **H. Wilson:** None. **R. Hess:** None. **M. Yasvoina:** None. **M. Tu:** None. **D.M. Menichella:** None. **R.J. Miller:** None. **A. Paller:** None. **P. Ozdinler:** None.

Poster

796. Motor Neuron Disease Mechanisms

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Title: Importance of UCHL1 function for the motor neuron circuitry and the health of the corticospinal motor neurons

Authors: *B. GENC[1], J. H. JARA[1], E. ULUPıNAR[2], M. MANUEL[3], G. A. COX[4], M. C. BOHN[5], C. J. HECKMAN[3,6], R. P. ROOS[7], J. D. MACKLIS[8,9], C. J. DIDONATO[10,11], P. ÖZDINLER[1,12,13];
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Abstract: Corticospinal motor neurons (CSMN) receive, integrate and relay cerebral cortex's input towards spinal targets to initiate and modulate voluntary movement. CSMN degeneration is central for numerous motor neuron disorders and neurodegenerative diseases, but the cellular and molecular basis of CSMN vulnerability remains unknown. Mutations in the ubiquitin C-terminal hydrolase-L1 (*UCHL1*) gene have been detected in patients with neurodegenerative disease that affect motor function, and recently three siblings displayed early neurodegeneration, including upper motor neuron dysfunction. There is a need for the generation and characterization of novel

mouse models that recapitulate upper motor neuron loss in patients. Here we report a unique function of UCHL1 in maintaining CSMN viability and cellular integrity. *Uchl1*^{nm3419} (UCHL1 -/-) mice, which lack all UCHL1 function, display motor neuron circuitry defects. Even though spinal motor neurons remain intact with subtle dysfunction, CSMN show early, selective, progressive and profound cell loss. CSMN degeneration is mediated via increased ER stress and becomes evident at pre-symptomatic stages by cytoarchitectural defects primarily involving the apical dendrites. To reveal the importance of UCHL1 function for the motor neuron circuitry, we now generated novel conditional mutant mice in which UCHL1 function is removed either from the corticospinal or the spinal motor neurons, respectively.

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Poster

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TARGET-ALS

Fondation Thierry Latran "OHEX project"

Title: The electrophysiological properties of the different motor unit subtypes are not equally affected in adult SOD1-G93A mice

Authors: *M. MANUEL[1], C. HECKMAN[2], D. ZYTNICKI[1];
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Abstract: One of the proposed mechanism to explain the death of motoneurons (MNs) in ALS is an excitotoxic process, i.e. an excess electrical activity leading to an overload of intracellular calcium triggering apoptotic death. However, we have recently shown (Delestrée et al., J Physiol

2014) that spinal MNs of adult mice are not intrinsically hyperexcitable in SOD1-G93A mice. Instead, we found that a subpopulation of MNs lost their ability to fire repetitively in response to a stationary input. Yet, at the time, we could not determine whether this hypo-excitability was restricted to a specific physiological type of motor unit. We have developed an *in vivo* adult mouse preparation allowing simultaneously recording intracellularly spinal MNs and recording the force developed by their motor unit. We characterized the properties of motor units (MUs) from the Triceps Surae muscles of SOD1 mice and their non transgenic controls (WT) at the stage preceding their denervation (P35-P65). We classified the motor units in physiological types ("Slow" - S, "Fast fatigue-Resistant" - FR and "Fast Fatigable" - FF) on the basis of their contractile properties (contraction time, twitch force, fatigability, sag in unfused tetanus). During the time frame studied, the contractile properties of the MUs are not affected by the disease, and we could use the same criteria to classify MUs as in WT mice. We found that many electrical properties of SOD1 MNs are unchanged. For example, there was no differences between SOD1 and WT MNs in term of input conductance or rheobase in each of the physiological types taken separately. However, in keeping with our earlier results, we found that a large proportion of MNs (33% vs. 9% in WT mice) lost the ability to produce a sustained firing in response to a stationary input. Interestingly, all of these MUs had a fast contraction time (mean ± stdev 13 ± 3 ms, N=13), and produced twitch forces ranging from 0.4 to 34 mN. Based on their various contractile properties, these MUs were classified as FF (4 MUs out of 6) or FR (9 MUs out of 18). We were therefore able to demonstrate that FF and FR MUs (that are vulnerable in ALS), but not the S type MUs (that are resistant in ALS), become progressively hypoexcitable before they lose their connections to their muscle fibers. We are now investigating if an excitotoxic process could nevertheless arise from changes in excitatory and inhibitory inputs to MNs.

Disclosures: **M. Manuel:** None. **C. Heckman:** None. **D. Zytnicki:** None.

Poster

796. Motor Neuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: *In vivo* characterization of G93A-SOD1 mouse model of amyotrophic lateral sclerosis by magnetic resonance imaging (MRI) and spectroscopy (MRS)

Authors: K. LEHTIMÄKI, J. OKSMAN, T. LAITINEN, T. AHTONIEMI, A. NURMI, *S. HÄTINEN;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a human neurodegenerative disorder that progressively leads to paralysis and death. It is caused by the loss of motor neurons especially in the spinal cord, but brainstem is also affected. In this study, G93A-SOD1 mouse model of ALS was characterized with magnetic resonance imaging (MRI) for brainstem neurochemical metabolite levels (1H-MRS) and for transverse relaxation time changes (T2 MRI) in brainstem nuclei. Non-treated G93A-SOD1 and WT male and female mice, at the age of 110 days, were subjected to MR scanning with 11.7T Bruker system. Brainstem 1H metabolites were acquired using a single-voxel PRESS sequence. Transverse relaxation time (T2) in the brainstem nuclei (Nc. Facialis, Nc. Hypoglossus and Nc. Trigeminus) was determined using a MSME sequence. Additionally, brainstem volume was assessed from MR images at range -8.00 and -5.40 mm from bregma. To confirm disease progression, motor performance and clinical score were evaluated until the imaging end-point. Significant volume decrease (-11.2%) in the brainstem of SOD1 mice was detected as compared to WT mice. This volume decrease was associated with significant T2 relaxation time increase brainstem nuclei Nc. Facialis (+8.9%), Nc. Hypoglossus (+8.1%) and Nc. Trigeminus (+7.5%). Decreased levels of alanine (-40.5%), gamma-aminobutyric acid (GABA, -27.8%), phosphocholine (-45.0%) and N-acetyl acetate (NAA, -25.3%) were detected. In contrast, glutamine (+22.3%), myo-inositol (+11.3%) and taurine (+21.2%) revealed increased concentrations. Our results show significant T2 increase in studied brainstem nuclei in SOD1 mice, which is considered to be associated with appearance of fluid filled vacuoles in place of degenerated motor neurons (Bucher et al. 2007). In addition to T2 changes, reduction in total volume of brainstem in SOD1 mice likely reflects the degeneration of motor neurons. *In vivo* neurochemical fingerprint for affected brainstem revealed several changes from which the reduction of NAA (neuronal health/number) and GABA (GABAergic neurotransmission) has been previously reported in G93A SOD1 model by use of high-resolution *in vitro* 1H-MRS (Niessen et al. 2007). Results obtained in this study highlight the importance of non-invasive *in vivo* imaging modalities. MR imaging parameters shown here can complement longitudinal drug investigational studies in G93A SOD1 mouse model that conventionally have concentrated on motor behavior, survival, biochemical and histological read-outs.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Title: Characterization of the astroglial gap junction protein Connexin 43 in Amyotrophic Lateral Sclerosis

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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a primary motor neuron (MN) disease manifesting with progressive muscle atrophy and paralysis. Astrocytes in ALS can exert toxicity on MNs both in *in vitro* and *in vivo* model systems. Astrocytes form a highly coupled intercellular network in the central nervous system (CNS) through gap junctions. Gap junction in astrocytes, specifically connexin43 (Cx43) conducts important homeostatic functions in the nervous system such as regulation of the excitatory neurotransmitters, providing metabolic support to neurons, regulation of neurovasculature and synaptic events. However, under pathological conditions connexins are altered and their functions are compromised as observed in numerous neurological trauma/diseases such as spinal cord injury Alzheimer's disease, Parkinson's, ischemia, and others. This prompted us to investigate if the expression level of the major astrocyte connexin, Cx43 is altered in patients with ALS as well as animal models. We characterized the expression level of Cx43 in the SOD1G93A mice and notably in human post-mortem tissue from ALS patients. We examined Cx43 protein levels in the cervical, thoracic and lumbar spinal cord segments in the endstage SOD1G93A mice and observed a significant increase in Cx43 expression levels in all three regions compared to their wild-type litter mates. The histological study revealed Cx43 increase predominantly in the gray matter co-localizing mainly with astrocytes and a few microglia. Interestingly, when we examined the human post-mortem tissue for total Cx43 protein levels from ALS patients compared to their age-matched controls, we observed a significant increase in the motor cortex and cervical and lumbar spinal cord segments. Similar to the mouse model, Cx43 localized primarily to astrocytes and in the gray matter of human cervical spinal cord. This finding potentially implicates aberrant connexin biology in ALS either as an astrocytic response to neuronal injury or possibly as a mediator of disease. Future studies to investigate these contributions may be helpful in understanding glial responses in motor neuron disease.

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Poster

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Title: Presymptomatic dysfunction in glial cells at the NMJ may impact reinnervation in SOD1G37R mice

Authors: *E. MARTINEAU, R. ROBITAILLE;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of motoneurons. Glial cells are known to play a major role in disease progression. However, the contribution of Perisynaptic Schwann cells (PSCs), glial cells at the neuromuscular junction (NMJ), is still ill-defined in ALS despite that they regulate both synaptic and structural plasticity of the healthy NMJ. We previously reported an alteration of PSC properties at a presymptomatic (P120) and pre-onset (P370) stage of the disease in the Soleus, a muscle exhibiting a delayed progression. These properties were consistent with an impaired repair ability of PSCs. Since fast-twitch muscles are more vulnerable in ALS, we tested whether PSC properties were also altered in the fast-twitch Sternomastoid muscle. Using Ca2+ imaging on isolated nerve muscle preparations, we found that glial Ca2+ responses to endogenous neurotransmitter release evoked by motor nerve stimulation were unaltered at P120 but greatly diminished at P180. Furthermore, PSCs on a single NMJ displayed heterogeneous responses suggesting that PSCs are unable to accurately decode synaptic activity. PSCs displayed an improper balance between purinergic and muscarinic signals as revealed by selective pharmacological agonists and antagonists of PSC receptors. Since adequate PSC decoding of synaptic activity is required for proper maintenance of the NMJ, these presymptomatic glial abnormalities suggest that PSCs may not respond adequately to denervation in symptomatic animals. Unlike wild-type littermates, and consistent with this possibility, PSCs at denervated NMJs of end-stage animals failed to upregulate Mac-2, a marker of glial activation, while PSCs

at innervated NMJs upregulated it. Furthermore, fewer process extension by PSCs at denervated NMJs and fewer nerve terminal sproutings at innervated NMJs were observed. These features are essential for reinnervation and are modulated by PSCs. Interestingly, these defects were less pronounced in the Soleus muscle which is consistent with the partial resistance of slow-twitch muscles. Together, these results show that neuron-glia communication is altered early in ALS which may result in a disorganised glial response to denervation during disease progression.

Disclosures: E. Martineau: None. **R. Robitaille:** A. Employment/Salary (full or part-time):; Université de Montréal.

Poster

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JSPS KAKENHI Grant 26830046

Title: The role of the infiltrating immune cells attracted by activated microglia in amyotrophic lateral sclerosis mice

Authors: O. KOMINE[1], H. YAMASHITA[3], N. FUJIMORI-TONOU[4], Y. MORIWAKI[5], F. ENDO[1], S. WATANABE[1], H. MISAWA[5], *K. YAMANAKA[2];
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Abstract: Recent evidence suggests that not only immune reactions but also glia-immune interactions contribute to disease processes of neurodegenerative diseases. Microglial cells are responsible for innate immune responses against a traumatic injury and a bacterial infection in the central nervous system. Although adaptive immune system was shown to be involved in disease process of amyotrophic lateral sclerosis (ALS), the role of innate immune system in ALS was unclarified. We have demonstrated that the innate immune pathway was activated in inherited ALS model mice (mSOD1 mice) and TRIF, an essential adaptor for Toll-like receptor

signaling, was identified as determinant of disease progression in mSOD1 mice. TRIF-deficient mSOD1 mice exhibited the acceleration of disease progression with decreased expression level of chemokines in microglia and fewer infiltrating immune cells in the spinal cord. We also show that the activation of TRIF signaling in microglia serves as a bridge between innate immunity and adoptive immunity and the infiltrating immune cells attracted by microglia actively involve in neuroprotection in the mSOD1 mice spinal cords. Finally, in contrasts to the previous reports, we identified that infiltrating immune cells, mainly cytotoxic T-lymphocytes, natural killer T-lymphocytes, and natural killer cells, confer novel role in maintaining glial environment surrounding the motor neurons.

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Poster

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University Medical Center Giessen and Marburg (UKGM)

Title: Early-onset neuropathology and neuroinflammation in motor and sensory brain areas and their association with CGRP abundance in the SOD1-G93A mouse model of amyotrophic lateral sclerosis

Authors: *B. SCHUETZ[1], C. RINGER[2], H. SCHWARZBACH[1], S. TUNE[2], E. WEIHE[1]; [1]Philipps Univ., Marburg, Germany; [2]Dept. of Neurol., Univ. of California, Irvine, CA

Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease affecting primary motor neurons in cortex, and secondary motor neurons in brain stem and spinal cord. Progressive loss of these motor neurons results in denervation of skeletal muscles, paralysis, and finally death. Neuronal vacuolization accompanied by pronounced neuroinflammation is the neuropathological hallmark in the superoxide dismutase 1-G93A (SOD1-G93A) mouse model of ALS. Methods and results: using histological staining (Giemsa) and immunohistochemistry for vacuolization (human SOD1), astrocytes (GFAP), microglia

(Iba1), and neuropeptides (calcitonin gene-related peptide, CGRP) we discovered that vacuolization begins in the pre-symptomatic stage at post-natal day 50 in diverse motor and non-motor brain areas, including olfactory bulb and retina. In the olfactory bulb vacuoles were restricted to the external plexiform layer and apparently originated from satellite-like neurons. In the retina vacuoles were confined to the internal plexiform layer and most likely originated from amacrine cells. In the brainstem, cholinergic, dopaminergic and noradrenergic neurons were affected. Following vacuolization with a delay of 10-20 days, astrogliosis also started pre-symptomatically and simultaneously throughout the brain. Only in the pyramidal motor system a dying forward progression to cortical primary motor neurons was observed, where vacuolization and astrogliosis in the motor cortex emerged not until symptomatic disease stages. Across all brain regions analyzed, the degree of astrogliosis did not correlate with the number and size of vacuoles, but with CGRP abundance. Conclusion: SOD1-G93A-related ALS vacuolization pathology prominently affects limbic, autonomic and other non-motor areas including sensory circuits in the olfactory bulb and the retina lending support to the concept that ALS is a multisystem disorder well beyond the motor system. The observed correlation between astrogliosis and CGRP abundance in most affected regions suggests that neuroglial CGRP signaling is involved in ALS disease progression. We propose to test CGRP receptor antagonists to mitigate ALS disease progression.

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Poster

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Title: Oxidative stress expands the repertoire of alternatively spliced transcripts of spinal muscular atrophy gene

Authors: *J. SEO, S. SIVANESAN, M. D. HOWELL, E. W. OTTESEN, N. N. SINGH, M. SHISHIMOROVA, R. N. SINGH*;
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Abstract: Loss of Survival Motor Neuron 1 (SMN1) gene coupled with the skipping of SMN2 exon 7 causes spinal muscular atrophy (SMA), a leading genetic cause of infant mortality. With the consequence to the increased severity of SMA, recent cell-based studies have revealed oxidative stress (OS)-induced enhanced skipping of SMN2 exons. However, effect of OS on tissue specific splicing regulation of SMN2 remains unknown. Taking advantage of a transgenic mouse expressing SMN2, here we report a comprehensive analysis of SMN2 transcripts expressed in different tissues under normal and OS conditions. Our findings supported by exon-trapping PCR, quantitative real-time PCR and a multi-exon skipping detection assay (MESDA) capture tissue-specific signatures of splicing events of SMN2. Our results underscore that the occurrence of co-skipping of SMN2 exons 5 and 7 in response to OS is a signature event that climaxes during the first 24 h of a high-dose treatment of paraquat (PQ). Regarding tissue-specific differences in response to OS, we observed a relatively high proportion of Δ5,7 transcripts in brain and spinal cord followed by kidney, liver, lung, muscle and heart. Notably, proportion of Δ7 transcripts remained unchanged during PQ-induced OS in liver, lung and muscle. Brain and kidney showed an OS-induced decrease in levels in Δ7 transcripts, whereas, heart and spinal cord showed a slight increase in the proportion of Δ7 transcripts in response to OS. Levels of Δ5 splice variant remained very low during OS in all tissues examined. We also observed differential expression of several exons including co-skipping of exon 3 with other exons. Interestingly, effect of OS on testis was found to be distinct from other tissues. We examined effect of OS on levels of several proteins including human SMN, mouse SMN, TIA1 and TIAR. Our results suggest a unique role of the alternatively spliced transcripts in regulation of SMN translation.

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Poster

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Title: Direct conversion of spinal astrocytes into functional neurons with defined transcription factors and small molecules

Authors: *L. ZHANG[1], J. YIN[2], H. YEH[2], N. MA[2], G. LEE[2], G. WU[2], G. CHEN[2];
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Abstract: Neurons can be induced from glial cells by overexpression of proneuronal genes. Neurogening 2 (Ngn2) and NeuroD1 have been reported to convert glial cells into glutamatergic neurons both *in vitro* and *in vivo*. Here we report that defined small molecules facilitated Ngn2 to reprogram cultured mouse spinal astrocytes directly into functional motor neurons. The converted neurons were immunopositive for motor neuron markers Hb9, Isl1 and cholinergic markers CHAT and VACHT. Electrophysiological analysis demonstrated that these converted neurons were fully functional as they were capable of firing repetitive action potentials and showed large ionic currents. In addition, small molecules could facilitate Ngn2 to convert cultured human astrocytes into functional motor neurons. Interestingly, a defined combination of small molecules was able to convert cultured human astrocytes into functional neurons without overexpression of proneural genes. Taken together, our studies suggest that defined combination of small molecules can facilitate glia-neuron conversion and help to guide the converted neurons into specific subtype.

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Poster

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Project A.L.S. Selective vulnerability of motor neurons in ALS, 2013-03

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Title: Emerging roles of RBFOX1 and motor neuron hyperexcitability in an iPSC model of ALS

Authors: *E. R. LOWRY[1,2], D. J. WILLIAMS[3], Q. YAN[4], D. OAKLEY[5], K. EGGAN[6], H. WICHTERLE[5], C. E. HENDERSON[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly fatal adult-onset neurodegenerative disorder with no clear etiology. Patients experience worsening symptoms of weakness as the motor neurons (MNs) that govern voluntary movement degenerate, and typically die within 3-5 years of diagnosis. Most cases of ALS are sporadic; however, a minority of patients develop a familial form involving a disease-triggering mutation in one of several known genes, including C9ORF72, SOD1, TDP-43, FUS, and ANG. The downstream events that culminate in MN degeneration are largely unknown, but there are early increases in MN excitability that make ALS MNs more likely to respond to incoming stimulation from the excitatory neurotransmitter glutamate. Accordingly, by comparing iPS-derived MNs from familial ALS patients with isogenic control lines, where the disease-causing mutation has been corrected by TALENS, we have shown that ALS MNs respond more strongly to and are slower to recover from glutamatergic stimulation than controls. One possibility is that hyperexcitability could make MNs more vulnerable to excitotoxic cell death, in which excessive glutamatergic stimulation causes fatal Ca2+ imbalances within the cell. In several models of ALS, we have uncovered selective down-regulation of RBFOX1, an RNA-binding protein that is known to regulate neuronal excitability. We have also found that mice lacking RBFOX1 showed robust motor impairments that mirror those in ALS mice. Importantly, they also exhibited hyperexcitability in the MNs involved in hindlimb movement. The goal of this project is to evaluate the causal relationship between changes in RBFOX1 expression and changes in MN excitability, and to determine how changes in MN excitability ultimately lead to MN death. To accomplish these goals, we are taking advantage of a broad range of iPS lines, enabling high-throughput analyses in live human ALS MNs that would otherwise be inaccessible in patients.

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Characterization of β-methylamino-L-alanine (BMAA) as a tool to study environmental-linked sporadic amyotrophic lateral sclerosis

Authors: *B. IKIZ[1], S. D. CROLL[2], A. J. MURPHY[2], L. E. MACDONALD[2], M. L. LACROIX-FRALISH[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset paralytic disorder, characterized by the loss of upper and/or lower motor neurons through a pathological mechanism that is not completely understood. While ALS is mostly a sporadic disease, for about 90% of the cases, the majority of the research done in the field had focused on modeling the familial form, which necessitates tools to model the sporadic form of ALS. β-methylamino-L-alanine (BMAA) is an environmental neurotoxin that has been linked to a significantly higher ALS incidence in a population in Guam. To test whether BMAA can be used to study environmental-linked sporadic ALS *in vitro*, we generated mouse embryonic stem cell-derived motor neurons and exposed these neurons to differing concentrations of BMAA over 14 days in culture. In these studies, we have found that BMAA induces motor neuron death in a dose-dependent manner after seven days in culture and this degeneration is not reversible even after the BMAA is removed from the neuronal cultures and replaced with fresh medium. We have also observed that the exposure to BMAA results in early oxidative stress in motor neurons. Moreover, given that the addition of AMPA/kainate receptor antagonist (NBQX), but not NMDA receptor antagonist (MK-801), to culture rescues motor neurons, we believe that the AMPA/kainate receptor-mediated glutamate excitotoxicity might be involved in this BMAA-induced motor neuron degeneration. These findings suggest common pathogenic pathways between the familial and BMAA-induced ALS, which may provide further information on the disease mechanisms involved in sporadic ALS.

Disclosures: **B. Ikiz:** A. Employment/Salary (full or part-time):; Regeneron. **S.D. Croll:** A. Employment/Salary (full or part-time):; Regeneron. **A.J. Murphy:** A. Employment/Salary (full or part-time):; Regeneron. **L.E. Macdonald:** A. Employment/Salary (full or part-time):; Regeneron. **M.L. Lacroix-fralish:** A. Employment/Salary (full or part-time):; Regeneron.

Poster

796. Motor Neuron Disease Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 796.22/O11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Thierry Latran Foundation

Title: Characterization of angiogenin-induced, trna-derived stress-induced rnas

Authors: *S. SUSDALZEW, M. C. HOGG, J. H. M. PREHN;
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Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disorder affecting motoneurons. Mutations in angiogenin, encoding a member of the pancreatic RNase A superfamily, segregate with ALS. We have previously shown that angiogenin is secreted from motoneurons under stress conditions and is taken up by astrocytes where it induces RNA cleavage and mediates neuroprotection in paracrine. Under stress conditions, Angiogenin has been shown to cleave tRNAs within the anticodon loop to produce tRNA-derived, stress-induced RNAs (tiRNAs). In reconstitution assays, tiRNAs have been shown to suppress cap-mediated global protein translation, but not internal ribosome entry sequence (IRES)-mediated translation which is often used by pro-survival and anti-apoptotic genes. 5' tiRNAs have also been shown to promote the assembly of stress granules (SGs), cytoplasmic foci at which untranslated mRNPs are transiently concentrated. We identified several tRNAs produced by Angiogenin in human astrocytoma cells. To characterise the effect of tiRNAs generated by Angiogenin, corresponding synthetic tRNA fragments (tiRNAs) labeled with a fluorophore were transfected in a neuronal cell line (SH-SY5Y), and their ability to protect against stress conditions analyzed in survival assays via flow cytometry. A broad range of stress treatments was chosen: Epoxomycin (proteasomal stress), Thapsigargin (ER stress and Ca2+ depletion) and sodium arsenite (oxidative stress), a commonly used stressors which induce apoptosis and lead to the formation of SGs. We verified that the transfected tiRNAs were internalised using confocal microscopy. However we did not detect significant neuroprotection induced by 5' or 3' aspartic acid or 5' alanine tiRNA in SH-SY5Y cells. Collectively these data suggest that 5' nor 3' tiRNAs generated by Angiogenin are not cytoprotective in this setting.

Disclosures: S. Susdalzew: None. **M.C. Hogg:** None. **J.H.M. Prehn:** None.

Poster

796. Motor Neuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R01-NS074886 (DT)

F31NS080539 (MRJ)

Title: Astrocytes derived from Amyotrophic Lateral Sclerosis patients upregulate ABC drug efflux transporters at the endothelial cell layer

Authors: *M. R. JABLONSKI, S. MARKANDAIAH, P. PASINELLI, D. TROTTI; Thomas Jefferson Univ., PHILADELPHIA, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a slowly progressing neurodegenerative disease, characterized by motor neuron degeneration. ALS is characterized by non-cell-autonomous components with astrocytes contributing to toxicity and selective loss of motor neurons. The blood-brain barrier (BBB) is formed by endothelial cells in association with pericytes and astrocytes, forming the neurovascular unit. Astrocytic end-feet encapsulate approximately 90% of endothelial cells and maintain homeostasis of the barrier. ABC drug efflux transporters, highly localized in endothelial cells, prevent a wide range of neurotoxicants and therapeutics from entering the CNS. We recently reported a disease-driven increase in expression and function of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in spinal cord capillaries of the SOD1-G93A mouse model of ALS and expression increases in human spinal cord tissue (Jablonski et al. 2012; Neurobiol Dis), suggesting these transporters could cause pharmacoresistance in ALS. The ability to produce neuronal cell types from induced pluripotent stem cells (iPSCs) allows the differentiation of astrocytes and endothelial cells from both ALS patients and controls. We hypothesized that astrocytes derived from iPSCs impart alterations in ABC drug efflux transporters in iPSC-derived endothelial cells. To examine this hypothesis, we implemented a co-culture system whereby iPSC-derived endothelial cells were plated on a transwell above a layer of iPSC-derived astrocytes from ALS patients or controls. P-gp protein expression is increased in endothelial cells, which are co-cultured with astrocytes differentiated from ALS patient compared to iPSC-derived control astrocytes. Furthermore, NFkB inhibition (SN50) in endothelial cells prevented the endothelial P-gp expression increases seen from iPSC-derived ALS astrocytes. These results are an important step towards understanding drug efflux transporter regulation in ALS.

Disclosures: **M.R. Jablonski:** None. **S. Markandaiah:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

796. Motor Neuron Disease Mechanisms

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Program#/Poster#: 796.24/P1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R01-NS044292 (DT)

Title: Aberrant release of netrin-1 by astrocytes is toxic to motor neuron in ALS

Authors: ***L. T. ROSENBLUM**[1], A. BOGUSH[2], X. WEN[1], P. PASINELLI[1], D. TROTTI[1]; [1]Thomas Jefferson Univ., Philadelphia, PA; [2]Temple Univ., Philadelphia, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and fatal neurodegenerative disease primarily affecting the corticospinal tract. In ALS, astrocytes cause toxicity to upper and lower motor neurons in a process known as non-cell autonomous toxicity. It has previously been shown that accumulation of a sumoylated C-terminal fragment (CTE-SUMO1) derived from caspase-3 cleavage of EAAT2 occurs in spinal cord astrocytes of the SOD1-G93A mouse model of ALS as disease progresses. Furthermore, mimicking the *in vivo* astrocytic accumulation, expression of an artificialCTE-SUMO1 construct by astrocytes causes secretion of one or more factors that are toxic to motor neurons in *in vitro* experiments. Astrocytes expressing CTE-SUMO1 substantially upregulate Ntn-1 and the corresponding secreted axonal guidance protein, Netrin-1. In addition to its extensive developmental functions, Netrin-1 functions in local axonal homeostasis, cell-cell contact, and myelin organization and has recently been implicated in neurodegenerative diseases. We present evidence that astrocytes expressing either the ALS-causative mutation SOD1-G93A or the CTE-SUMO1 construct are toxic to motor neurons via netrin-1 secretion and indications that astrocytic netrin-1 secretion is a pathogenic mechanism in ALS. Netrin-1 is expressed by astrocytes only during the symptomatic stage in mice, increasing with disease course, and in post-mortem samples of affected areas of the human CNS. Furthermore, Netrin-1 is directly toxic to primary motor neurons, as measured by decreased neurite length and by increased death, but is not toxic to non-ALS susceptible cortical neurons. We also show that Netrin-1 mediates the toxicity of astrocytes expressing theCTE-SUMO1 construct or the ALS-causative SOD1-G93A mutation, both in a co-culture cell

model and from astrocyte-conditioned media, as shown with the addition of neutralizing antibodies.

Disclosures: **L.T. Rosenblum:** None. **A. Bogush:** None. **X. Wen:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

796. Motor Neuron Disease Mechanisms

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Program#/Poster#: 796.25/P2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Connecticut Stem Cell Research Grant (11SCB24)

Spastic Paraplegia Foundation

Title: Modeling disease-specific axonal defects of hereditary spastic paraplegia with human pluripotent stem cells

Authors: ***K. R. DENTON**[1], L. LEI[1], J. GRENIER[1], V. RODIONOV[2], C. BLACKSTONE[4], X.-J. LI[1,3];
[1]Neurosci., [2]Ctr. for Cell Analysis and Modeling and Dept. of Cell Biol., [3]The Stem Cell Inst., Uconn Hlth. Ctr., Farmington, CT; [4]Cell Biol. Section, Neurogenetics Br., Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Hereditary spastic paraplegias (HSPs) comprise a large and diverse group of genetic neurodegenerative disorders (SPG1-71). The hallmark of all HSP subtypes is the length-dependent distal axonopathy, mainly affecting long projection neurons, especially cortical spinal motor neurons (CSMNs). The most common form of HSP is SPG4, which is caused by dominant mutations to the SPAST gene that encodes the microtubule-severing ATPase spastin. Here, we generated a model of SPG4 using human induced pluripotent stem cells (iPSCs) and examined differentiated forebrain glutamatergic neurons. The SPG4 neurons displayed a significant increase in axonal swellings, which stained strongly for mitochondria and tau, indicating the accumulation of axonal transport cargoes. Analysis of fast axonal transport revealed a significant reduction in mitochondrial transport indicating that these patient iPSC-derived neurons recapitulate disease-specific axonal phenotypes. Interestingly, spastin protein levels were

significantly decreased in SPG4 neurons, supporting a haploinsufficiency mechanism. Furthermore, cortical neurons derived from spastin-knockdown human embryonic stem cells (hESCs) exhibited similar axonal swellings, confirming that the axonal defects can be caused by loss of spastin function. Vinblastine, a microtubule-destabilizing drug, rescued this axonal swelling phenotype in neurons derived from both SPG4 iPSCs and spastin-knockdown hESCs. Thus, this study demonstrates the successful establishment of human pluripotent stem cell-based models of SPG4, which will be valuable for dissecting the pathogenic cellular mechanisms and screening compounds to rescue the axonal degeneration in HSP.

Disclosures: K.R. Denton: None. **L. Lei:** None. **J. Grenier:** None. **V. Rodionov:** None. **C. Blackstone:** None. **X. Li:** None.

Poster

796. Motor Neuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS-RO1 NS051488

Farber Family Foundation

Title: Amyotrophic lateral sclerosis linked mutations in fused in sarcoma (fus) cause non-cell autonomous toxicity to motor neurons

Authors: *A. T. KIA, K. MCAVOY, K. KRISHNAMURTHY, S. SHAMAMANDRI MARKANDAIAH, D. TROTTI, P. PASINELLI;
Jefferson Hosp. of Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: It has become well established that astrocytes contribute to motor neuron degeneration and disease progression in Amyotrophic Lateral Sclerosis (ALS), an incurable neurodegenerative disease. Mutations in fused in sarcoma/translocated in liposarcoma (FUS/TLS, FUS) account for approximately 4% of familial (fALS) cases and FUS-positive pathological inclusions have been identified in sporadic ALS (sALS) cases as well. It is important then to elucidate what role is played by mutant FUS in driving astrocytic toxicity to motor neurons. Using primary astrocytes infected with N-terminally tagged GFP-FUS (WT or R521G), we show that R521G expressing astrocytes, but not controls, are in fact toxic to wild-

type motor neurons. Further, we show that conditioned medium from these astrocytes is sufficient to induce a neurotoxic effect on motor neurons. We identified one of the toxic molecules released in the medium of FUS-containing astrocytes and show that neutralizing that molecule affords protection to motor neurons. We also show that mutant FUS infected astrocytes display classical cytoplasmic inclusions seen in patients and, while they undergo changes in cellular phenotype, they do not die. Thus, like for SOD1-ALS but not for TDP-43-ALS (Serio et al., PNAS 2012), our data highlight a non-cell autonomous glial effect in disease and provide a viable *in vitro* system to further understand how mutations in different genes differentially affect glia-neurons interactions. This work was supported by NINDS-RO1 NS051488 and the Farber Family Foundation.

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Poster

796. Motor Neuron Disease Mechanisms

Location: Halls A-C

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Program#/Poster#: 796.27/P4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The involvement of glial cells in FUS-related ALS/FTLD pathophysiology -FUS knock-down glial cells have a neuro-protective effect.-

Authors: *Y. FUJIOKA[1], S. ISHIGAKI[1], T. SHIROMIZU[2], T. UDAGAWA[1], D. HONDA[1], S. YOKOI[1], K. IKENAKA[1], M. KATSUNO[1], T. TOMONAGA[2], G. SOBUE[1];
[1]Nagoya Univ., Nagoya, Japan; [2]Natl. Inst. of Biomed. Innovation, Osaka, Japan

Abstract: Amyotrophic lateral sclerosis (ALS) is one of the most common neurodegenerative disorders, characterized by selective motor neuron degeneration in the primary motor cortex, brainstem and spinal cord. FUS is the causative gene of familial ALS and pathologically linked to sporadic ALS and frontotemporal lobar degeneration (FTLD). FUS is an RNA binding protein which was reported to function in transcription, RNA splicing, and RNA transport in various cells including neurons and glial cells. Recently, non-cell autonomous toxicity has been thought to be involved in the pathophysiology of SOD1-related ALS. However, it has not been clear whether glial cells have effects on neurons in FUS-related ALS/FTLD pathology. To elucidate the effects of FUS-silenced glial cells to neurons, the neurite outlength of primary cortical neurons (PCN) co-cultured with glial cells with/without FUS was measured by the autoimage

scanner. Surprisingly, the neurite outlength of PCN were elongated after the co-culture with FUS knock-down glial cells, indicating that FUS knock-down glial cells have a protective effect. Next, we identified some proteins specifically elevated in the culture supernatant of FUS-silenced glial cells using LC-MS/MS analysis as candidates for protective factors. Thus, glial cells which lost the function of FUS countervail the progression of the pathology of FUS related ALS/FTLD through the expression of protective factors.

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Poster

796. Motor Neuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: European Research Council

Title: Impact of *in vitro* and *in vivo* modulation of ALS C9ORF72 gene

Authors: ***P. J. MULCAHY**, A. HIGGINBOTTOM, G. HAUTBERGUE, P. J. SHAW, M. AZZOUZ;
Neurosci., Sheffield Inst. For Translational Neurosci., Sheffield, United Kingdom

Abstract: Theme: **C.04. Neurodegenerative Disorders and Movement Disorders** C.04.g. Motor neuron disease: *in vitro* studies **Rationale & Objectives:** Since the recent discovery of the relationship between the C9ORF72 gene and Amyotrophic Lateral Sclerosis (ALS), much research efforts have focused on understanding the role of this particular protein and modelling the disease. Repeat GGGGCC expansions located in the intronic region have been linked to the disease in familial ALS cases. Here, we attempt to elucidate the role of C9ORF72 in terms of both its role in normal function and also in ALS. The function of the C9ORF72 was assessed by gene silencing both *in vitro* and *in vivo*. Repeat expansion induced cellular disruptions were also investigated to determine the mechanistic pathways involved with the aim of potential gene therapy development. **Methodology:** To determine the role of C9ORF72, knockdown miRNA sequences were designed to target mouse gene and were cloned into lentiviral and adeno-associated viral vectors. Transduction of cell lines and primary neurons were carried out and

C9ORF72 silencing was assessed at mRNA and protein levels. To assess the effect of GGGGCC repeat expansions, lentiviral and adeno-associated viral vectors with repeat sequences from 10 to 102 repeats were generated. Transduction assays were carried out using cell lines and primary neuronal cells (cortical neurons and spinal motor neurons). Cell viability and apoptosis were assessed and C9ORF72 associations with RNA binding proteins analysed. *In situ* evaluation of RNA foci development, as noted in the human condition, following viral expansion transduction was carried out using both sense and antisense probes. To examine the effects associated with C9ORF72 knockdown or repeat expansion *in vivo*, neonatal mice were infused with the viral vectors and behavioural testing was performed. **Findings:** Results have shown that viruses generated to silence C9ORF72 showed significant knockdown following cell transduction compared to scrambled control *in vitro*. RNA foci development following transduction with lentiviral-mediated repeat expansions has been observed in neurons. Behavioural and histological data from animals injected with AAV9 expressing GGGGCC are being analysed. Our current studies will allow many avenues to open up in terms of C9ORF72 research into the causes and potential treatments for ALS.

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Poster

796. Motor Neuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (C) 15590908

Keio Gijuku Academic Development funds

Title: SUMO3 accelerates aggregation of ALS-linked SOD1 mutants

Authors: *T. NIIKURA[1], Y. KITA[2], Y. ABE[3];
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Abstract: Mutations in superoxide dismutase 1 (SOD1) are the major cause of familial amyotrophic lateral sclerosis (FALS) and have been widely used for *in vitro* and *in vivo* models to investigate the pathomechanisms of ALS. FALS-linked mutant SOD1 proteins are misfolded and aggregated into intracellular inclusions both *in vitro* and *in vivo*. It is generally accepted that the propensity for aggregation is associated with the pathobiology of SOD1 mutants. Sumoylation, a post-translational protein modification, involves covalent attachment of small ubiquitin-like modifier (SUMO) proteins to target proteins. The astrocytic expression of sumoylated EAAT2 fragment induces cytotoxicity in motoneuronal NSC34 cells and primary motor neurons, suggesting the involvement of sumoylation in ALS pathogenesis. Here, we investigated the effect of sumoylation on ALS-linked mutant SOD1 proteins in motoneuronal NSC34 cells. We found that both SUMO1 and SUMO2/3 modify ALS-linked SOD1 mutant proteins at lysine75 in these cells. The modification of ALS-linked SOD1 mutant proteins by SUMO3, rather than by SUMO1, significantly increased the stability of the proteins and accelerated intracellular aggregate formation. These findings suggest the contribution of sumoylation, particularly by SUMO3, to the protein aggregation process underlying the pathogenesis of ALS.

Disclosures: **T. Niikura:** None. **Y. Kita:** None. **Y. Abe:** None.

Poster

796. Motor Neuron Disease Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 796.30/P7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Target ALS

Title: Mechanistic study of oligodendrocyte degeneration in ALS *in vitro*

Authors: ***Y. LI**[1], B. DWIGHT[2], J. D. ROTHSTEIN[2];
[1]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]the Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult onset degenerative disease characterized by the death of motor neurons. Both macroglia and microglia play a role in disease development. However, oligodendrocyte (OL) lineage, in contrast to other glial types, has been found to significantly affect disease onset in mouse models. OLs indeed die at pre-symptomatic

stage in animal models. Remarkably, OLs degeneration is common in ALS patients, including both familial and sporadic cases. All these data indicate that OL lineage cells play a critical role in ALS pathogenesis. To further investigate the mechanisms involved in OL degeneration and how it affects motor neuron survival, we generated *in vitro* models. In two-dimensional (2-D) pure mouse OPC (msOPC) culture, no differences in cell proliferation and death have been seen between SOD1-msOPCs and wild type (WT)-OPCs, while SOD1-msOPCs showed slightly enhanced differentiation to myelinating OLs after triiodothyronine (T3) treatment. As similar to 2-D culture, when msOPCs were cultured on nanofibers (3-D), no difference in cell proliferation was observed. In contrast, T3 treatment induced more myelinating SOD1-OL differentiation and death compared to WT-OLs, indicating that overexpression of mutant SOD1 affects mouse OL differentiation and survival. We further cocultured mouse SOD1-OLs with human neurons that were differentiated from human control- or ALS-specific induced pluripotent stem cells (iPSCs). Abnormal OL differentiation was seen in the co-cultures with mouse SOD1-OLs as indicated by abnormal myelin basic protein (MBP) expression. Those OLs with abnormal MBP expression had cleaved caspase 3 expression or condensed nuclei, indicating they were degenerating. Given that human OPCs (huOPCs) have reactive morphology and gray matter OL degeneration are common in ALS patients, we further set out to investigate human ALS-specific oligodendrocytes. We differentiated ALS-specific iPSCs to OPCs, myelinating OLs, and also cocultured differentiated huOPCs with human neurons. We did not see significant differences in huOPC differentiation between control- and ALS-iPSCs. When human neurons were cocultured with human oligospheres, axonal degeneration was readily seen in the cultures with ALS-oligospheres compared to that with control-oligospheres. These *in vitro* models are being used to study the mechanisms involved in OL degeneration in ALS.

Disclosures: Y. Li: None. **B. Dwight:** None. **J.D. Rothstein:** None.

Poster

797. Other Neurodegenerative Disorders II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.01/P8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR

Title: Selective Neurodegeneration in Alzheimer’s Disease and Parkinson’s Disease

Authors: *J. WANG, W. SONG;
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Abstract: A hallmark of Alzheimer's Disease (AD) is neuritic plaques, primarily consist of extracellular deposits of amyloid β (Aβ) cleaved from β-amyloid precursor protein (APP). Parkinson's Disease (PD) is characterized by intracellular Lewy bodies (LBs), primarily consist of aggregated alpha-synuclein protein (αSyn), encoded by SNCA gene. Both AD and PD are characterized by extensive neuronal loss within certain brain regions. An invariant feature of AD is cholinergic dystrophy and dopaminergic neurodegeneration in substantia nigra is characteristic in PD. Previous studies suggested that AD-associated Swedish APP mutation promoted Aβ generation and neuronal loss, whereas PD-associated SNCA A53T mutation caused αSyn more prone to aggregate. In this study, we used cholinergic SN56 cells and dopaminergic MN9D cells to stably overexpress wildtype (WT)/ mutant APP or WT/ mutant SNCA gene, followed by performing whole-genome expression profiling in these stable cell lines as well as two background cell lines. There were 213 probes identified as having an interaction between APP mutation's effect and cell line's effect. There were 1422 probes having an interaction between SNCA mutation's effect and cell line's effect. As for gene set enrichment analysis, the difference of Swedish APP gene overexpressing in SN56 and MN9D cell lines has the strongest effect on ECM-receptor gene expression, while the difference of overexpressing SNCA A53T gene in two cell line has the strongest effect on spliceosome gene expression. Our data supports the hypothesis that APP and SNCA mutations change gene expression in a cell type-dependent way, which may contributes to selective neurodegeneration in AD and PD.

Disclosures: J. Wang: None. **W. Song:** None.

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Hawaii Community Foundation Alan M. Krassner Fund 1ADVC-49233

NIH National Institute of General Medical Sciences 8 P20 GM 103466-11

Title: Selenoprotein P mediates modification of synaptic physiology and plasticity

Authors: *E. D. NGUYEN-WU;
Cell and Mol. Biol., Univ. of Hawaii At Manoa, John A. Burns Sch. of Med., Honolulu, HI

Abstract: Selenoprotein P (Sepp1) is an extracellular protein with multiple selenocysteine residues involved in interorganal selenium transport. Sepp1 is part of the unique family of selenoproteins obtained mainly through dietary selenium, an essential micronutrient. Association of Sepp1 with pathology in neurodegenerative disorders and neurological impairments in Sepp1 knockout (Sepp1 KO) animals highlight the importance of Sepp1 for brain function. However, the direct actions of Sepp1 in brain remain poorly understood. We investigated expression of Sepp1 in the cortex, substantia nigra and putamen, as well as cuboidal cells of the choroid plexus in human postmortem Alzheimer's and Parkinson's disease brain samples. Our published data demonstrated for the first time, that Sepp1 is associated with both Alzheimer's and Parkinson's brain pathology. These neurodegenerative diseases involve memory loss and motor dysfunction similarly exhibited by Sepp1 KO mice. We are currently investigating the role of Sepp1 in synaptic physiology using Sepp1 KO mice. Deletion of Sepp1 results in bidirectional changes in synaptic plasticity, learning and memory deficits, and neuromotor defects. Long-term potentiation (LTP) is a well-established cellular model for learning and memory. LTP deficits observed in Sepp1 KOs can be reversed by applying Sepp1 directly onto the hippocampus. Altogether, these studies underscore the importance of Sepp1 in maintaining normal synaptic integrity and plasticity. These novel findings strongly support our hypothesis that Sepp1 has direct actions on neuron physiology besides its role in transporting selenium.

Disclosures: E.D. Nguyen-Wu: None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Introducing MAPT mutation into human iPSC-derived neural stem cells using CRISPR/Cas systems

Authors: *Y. IMAIZUMI, H. OCHIAI, K. YAMAZAKI, K. SAGANE, M. ITO;
Eisai Co., Ltd., Ibaraki, Japan

Abstract: To understand the pathogenic mechanisms in patients with neurological disorders, induced pluripotent stem cell (iPSC)-based human neuronal disease models could be helpful application tools. For more precise analysis, recent development of the genome editing technology, such as zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN), enabled us to generate monogenic iPSCs from wild-type iPSCs and rescue disease phenotypes on patient derived-iPSCs. However, use of ZFNs or TALENs on iPSCs is still a challenge, because of low efficiency of genome editing. Here, we used a novel genome editing technology "clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems" on human iPSC-derived neural stem cells (NSCs) due to the following two reasons. (1) Recently, Ding *et al.,* demonstrated that efficiency of iPSC genome editing was enhanced using CRISPR/Cas systems compared with TALENs (Ding, *et al.*, 2013). (2) NSCs might show higher viability and transfection efficacy, and shorter neural differentiation period, compared with iPSCs. NSCs were induced from 201B7 iPSCs (iPSC from a healthy donor) by using Gibco's NSC induction medium. Double-strand DNA repair template, guide RNA (gRNA) and Azami green fused Cas9 vector were designed to generate microtubule-associated protein tau (MAPT) mutation. After 48 hrs from transfection, Cas9-activated NSCs were sorted by fluorescence-activated cell sorting (FACS) system and expanded. Then, some NSC clones with MAPT homozygous mutation were established. Finally, MAPT NSCs were able to differentiate into neurons and showed marked accumulation of phosphorylated tau. In conclusion, we succeeded to generate human iPSC-derived NSCs with MAPT mutation using CRISPR/Cas systems. Moreover, this study suggests that NSCs will be one of good host cells for genome editing to establish neurological disease models efficiently.

Disclosures: Y. Imaizumi: A. Employment/Salary (full or part-time):; Eisai Co., Ltd. **H. Ochiai:** A. Employment/Salary (full or part-time):; Eisai Co., Ltd. **K. Yamazaki:** A. Employment/Salary (full or part-time):; Eisai Co., Ltd. **K. Sagane:** A. Employment/Salary (full or part-time):; Eisai Co., Ltd. **M. Ito:** A. Employment/Salary (full or part-time):; Eisai Co., Ltd..

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R15 NS070774

Title: Targeted induction of endogenous TRPML2 (Mucolipin-2) or TRPML3 (Mucolipin-3) expression for use as potential therapy for Mucolipidosis type IV

Authors: J. A. VALADEZ, *M. P. CUAJUNGCO;
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Abstract: There is currently no therapy or cure for Mucolipidosis type IV (MLIV). A previous study in *Caenorhabditis elegans* has shown that the loss of CUP5 protein, a Mucolipin-1 (TRPML1) orthologue, could be rescued by genetic complementation using human TRPML1 or TRPML3. The high degree of sequence and functional similarities between TRPML1 and its related proteins, TRPML2 and TRPML3, make them an excellent candidate to potentially substitute for the loss of TRPML1 function in MLIV. In order to consider this possibility, we first focused on studying the mechanisms that control the expression of *TRPML2* (*Mucolipin-2*) gene. We cloned DNA regions of varying size upstream of the *TRPML2* gene transcriptional start site (TSS) into a dual luciferase reporter (DLR) assay. We identified a putative core promoter region between -320 and -287 of the TSS containing a consensus sequence for six transcription factors (TFs). Heterologous expression of the candidate TFs conferred an effect on the expression of endogenous *TRPML2* transcripts compared to controls. To determine post-transcriptional regulators of endogenous *TRPML2* transcripts, we screened and identified a micro-RNA that modulates the expression of endogenous *TRPML2* transcripts using real-time quantitative PCR (qPCR) technique. Meanwhile, we use the DLR assay to screen a drug library to identify compounds that up-regulate the expression of *TRPML2* and *TRPML3* genes. Using real-time qPCR, we found several candidate drugs that significantly increased the transcript levels of endogenous *TRPML2* and *TRPML3* genes. These results show that we could induce the expression of the *TRPML2* and *TRPML3* genes through exogenous manipulation of transcriptional activators, or post-transcriptional regulators. Understanding the mechanism of TRPML2 or TRPML3 protein expression brings us a step closer to our goal of manipulating the expression of either protein as a potential therapeutic substitute for the loss of TRPML1 function in MLIV disease.

Disclosures: J.A. Valadez: None. **M.P. Cuajungco:** None.

Poster

797. Other Neurodegenerative Disorders II

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ML4 Foundation Grant

Title: Evidence for lysosomal membrane permeability and protein aggregate formation in Mucolipidosis type IV

Authors: *L. C. BOUDEWYN, M. C. MICSENYI, S. U. WALKLEY;
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Abstract: Mucolipidosis type IV (MLIV) is a rare autosomal recessive lysosomal disease that presents early in life with progressive neurological symptoms, including intellectual disability, delayed developmental and motor milestones, ophthalmologic abnormalities, and premature death. There is currently no cure or corrective therapy. MLIV stems from mutations in the MCOLN1 gene, which encodes the late endosomal/lysosomal transient receptor potential channel, mucolipin-1. Its dysfunction leads to a host of metabolic abnormalities, most notably the accumulation of glycosphingolipids and acid mucopolysaccharides in neurons and other cells throughout the central nervous system (CNS) and periphery. In addition to the accumulation of storage material in Mcoln1-/- mice, our lab has previously reported the presence of large inclusion bodies comprised in part of the autophagic adaptor protein p62. Confocal microscopy has revealed that these inclusions are in fact lysosomal storage bodies where p62 is localized in a shell-like pattern around the periphery of lysosomal membranes. Our lab recently identified that p62 responds to a novel pathophysiological event known as lysosomal membrane permeability (LMP) in the lysosomal disease, late-infantile neuronal ceroid lipofuscinosis. We found that LMP, a known mechanism of cell death, stimulates a coordinated response by macroautophagy adaptor proteins including p62 to sequester damaged lysosomes and their content as cytosolic protein aggregates. Our recent findings of p62-positive aggregates in MLIV disease and the distinct localization of p62 to the periphery of lysosomal storage bodies in both neuronal and glial cell types suggests LMP and a similar response may be occurring. Notably, recent reports have shown that LMP induction stimulates an autophagy mechanism known as lysophagy. Lysophagy involves the recruitment of ubiquitin, p62 and LC3-II to lysosomes undergoing LMP to engulf these damaged organelles within nucleating autophagosomes to be redelivered to intact lysosomes. This mechanism prevents the release of lysosomal cathepsins and lysosomal-mediated cell death. Evidence in MLIV and other lysosomal diseases for p62 sequestration of lysosomes and lysosomal storage material suggests that LMP is occurring in these disorders. Importantly, the persistent presence of p62-aggregates in these diseases may indicate a failure in lysophagy to adequately respond to LMP. Here we are using Mcoln1-/- mice to clarify the pathophysiological events that occur in MLIV disease progression. Addressing this issue is central to understanding the cellular abnormalities leading to MLIV's clinical manifestations.

Disclosures: **L.C. Boudewyn:** None. **M.C. Micsenyi:** None. **S.U. Walkley:** None.

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant

FDA Grant 1R01FD004127-01

Title: Deoxysphingolipids: A new biomarker in peripheral neuropathy?

Authors: ***E. A. JOHNSON**[1], N. WEI[1], T. HORNEMANN[2], F. EICHLER*[1];
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Abstract: Deoxysphingolipids (deoxySLs) are neurotoxic lipids that have been shown to accumulate as a byproduct of aberrant sphingolipid synthesis (Penno et al 2010). It was originally recognized that they were toxic in various cancer cell lines (Cuadros et al 2000) but their role in normal physiology and disease remained poorly understood. Interestingly, *in vitro* studies also showed that dSL are toxic to cultured sensory neurons. Our recent studies examined the accumulation of dSL *in vivo* in patients with Hereditary Sensory Autonomic Neuropathy Type I (HSAN1) as well as in diabetic patients (T1DM and T2DM, with and without neuropathy). Studies were conducted on both retrospectively and prospectively collected samples. For sphingoid base analysis the samples were shipped on dry ice overnight to the University of Zurich. The sphingoid base profile was analyzed by LC-MS after hydrolyzing the N-acyl and O-linked headgroups as described earlier (Zitomer et al 2009). Marked accumulation of two atypical deoxy-sphingoid bases (1-deoxy-sphinganine and 1-deoxymethyl-sphinganine) were found in HSAN1 patients (N=18) consistent with our prior reports (Garofalo et al., 2010). Next we compared the long chain base profile in plasma from individuals with T1DM (N=27), T2DM (N=30) and healthy controls (N=23). 1-deoxySLs were significantly higher in the groups with T2DM but not different between T1DM and controls. In contrast to patients with T2DM, 1-deoxSL levels are not elevated in T1DM. Our studies provide further evidence that aberrant dSL are a biomarker for particular metabolic disorders that cause neuropathy. It appears that in diabetes the 1-deoxySL formation is not per-se caused by hyperglycemia but rather specifically

associated with metabolic changes in T2DM. Ongoing studies are assessing whether the degree of neurologic impairment is correlated with dSL levels in both HSAN1 patients and T2DM diabetic patients.

Disclosures: E.A. Johnson: None. **N. Wei:** None. **T. Hornemann:** None. **F. Eichler*:** None.

Poster

797. Other Neurodegenerative Disorders II

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Program#/Poster#: 797.07/Q2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Dynamic palmitoylation of H+-transporting V-ATPase subunit a1 regulates lysosomal acidification and is disrupted in Ppt1-/- mice

Authors: M. B. BAGH[1], G. CHANDRA[3], Z. ZHANG[1], S. PENG[2], *A. B. MUKHERJEE[4]; [1]NICHD (National Inst. of Child Hlth. and Human Development), [2]NINDS (National Inst. of Neurolog. Disorders and Stroke), NIH, Bethesda, WA; [3]Dept. of Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL; [4]NICHD (National Inst. of Child Hlth. and Human Development), Natl. Inst. of Hlth., Bethesda, MD

Abstract: Neuronal ceroid lipofuscinoses (NCLs) are the most common childhood neurodegenerative lysosomal storage disorders (nLSDs). The infantile NCL (INCL) is caused by mutations in the CLN1 gene encoding palmitoyl-protein thioesterase-1(PPT1). Dynamic palmitoylation (palmitoylation-depalmitoylation) is critical for regulating the functions of many proteins, especially in the brain, and involves palmitoylation by palmitoyl-acyltransferases (PATs) as well as depalmitoylation by palmitoyl-protein thioesterases (PPTs). PPT1-deficiency impairs degradation of palmitoylated proteins (constituents of ceroid) by lysosomal hydrolases causing lysosomal ceroid accumulation leading to INCL. However, lysosomal hydrolases require acidic pH for enzymatic activity and in virtually all LSDs the lysosomal pH is elevated and the molecular mechanism(s) of this defect remains unexplained. The vacuolar H+-transporting ATPase (V-ATPase) is a multi-subunit protein-complex that regulates endosomal/lysosomal pH. It is suggested that the reversible assembly of its membrane-bound V0 sector and a cytosolic V1 sector regulate the acidification of intracellular compartments including the lysosomes. Accordingly, we tested a hypothesis that one or more subunits of V-ATPase require dynamic

palmitoylation for lysosomal targeting and are disrupted in INCL. Using Ppt1-/- mice, a reliable animal model of INCL, we found that the a1 subunit of V0-ATPase (V0a1) requires palmitoylation on Cys-25 for localization to endosomal/lysosomal membranes. Unexpectedly, we found that in brain tissues of Ppt1-/- mice V0a1 is mis-targeted to the plasma membrane instead of lysosomal membrane.Moreover, V0a1 is transported via clathrin/AP2-dependent pathway from plasma membrane to early endosomes where lack of PPT1 suppressed depalmitoylation impairing dissociation of V0a1 from clathrin/AP2/V0a1 complex. Failure of V0a1 dissociation prevented its repalmitoylation essential for binding AP3, required for its transport to lysosomes. Consequently, clathrin/AP2/V0a1 complex was transported to the plasma membrane via the recycling endosomes. This defective trafficking of V0a1 disrupted V0V1 assembly on lysosomal membrane, essential for pH regulation. Importantly, a PPT1-mimetic small molecule, NtBuHA, which we recently identified, ameliorated these defects. Our findings for the first time demonstrate that PPT1-deficiency impairs dynamic palmitoylation (palmitoylation-depalmitoylation) of V0a1 which elevates lysosomal pH in INCL and suggest that varying mechanism(s) impairing V0ATPase may underlie elevated lysosomal pH in other LSDs.

Disclosures: **M.B. Bagh:** None. **G. Chandra:** None. **Z. Zhang:** None. **S. Peng:** None. **A.B. Mukherjee:** None.

Poster

797. Other Neurodegenerative Disorders II

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Title: Intrathecal or intravenous recombinant human alpha-iduronidase improve brain myelination in canine mucopolysaccharidosis I

Authors: *P. DICKSON[1], J. M. PROVENZALE[2], S. CHEN[2], I. NESTRASIL[3], J. YEE[4], S.-H. KAN[4], S. Q. LE[4], J. JENS[5], E. SNELLA[5], M. A. GUZMAN[6], C. VITE[7], E. G. SHAPIRO[3], N. ELLINWOOD[5];
[1]Harbor-Ucla, Torrance, CA; [2]Radiology, Duke Univ., Durham, NC; [3]Pediatrics, Univ. of Minnesota, Minneapolis, MN; [4]Pediatrics, Harbor-UCLA/LA BioMed, Torrance, CA; [5]Animal Sci., Iowa State Univ., Ames, IA; [6]Pathology, St. Louis Univ., Saint Louis, MO; [7]Sch. of Vet. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Objectives: Children with mucopolysaccharidosis I (MPS I) show reduced fractional anisotropy (FA, a measure of white matter integrity) on diffusion tensor imaging (DTI) of the corpus callosum (CC) that correlates with diminished attention on neurocognitive tests (Shapiro et al., 2012). Our neuroimaging and neuropathological studies in MPS I dogs suggest that reduced volume and FA in the CC are caused by abnormal myelination (Provenzale et al., in preparation). We therefore studied the impact of enzyme replacement therapy with recombinant human alpha-L-iduronidase (rhIDU) on abnormal myelination in the canine CC. Methods: MPS I dogs received intrathecal (IT) rhIDU 0.05 mg/kg every 3 months and/or intravenous (IV) rhIDU 0.58-2.0 mg/kg weekly. Untreated MPS I and unaffected carriers were used as controls. Dogs underwent brain magnetic resonance imaging and DTI. CC was evaluated using sandwich ELISA for myelin basic protein (MBP), RT-PCR for myelin-related gene expression, lipidomics, and electron microscopy. Results: Mean whole CC volumes were lower in MPS I dogs vs. carriers ($p=0.02$). CC volume was preserved with IT rhIDU ($p=0.006$ vs. untreated MPS I). FA was reduced in MPS I dogs vs. carriers ($p=0.004$). MBP in CC of treated MPS I, untreated MPS I, and carrier dogs correlated with FA ($r2=0.56$, $p=0.01$) and inversely correlated with radial diffusivity ($r2=0.51$, $p=0.02$), demonstrating a relationship between CC myelination and DTI. We found decreased myelination in MPS I dogs ≥6 weeks. Myelin composition was abnormal in MPS I dogs. Neuroimaging findings, MBP and myelin lipid composition in the CC was improved with administration of IT rhIDU, even if started at 4 months. Expression of myelin-related genes in CC improved in MPS I dogs treated with IT and/or IV rhIDU beginning at ≤30 days of age. Conclusions: Reduced CC volume and FA in MPS I dogs may be caused by abnormal myelination. IT and/or IV rhIDU improves abnormal myelination in the CC, especially if treatment begins early.

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Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 5R01NS038850

Title: The mechanism of HspB1 and its mutants on the regulation of RhoA activity

Authors: *X. SUN[1,2], D. J. FINK[1,2], M. MATA[1,2];
[1]Neurol., Univ. of Michigan, Ann Arbor, MI; [2]VA Ann Arbor Healthcare Syst., Ann Arbor, MI

Abstract: Heat shock protein B1 (HspB1) is an ATP-independent molecular chaperone belonging to a family of small heat shock proteins with a conserved α-crystalline domain in the C-terminalregion. A number of mutations in HspB1 have been identified in families with Charcot Marie Tooth type IIF (CMT-IIF) disease or with distal hereditary motor neuropathy II (dHMN II). CMT is a clinical heterogeneous group of diseases characterized by progressive nerve degeneration causing distal muscle weakness and atrophy and sensory loss; in dHMNII phenotype the degeneration affects motor nerves but sensory abnormalities are absent. Mutations outside the crystalline domain (P39L, G84L, P128S, P182L) appear to associate with dHMN, while mutations within the crystalline domain may associate with either phenotype We previously observed that wild type HspB1 reduces RhoA activation by translational inhibition of PDZ-RhoGEF, a RhoA specific GEF. Decreased expression of PDZ-RhoGEF by HspB1 was accomplished by enhancing expression of specific microRNAs (miR20a and miR128) that inhibit the translation of PDZ-RhoGEF and resulted in an increase neurite growth response in cortical neurons. To determine whether this signaling pathway may be altered in HspB1

mutations causing disease, we studied three different mutations (S135F, R136W and R127W) that are closely localized in the α-crystalline domain of HspB1 and that are associated with an AD CMT-IIF phenotype. Mutants R136W and R127W behave as wt HspB1 in the regulation of RhoA activity by translation inhibition of PDZ-RhoGEF, but mutant S135F did not increase expression of miR20a and miR128 to modify PDZ-RhoGEF levels nor decrease RhoA activity in Neuro-2a cell line and cortical neurons Our results suggest that either these HspB1 mutations alter independent cellular mechanisms or that their effects may converge downstream of RhoA GTPase signaling to cause similar disease phenotype.

Disclosures: X. Sun: None. **D.J. Fink:** None. **M. Mata:** None.

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Investigating SPTLC1 mutations on protein profiles in HSN-I patient lymphoblasts

Authors: *S. STIMPSON, J. COORSSEN, S. MYERS;
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Abstract: Axonal degeneration is the final common path in many neurological disorders. Subsets of neuropathies involving the sensory neuron are known as hereditary sensory neuropathies (HSN). Hereditary sensory neuropathy type I (HSN-I) is the most common subtype with autosomal dominant inheritance. It is characterized by the progressive degeneration of the DRG and an onset of clinical symptoms between the second or third decade of life. Heterozygous mutations in the serine palmitoyltransferase (SPT) long chain subunit 1 (SPTLC1) were identified as the pathogenic cause of HSN-I. Previous studies have shown that in HSN-I patient lymphoblasts, mitochondria play a role in HSN-I. Transmission electron micrographs studies have shown that the mitochondria in the HSN-I mutant cells are morphologically challenged, cluster to the perinucleus and are wrapped by the endoplasmic reticulum (ER). This investigation has shown that mutant SPTLC1 alters the expression of and potentially interacts with a set of proteins that associate with the mitochondria and ER. Using mitochondrial and ER protein isolates from control and patient lymphoblasts, via 1 and 2-dimensional gel electrophoresis, we have confirmed up regulation of a number of proteins by mass spectrometry caused by the SPTLC1 mutations. In conclusion, previous studies have shown alterations to the

mitochondrial and ER protein profiles in patient-derived lymphoblasts, with this investigation confirming changes and has identified novel proteins which may help further the understanding of HSN-I and establish a basis for treatment. The regulation of these proteins may prove a new route to understanding the cellular mechanisms underlying HSN-I.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR Grant MOP-57851

Title: Axonal transport disruption and cytoskeletal restructuring in retinal ganglion cells following elevated intraocular pressure

Authors: *A. NUSCHKE[1], X. WANG[4], C. SMITH[2], V. DANTHUREBANDARA[3], N. O'LEARY[5], B. CHAUHAN[2];
[2]Physiol. and Biophysics, [3]Ophthalmology and Visual Sci., [1]Dalhousie Univ., Halifax, NS, Canada; [4]Natl. Eye Institute, Natl. Inst. of Hlth., Bethesda, MD; [5]Ctr. for Biostatistics, Inst. of Population Hlth., Univ. of Manchester, Manchester, United Kingdom

Abstract: **Introduction:** Disruption of axonal transport (AT) and changes in structural proteins within the retinal ganglion cell (RGC) axon are associated with RGC response to injury, both in human optic neuropathies and experimental models of RGC loss. A time-course of such changes following a transient injury is not well characterized. Here we examine AT and the expression of cytoskeletal proteins within the RGC axon following transient retinal ischemia induced by elevated intraocular pressure (IOP). Responses to an IOP elevation for durations known to cause (90 mins) and not cause (30 mins) progressive RGC loss are compared. **Methods:** The anterior chamber of right eyes of Brown Norway rats was cannulated with a 30 G needle attached to a saline reservoir, which was raised to induce an IOP of 120 mmHg. The left eye was used as control. Anterograde AT was monitored via injection of the tracer cholera-toxin B-subunit (CTB) Alexa488 conjugate into the vitreous of the eye immediately prior to IOP increase. Elevated IOP was maintained for 30 or 90 mins. Rats were sacrificed at 3, 6 and 24 hours following IOP increase for AT analysis, and 1 week for quantification of RGC loss. Average

fluorescence within optic nerves (ONs) was measured from confocal images of longitudinal ON sections along their entire length. These data were analysed using a linear mixed-effects model. RGC loss was quantified in retinal wholemounts using the RGC marker Brn3a. Immunohistochemistry for phosphorylated and non-phosphorylated neurofilament heavy, as well as markers of glial activation (GFAP and Iba-1) was performed in longitudinal sections of the optic nerve head (ONH). **Results:** A 90-min increase in IOP caused 98±0.9% (N=2, P=0.004) RGC loss 1-week post injury, as well as retrograde accumulation of CTB in the ONH. In ON sections there was a mean 56% (N=12, P<0.001) decrease in AT compared to control. The 30-min injury caused no detectable RGC loss (1.2±2.5%, N=2, P=0.002) and no evidence of AT blockade at the ONH. However, analysis of ONs after the 30-min injury demonstrated a mean 8% (N=12, P<0.001) decrease in AT. There was no effect of recovery time following either injury. Analysis of neurofilament expression suggested an increase in phosphorylated neurofilament at 6 hours following 90 mins of IOP elevation, while no change is observed following 30 mins. **Conclusions:** Despite no detectable RGC loss following 30 mins of elevated IOP, there was a significant blockade in AT evident in the ON. Evidence of cytoskeletal restructuring was only present in the case of severe injury causing RGC loss. It is possible that mild disruptions of AT are less determining than cytoskeletal modifications in RGC survival.

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Poster

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The Bluefield Project to Cure FTD

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UCSF Resource Allocation Program

UCSF Alzheimer's Disease Research Center

Alzheimer's Drug Discovery Foundation

Title: Progranulin haploinsufficiency causes pre-clinical retinal thinning and NCL-like pathology in humans

Authors: *M. E. WARD[1], R. CHEN[2], A. TAUBES[2], S. MINAMI[1], Y. LI[1], B. MILLER[2], B. SEELEY[2], H.-Y. HUANG[2], H. BOUDIN[2], E. HUANG[2], S. COTMAN[3], J. STAROPOLI[3], A. GREEN[2], L. GAN[1];
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Abstract: Reduced expression of progranulin (PGRN) via heterozygous mutations in the *GRN* gene causes frontotemporal dementia (FTD), a neurodegenerative disorder typified by behavioral and/or language abnormalities. Complete loss of PGRN expression due to homozygous *GRN* mutations was recently discovered in two siblings. Unexpectedly, these siblings developed symptoms and pathologic features consistent with Neuronal Ceroid Lipofuscinosis (NCL), a group of related pediatric neurodegenerative disorders characterized by vision loss, retinal atrophy, seizures, and the presence of autofluorescent intracellular storage material. Here, we provide the first evidence of NCL-like clinical and pathological features in humans with heterozygous *GRN* mutations. Using optical coherence tomography, we measured retinal thickness in 12 living *GRN* mutation carriers and 24 age- and sex-matched controls. We discovered substantial retinal thinning in *GRN* mutation carriers, including those who were cognitively asymptomatic. Autofluorescent aggregates were also present in the retinas of several *GRN* mutation carriers. Electron microscopy (EM) studies of post-mortem brain tissue from *GRN* mutation carriers revealed a significant increase in electron-dense intraneuronal storage material. Aggregates of mitochondrial subunit C, characteristic findings in NCL, were present in post-mortem brain tissue from *GRN* mutation carriers. EM analysis of human lymphoblasts isolated from five separate *GRN* mutation carriers showed significant increases in NCL-like storage material compared to non-mutation sibling controls. This storage material phenotype could be rescued by overexpression of PGRN. Our findings indicate that heterozygous *GRN* mutations cause early retinal thinning, a clinical feature of NCL, and NCL-like pathology in humans. Taken together, they suggest that *GRN*-associated FTD and the NCLs may share similar mechanisms.

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Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Identification of transcription factors necessary for re-establishing retinotectal connections after optic nerve transection in zebrafish

Authors: *I. VENKATESH, R. TEAL, A. UDVADIA;
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Abstract: Damage to the central nervous system (CNS) circuitry of adult mammals results in permanent disability. In contrast, the ability to regenerate damaged CNS nerves and achieve functional recovery occurs naturally in fish. The ability of fish to successfully regrow damaged CNS nerves is in part a consequence of their ability to re-express key neuronal growth-associated genes in response to CNS injury. However, our knowledge of the molecular mechanisms regulating growth-associated gene expression during successful CNS regeneration in fish remains limited. We have focused our initial efforts on a well-charaterized growth associated gene encoding Growth-Associated Protein-43 (GAP-43), a protein highly enriched in axonal growth cones during CNS development and regeneration. Our studies demonstrate that knockdown of *gap43* gene expression during optic nerve injury in fish obstructs regenerative axon outgrowth *in vivo.* This prompted us to investigate regulatory pathways responsible for *gap43* gene activation during CNS regeneration in fish. Using *in vivo* reporter assays, we identified select transcription factors involved in activation of *gap43* transcription during optic nerve regeneration in fish. We have also utilized *in vivo* regeneration assays to determine the impact of these same transcription factors on the ability of damaged retinal axons to re-establish tectal connections after optic nerve transection. We are currently performing chromatin immunoprecipitation (ChIP) assays in regenerating zebrafish retina, to determine if the identified candidate transcription factors directly regulate *gap43* expression by binding to the previously identified regeneration-specific promoter regions during optic nerve regeneration. Using *gap43* as a model we have identified transcription factors necessary for re-activation of growth-associated gene expression and axon regrowth during CNS regeneration in fish, thereby revealing potential targets for therapeutic strategies designed to improve regenerative capability in mammals

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Poster

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Support: NIH R01 EY022358

Title: Cytoskeletal alterations and abnormal protein accumulation in the primary visual projection of the DBA/2J mouse model of glaucoma

Authors: *S. D. CRISH, G. N. WILSON, M. A. SMITH, C. M. DENGLER-CRISH; Pharmaceut. Sci., NEOMED, Rootstown, OH

Abstract: In neurodegenerative disorders, cytoskeletal defects and abnormal protein accumulation in the brain are early pathologies that occur prior to neuronal loss. For example, hyperphosphorylation of neurofilament (pNF-H) and/or site-specific hyperphosphorylation of the microtubule associated protein tau are thought to destabilize the cytoskeleton, interrupting axonal transport. Additionally, abnormal accumulation of Amyloid-beta not only creates structural blockades that hinder transport but can also activate kinases that can drive pathological phosphorylation of tau and NF-H. We used ELISA to quantify pNF-H, Aβ(1-42), total tau, and two phosphoisoforms of tau: RZ3 (ptau231), and CP13 (ptauS396/404) in retina, optic nerve (ON), and superior colliculus (SC) of pre-glaucomatous, early, and late glaucomatous DBA/2J mice. To assess the relationship between protein concentration and axonal transport outcome, we injected the anterograde tracer, cholera toxin-B (CTB) into the eye to trace the retinofugal projection. The SC was microdissected and analyzed based on presence/ absence of CTB; corresponding retina and optic nerve were also analyzed based on transport outcome. Significant elevations in Aβ(1-42) were observed in the retina of all DBA/2J age groups, even the preglaucomatous age, while elevations were not present in the SC until early glaucomatous ages. Aβ(1-42) elevations occurred prior to transport loss. There were also significant age-dependent and tissue-specific increases in pNF-H in the retina, ON and SC of DBA/2J mice. While retinal pNF-H concentrations were not elevated until transport deficits were evident, a significant increase in pNF-H was observed in the SC and ON beginning at the earliest, pre-glaucomatous stage and persisting throughout all ages - providing support for a distal axonopathy model. Furthermore, elevated pNF-H in SC and corresponding projections with compromised/absent axonal transport was observed. Surprisingly, no clear relationship between total or phosphorylated tau levels and age or transport outcome was found. Understanding the progression of these structural alterations in glaucoma may present new therapeutic avenues for this debilitating disease.

Disclosures: **S.D. Crish:** None. **G.N. Wilson:** None. **M.A. Smith:** None. **C.M. Dengler-Crish:** None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.15/R1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JDRF

ADA

PA Lions

Title: The number of photoreceptors is reduced in postmortem human retinas after short durations of diabetes

Authors: *A. J. BARBER[1], S. MOHR[2], W. F. ROBINSON[1];
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Abstract: Objective: Diabetic retinopathy is a common complication of diabetes that causes vision loss in working-age adults. The disease is progressive and generally diagnosed between 5 and 15 years after diabetes onset. Clinical indications of retinopathy include macular edema due to blood-retinal barrier breakdown, and vascular lesions such as microaneurysms and vascular cell drop-out. People with diabetes often report vision loss such as reduced night vision and acuity, but the underlying cause is unclear. Evidence from animal models indicates that neurodegeneration leads to cell death in retinal ganglion cells and other neurons. This study examined the effect of diabetes on retinal thickness, as an indicator of neuronal cell number, as well as the abundance of photoreceptors, in postmortem eyes collected from human donors with relatively short durations of diabetes (3 to 5 years). Methods: Postmortem tissue was collected from donors with no diabetes (ND; n=7), non-insulin dependent diabetes ((-)Ins; n=6), or insulin-dependent diabetes ((+)Ins; n=6). Donor ages were closely matched and averaged 55 ± 12 years old. Retinal cell layer thickness was measured in H&E stained paraffin sections by bright field microscopy. The density of the nuclei in the outer nuclear layer was also measured in peripheral and central retina. Cone photoreceptor density was measured in these regions using

immunofluorescence to 7G6 (all cones) and red/green opsin (red and green cones). Vascular microaneurysms and acellular capillaries were counted in contralateral retinas by whole-mount elastase digest, to expose the entire vascular structure. Data were analyzed by one-way ANOVA with multiple comparisons test. Results: There was no significant difference in the thickness of retinal cell layers in (-)Ins and (+)Ins donors, compared to ND. The (-)Ins and (+)Ins retinas, however, contained significantly fewer nuclei in the outer nuclear layer of peripheral retina compare to the ND tissue ($p<0.05$). There was no significant difference in the number of cones in each group. Quantification of vascular lesions in the elastase-digested retinas suggested a trend towards more microaneurysms in the (+)Ins retinas compared to ND, but this was not a statistically significant difference. Conclusions: The results indicate that outer nuclear layer cells in the peripheral retina are depleted during the early stages of diabetic retinopathy, in the absence of gross vascular lesions. Since the number of cone photoreceptors was unchanged, the reduction in cell number must be due to a loss of rods. These data may explain some of the early vision loss in diabetes.

Disclosures: A.J. Barber: None. **S. Mohr:** None. **W.F. Robinson:** None.

Poster

797. Other Neurodegenerative Disorders II

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Program#/Poster#: 797.16/R2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CONACyT Grant 232956

Title: Aberrant homeostasis of the dopamine D2-like receptor subtypes mediating inhibition of the vasopressor sympathetic outflow in diabetic rats

Authors: *A. H. ALTAMIRANO-ESPINOZA, G. MANRIQUE-MALDONADO, E. RIVERA-MANCILLA, B. VILLANUEVA-CASTILLO, C. M. VILLALON; Farmacobiología, Dept. De Farmacobiología Cinvestav Sede Sur, Mexico DF, Mexico

Abstract: Dopamine plays an important role in the modulation of several physiological functions, including cardiovascular homeostasis, via dopamine D_1-like and D_2-like receptors. Based on the conjunction of structural, transductional and operational criteria, dopamine D_2-like receptors consist of the D_2, D_3 and D_4 subtypes. Our group has recently reported that activation of dopamine D_3 and, to a lesser extent, of D_4 receptor subtypes inhibit the vasopressor

sympathetic outflow in healthy pithed rats. Moreover, other studies have shown alterations in the expression and/or function of D_2-like receptors in the central nervous system in diabetic animals; nevertheless, it remains unknown whether these alterations encompass the perivascular sympathetic neurons innervating resistance blood vessels. Hence, this study investigated with selective antagonists the specific role of the dopamine D_2, D_3 and D_4 subtypes in the inhibition of the sympathetic vasopressor outflow in diabetic pithed rats. For this purpose, male Wistar rats were pretreated intraperitoneally with streptozotocin (50 mg/kg, i.p.; n=60) or vehicle (citrate buffer; 1 ml/kg, n=20) and kept for 4 weeks; then they were pithed and artificially respired with room air. Under these conditions, electrical stimulation (0.03, 0.1, 0.3, 1 and 3 Hz; 50 V and 2 ms) of the thoracic spinal cord (T_7-T_9) or i.v. bolus injections of exogenous noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg/kg) resulted in frequency-dependent and dose-dependent vasopressor responses, respectively. Moreover, i.v. continuous infusions of quinpirole (1 μg/kg.min) inhibited the electrically-induced, but not the noradrenaline-induced, vasopressor responses in both healthy and diabetic rats. This quinpirole-induced sympatho-inhibition in diabetic rats was: (i) unchanged after vehicle or L-741,626 (a D_2 receptor antagonist; 300 μg/kg); and (ii) partially blocked after SB-277011-A (a D_3 antagonist; 300 μg/kg), L-745,870 (a D_4 antagonist; 100 μg/kg) or the combination of SB-277011-A + L-745,870 (300 and 100 μg/kg, respectively). These doses of antagonists did not affect per se the sympathetically-induced vasopressor responses and were high enough to completely block their respective receptors, as previously reported in pithed rats. These findings in diabetic rats suggest: (i) differences in the pharmacological profile of the D_2-like receptor subtypes mediating inhibition of the sympathetic vasopressor outflow (as compared to healthy rats); and (ii) that quinpirole might interact with other receptors (probably α_2-adrenoceptors) besides the D_3 and D_4 subtypes.

Disclosures: A.H. Altamirano-Espinoza: None. **G. Manrique-Maldonado:** None. **E. Rivera-Mancilla:** None. **B. Villanueva-Castillo:** None. **C.M. Villalon:** None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.17/R3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Characterization of cerebrovascular function in mice developing cerebral small vessel disease

Authors: *M. BALBI, N. PLESNILA;
Inst. For Stroke and Dementia (ISD)University, Munich, Germany

Abstract: Background: CADASIL - cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy - is the most common form of hereditary small vessel disease. It is caused by mutations of the Notch 3 gene, located on chromosome 19 (1). Transgenic mice overexpressing mutated Notch3 (TgNotchR169C) replicate the disease and are therefore the only available model for small vessel disease. This study aims to identify early microvascular dysfunctions in the CADASIL model in order to facilitate the development of therapeutic strategies. Methods: Pial microvascular reactivity to CO2 (5 and 10%) was investigated in 7.5 (n=21) and 11 (n=16) months old TgNotchR169C and control mice using intravital fluorescence microscopy. Cerebral blood flow (CBF) was measured with a laser Doppler probe over the territory of the middle cerebral artery. Neurovascular coupling was investigated in a second group of 8.5 (n=25) and 12.5 (n=15) months old mice, via electrical stimuli to the forepaw and simultaneous recording of CBF over the associated somatosensory cortex. Results: Vessels of 20 to 30 micrometers in diameter were selected for analysis. All groups show an increase in vessel diameter in response to CO2. Estimating the cumulative increase in vessel diameter and CBF, significant differences were found: at 11 months, TgNotchR169C mice show increased vessel dilation at 5% CO2, and decreased CBF response at 10%. In the second group, CBF responses were analyzed sorting the first four and last six stimuli separately. While there was no significant difference in reaction between TgNotchR169C and control mice, older mice - 8 and 11.5 months old - exhibited a significant decrease in reaction over time compared to younger controls (n=7) - 6 weeks old. Conclusion: We conclude from these findings, that aging reduces neuro-vascular coupling, i.e. vasodilatation to neuronal activation, and that mice suffering from progressive small vessel disease develop endothelial dysfunction. Accordingly, aging and small vessel disease may result in completely different pathophysiologies. [1] Joutel A, Corpechot C, Ducros A, et al. (October 1996). "Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia". Nature 383 (6602): 707-10

Disclosures: M. Balbi: None. **N. Plesnila:** None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.18/R4

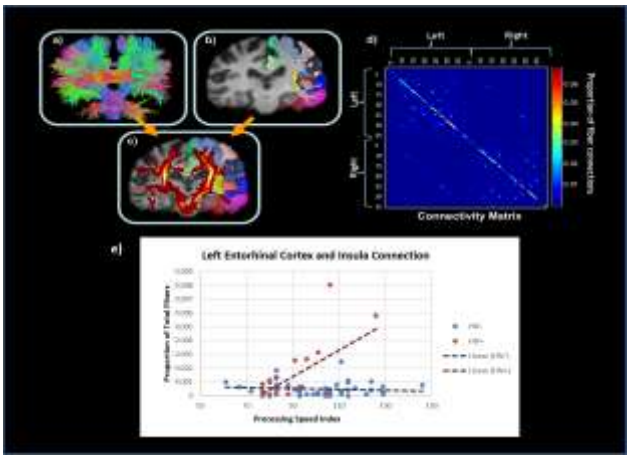
Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH

Title: Altered structural white matter patterns in children with perinatally acquired HIV are associated with processing speed

Authors: *T. M. NIR[1], N. JAHANSHAD[1], V. G. VALCOUR[2,3], W. PRASITSUEBSAI[4], A. DESAI[2], S. CATELLA[2], S. LERDLUM[5], P. VISRUTARATNA[5,6], K. PRUKSAKAEW[4], L. AURPIBUL[7], T. PUTHANAKIT[4,5], S. J. KERR[4], J. ANANWORANICH[4,3,8], P. THOMPSON[1]; [1]Inst. for Neuroimaging and Informatics, Neurol., USC Imaging Genet. Ctr., Marina del Rey, CA; [2]Neurol., UCSF, San Francisco, CA; [3]SEARCH, Bangkok, Thailand; [4]HIV-NAT, The Thai Red Cross AIDS Res. Ctr., Bangkok, Thailand; [5]Chulalongkorn Univ., Bangkok, Thailand; [6]Chiang Mai Univ., Chiang Mai, Thailand; [7]RIHES, Chiang Mai, Thailand; [8]US Military HIV Res. Program, Henry M. Jackson Fndn., Bethesda, MD

Abstract: Worldwide, about 330,000 infants are infected with HIV each year because they are born to mothers living with HIV. Cerebral atrophy and cognitive impairment are common in HIV-infected patients; however, the neurobiological consequences of perinatal HIV infection and ART exposure on brain development are not well understood, and the effect of HIV and ART on the brain's structural connectivity has yet to be studied. One of the most frequent cognitive abnormalities in HIV infection is slowing of mental processing speed. To identify whether impaired anatomical networks in HIV+ youth are associated with information processing speed index (PSI), we used 1.5T MRI-based cortical parcellations (Fig1a) and 25-direction whole-brain DTI-based tractography (Fig1b) to evaluate the white matter connection density in cortico-cortico connectivity maps (Fig1d); 46 HIV- children (mean age: 10.4+/-2.4; 23M/23F) and 18 HIV+ children (mean age: 10.8+/-1.8; 9M/9F) from Thailand were imaged. The fiber density between each pair of cortical regions was evaluated to identify connections that showed changes with respect to disease status, PSI, or their interaction (Dis*PSI). Linear regressions were used, adjusting for the linear and non-linear effects of age, sex, and their interactions. While PSI was significantly lower in HIV infected youth than uninfected youth ($p<0.02$), we found no overall effects of PSI or disease status on the connectivity network. However, an increase in the density of connections between the left entorhinal cortex and insula was significantly associated with an increased interaction score ($p<9.8*10^{-5}$). As seen in Figure 1d, this suggests that HIV status affects the relation of white matter connectivity and PSI, with *better* cognition being associated with *fewer* fiber connections in uninfected youth and *more* fiber connections in HIV+. Compensatory mechanisms in temporal lobe network connections, thought to mediate many cognitive functions like PSI, may allow for normal cognitive function. Ultimately, perinatal HIV infection may alter network developmental trajectories.



Disclosures: **T.M. Nir:** None. **N. Jahanshad:** None. **V.G. Valcour:** None. **W. Prasitsuebsai:** None. **A. Desai:** None. **S. Catella:** None. **S. Lerdlum:** None. **P. Visrutaratna:** None. **K. Pruksakaew:** None. **L. Aurpibul:** None. **T. Puthanakit:** None. **S.J. Kerr:** None. **J. Ananworanich:** None. **P. Thompson:** None.

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: A New Investigator Award from DC D-CFAR (NIH P30AI087714)

NIH Grant 5U01AI069494

Georgetown Unviersity Transitional Award

Title: Functional connectivity revealed an isolated cingulate cortex due to aging and HIV-infection

Authors: *X. JIANG[1], C. LIU[2], C. WANG[2], M. YOUNG[2];
[1]Dept Neurosci, [2]Dept Med., Georgetown Univ. Med. Ctr., WASHINGTON, DC

Abstract: **Background:** Despite the widening use of combination antiretroviral therapy (cART), HIV-associated neurocognitive disorders, or HAND, remains as one of the most common disorders in people with HIV infection. However, the neuropathogenesis of HAND is

still poorly understood. Recently, using a combination of a task-switching paradigm and functional magnetic resonance imaging (fMRI) technique, we have found that reduced executive function in HIV+ older adults is linked to reduced brain activations in the anterior cingulate cortex (ACC), suggesting that ACC might be one of key regions affected by HIV-infection (*Magnus et al., CROI, 2014*). Here we hypothesized that the disrupted activations in ACC might reflect a disconnection of ACC and posterior cingulate from other brain regions due to HIV-induced synaptodendritic injury (*Ellis et al., 2007*). To test this hypothesis, resting state functional connectivity between cingulate cortex and other brain regions was examined. **Method:** Twenty-three women (50.2±6.8 years old, 13 HIV+) participated in the study. They were recruited from the Washington DC site of the Women's Interagency HIV Study and had no major psychiatric disorders or other confounding health problems. One run of resting state fMRI data was collected from each subject. After standard MRI preprocessing, the nuisance factors that include head motion, signal from white matter and cerebrospinal fluid, global signal, and linear trend, were regressed out. We then obtained two sets of voxel-wise correlations: R_{within}, the mean pairwise correlations between voxels in the cingulate cortex; and $R_{between}$, the mean pairwise correlations between voxels in the cingulate cortex and voxels in the rest of brain (gray matter). The isolation of cingulate cortex from the rest of brain was measured as $I_{cc} = (R_{within} - R_{between}) / (R_{within} + R_{between})$. **Results:** Pearson's correlation analysis revealed a significant positive correlation between age and I_{cc}, ($r=0.60$, $p<0.005$), and two-sample t-test revealed that I_{cc} is higher in HIV+ women than in HIV-negative controls ($p<0.05$, one-tailed). **Conclusion:** Cingulate cortex, one of most interconnected regions with other parts of brain, is involved in many important cognitive functions, including executive function. Here we provided preliminary evidence suggesting that connections between cingulate cortex and the rest of brain are affected by both aging and HIV-infection. This may be due to age- and/or HIV-related synaptodendritic injury, leading to a more "isolated" cingulate cortex, which might be responsible for the high prevalence of reduced executive function with the aging HIV-positive populations in the cART era.

Disclosures: X. Jiang: None. **C. Liu:** None. **C. Wang:** None. **M. Young:** None.

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Global COE grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Grant from the Ministry of Health, Welfare and Labor of Japan

Title: Antemortem diagnosis of Neuronal intranuclear inclusion disease

Authors: *J. SONE[1], N. KITAGAWA[2], T. INAGAKI[1], Y. IWASAKI[3], M. YOSHIDA[3], F. TANAKA[4], G. SOBUE[1];
[1]Nagoya Univ. Sch. of Med., Nagoya / Aichi, Japan; [2]Neurol., Kosei Chuo Gen. Hosp., Tokyo, Japan; [3]Neuropathology, Inst. for Med. Sci. of Aging, Aichi Med. Univ., Nagakute/Aichi, Japan; [4]Neurol., Yokohama City Univ. Grad. Sch. of Med., Yokohama, Japan

Abstract: Neuronal intranuclear inclusion disease (NIID) is a progressive neurodegenerative disease characterized by widespread presence of eosinophilic hyaline intranuclear inclusions in neuronal cells and somatic cells in H&E slides. NIID has been considered to be a heterogeneous disease with highly variable clinical manifestations such as neuropathy, cerebellar ataxia and dementia etc, which may occur concomitantly in certain cases. Sporadic and familial cases have been reported, and the onset of disease varies from the infantile stages to late middle age. These factors made the antemortem diagnosis of NIID difficult. So, we investigated the capability of skin biopsy for antemortem diagnosis of NIID, and reported that skin biopsy is a useful antemortem diagnostic tool for familial neuronal intranuclear inclusion disease because it detects intranuclear inclusions in the dermal cells. Recently, some autopsies of sporadic NIID patients with leukoencephalopathy which showed high-intensity signal in MRI diffusion-weighted imaging (DWI) in the corticomedullary junction have been reported. So, we studied skin biopsy samples of normal volunteers and sporadic NIID suspected patients who presented leukoencephalopathy and DWI high-intensity signal, and investigated the capability of skin biopsy for antemortem diagnosis of sporadic NIID. Skin biopsy samples were collected under local anesthaesia. A 3-mm-diameter punch biopsy specimen was obtained at 10 cm above the lateral malleolus. Biopsy samples were fixed in 10% formalin and treated. We performed an immunohistochemical analysis using a Ventana DISCOVERY system with anti-ubiquitin antibody. The samples for electron microscopy were fixed with glutaraldehyde in cacodylate buffer and embedded in epoxy resin. In skin biopsy samples from sporadic NIID, intranuclear inclusions were found in sporadic NIID patients with leukoencephalopathy and DWI high-intensity signal. Inclusions were found in adipocytes, fibroblasts and sweat gland cells. Electron microscopy revealed that intranuclear inclusion in cutaneous cells have no limiting membrane and consist of haphazardly arranged filaments. These inclusions were positively stained with anti-ubiqutin antibody. These features of inclusion were identical with those of reported familial NIID inclusions in neuronal cells and dermal cells. In normal control and other neurodegenerative disease samples, no intranuclear inclusion was found in adipocytes, fibroblasts and sweat gland cells. Skin bipsy is available, less invasive and safe tool for the antemortem diagnosis of NIID.

Disclosures: **J. Sone:** None. **N. Kitagawa:** None. **T. Inagaki:** None. **Y. Iwasaki:** None. **M. Yoshida:** None. **F. Tanaka:** None. **G. Sobue:** None.

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIGMS 1SC3GM086323

CTSC #UL1-RR024996

G12 RR003037

G12 MD007599

Title: Proteasome impairment and protein aggregation in the nervous system of *Drosophila*

Authors: M. JANSEN[1], E. LEMPERT[1], R. ADEROGBA[2], *T. SCHMIDT-GLENEWINKEL[2]; [1]Biol. Sci., Hunter Col. and Grad. Ctr. of CUNY, New York, NY; [2]Biol. Sci., Hunter Col. of CUNY, NEW YORK, NY

Abstract: The etiology of aging and neurodegeneration is associated with impairment of the ubiquitin proteasome pathway and the accumulation of protein aggregates in the brain. Under normal circumstances the proteasome mediates the proteolysis of short-lived, mutated, miss-folded, and aberrantly-modified proteins. However, during the aging process and in the progression of neurodegenerative disorders, proteasome function is inexplicably inhibited, leading to the production of proteinaceous aggregates. To further elucidate the relationship between proteasome impairment and protein aggregate formation, we used the inducible binary Geneswitch system in *Drosophila* melanogaster to express an RNAi against the pros-β5 subunit of the proteasome. The β5 subunit confers the rate limiting chymotrypsin-like proteolytic activity to the proteasome, making it an ideal target for RNA interference in our proteasome disruption studies. We detected a significant decrease in proteasome activity accompanied by an increase in protein aggregates in the brains of our knockdown flies compared to the controls. To better characterize the mechanisms involved in aggregate formation, we will analyze protein aggregate formation in neurons, glia, and the fly neuropil. In addition we will employ mass spectrometry to identify proteins that are more susceptible to being miss-folded and included in aggregates.

Disclosures: **M. Jansen:** None. **E. Lempert:** None. **R. Aderogba:** None. **T. Schmidt-Glenewinkel:** None.

Poster

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Support: NIH Grant DA07304

NIH Grant DA035663

NIH Grant DA007097

Title: HIV-1 Tat activates a RhoA/ROCK signaling pathway to reduce NMDA-evoked calcium increases in hippocampal neurons via an actin-dependent mechanism

Authors: ***K. A. KROGH**, E. A. LYDDON, S. A. THAYER;
Univ. of Minnesota, Minneapolis, MN

Abstract: Almost half of the 33 million people currently infected with HIV will develop HIV-associated neurocognitive disorder (HAND). Cognitive decline in patients with HAND correlates with synaptodendritic damage and loss of dendritic spines. HIV-infected cells in the brain shed viral proteins, such as the transactivator of transcription (Tat), which causes synapse loss via an NMDA receptor (NMDAR)-dependent mechanism. Here we report that following Tat-induced potentiation of NMDARs, a RhoA/Rho-associated protein kinase (ROCK) signaling pathway is activated; a subsequent rearrangement of the actin cytoskeleton results in a reduction in NMDAR function. Tat-induced changes in NMDA-evoked increases in [Ca2+]i were measured using fura-2-based Ca2+ imaging of rat hippocampal neurons in culture. Ca2+ influx was triggered by the transient application of NMDA (10 μM) following 0 to 24 h exposure to Tat (50 ng/mL). Tat induced a time-dependent potentiation of the NMDAR that peaked by 8 h and then adapted back to control levels by 24 h. Tat-induced NMDAR potentiation was unaffected by inhibition of RhoA by C3 transferase (1 μM) or by inhibition of ROCK by Y27632 (10 μM) or H1152 (10 μM). However, inhibition of either RhoA or ROCK prevented adaptation of the NMDAR. Expression of dominant-negative RhoA (T19N) also prevented adaptation following Tat-induced NMDAR potentiation, confirming Tat-induced activation of this pathway. RhoA/ROCK

signaling is known to affect the actin cytoskeleton. Stabilization of actin with phalloidin (10 μM) or depolymerization of actin with cytochalasin D (10μM) had no effect on Tat-induced NMDAR potentiation, but prevented adaptation of NMDAR function. Preliminary data suggest that Tat causes loss of dendritic spines expressing EGFP-actin, which may arise following activation of RhoA/ROCK. Together, these findings indicate that Tat activates a RhoA/ROCK signaling pathway resulting in actin rearrangement with a subsequent reduction in NMDAR function. Adaptation of NMDAR function and loss of dendritic spines may be a mechanism to protect neurons from excessive Ca2+ influx and excitatory input and could reveal targets for the treatment of HAND.

Disclosures: **K.A. Krogh:** None. **E.A. Lyddon:** None. **S.A. Thayer:** None.

Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: KHIDI Grant A120202

Title: Effect of adipose derived mesenchymal stem cells administration on hypoglycemia-induced neuronal death

Authors: ***J. KIM**[1], H. KIM[1], B. LEE[1], B. CHOI[1], I. KIM[1], S. LEE[1], M. SOHN[2], S. SUH[1]; [1]Dept. of Physiol., Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [2]Inha Univ., InCheon, Korea, Republic of

Abstract: Diabetic patients who attempt tight control of blood glucose levels frequently experience hypoglycemia. Acute and prolonged hypoglycemia results in neuronal death and cognitive dysfunction. Recently, therapeutic stem cell transplantation has been highlighted because stem cells ability to promote tissue regeneration, neuroprotection, and improvement of endogenous repair. Human adipose derived mesenchymal stem cells (MSC) can be easily attained from liposuction tissue following plastic surgery. In a previous study, MSC have shown neuroprotective effects on ischemia-induced brain injury. MSC has never been tested for its therapeutic effect on hypoglycemia-induced brain injury. To investigate the therapeutic potency of MSC on hypoglycemia-induced neuronal death, 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. MSC (1x10^6) intravenous injection was started immediately after hypoglycemia. Histological test for neuronal death, microglia activation, oxidative injury and blood brain barrier (BBB) disruption were evaluated at 1 week after hypoglycemia. Hippocampal neuronal death was evaluated by Fluoro Jade-B. Activated microglia were evaluated by immunostaining for CD11b. IgG immunostaining was performed to detect BBB disruption and 4HNE immunostaining was performed to detect oxidative injury. Neuronal death, microglia activation, oxidative injury and BBB disruption were significantly reduced by MSC administration when evaluated at 1 week after hypoglycemia compared with vehicle treated group. The present study suggests that MSC may have therapeutic potential to reduce hypoglycemia-induced brain injury in diabetic patients.

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

Poster

797. Other Neurodegenerative Disorders II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Reta Lila Weston Institute

UCL Grand Challenge

ARUK

Title: Analysis of FET and stress granule related proteins in frontotemporal lobar degeneration (FTLD)

Authors: *R. BANDOPADHYAY, J. BRELSTAFF, C. PERSAD, T. LASHLEY;
Inst. of Neurol., London, United Kingdom

Abstract: The neuropathological hallmark of FTLD-FUS is extensive FUS aggregate pathology demonstrated by cytoplasmic and intranuclear inclusions. Transportin-1(TRN1) is a nuclear transporter that shuttles FET proteins (FUS/TLS, EWS and TAF15) and other cargoes back into the nucleus. We have recently shown that TRN1 is also present in the pathological lesions of FTLD-FUS cases (Brelstaff et al 2011). Here we have investigated the presence of FET proteins, EWS, TAF15 and NUP98 (a nuclear-pore complex protein) in our cohort of FTLD-cases along

with healthy control brain tissue from Queen Square Brain Bank using standard immunohistochemistry and immunoblotting protocols. We also examined the presence of FET proteins and TRN1 in Huntington's disease (HD) and spinocerebellar ataxia. We show EWS and TAF15 within a variety of morphological inclusions in the FTLD-FUS cases that mirrors those observed under FUS and TRN1 immunohistochemistry by our group (Lashley et al, 2011; Brelstaff et al 2011). Inclusions are observed in frontal cortex, hippocampal formation, medulla and the motor neurons of spinal cord. We also noted that NUP98 is present in the cytoplasmic inclusions in a NIFID case and which overlapped with FUS pathology. The stress granule markers, PABP1 and G3BP label a variety of cytoplasmic inclusions in FTLD. Cytoplasmic EWS and TAF15 levels are increased in FTLD-FUS cases compared to controls as seen by immunoblotting. We conclude that in our cohort of FTLD-FUS cases, the typical pathological lesions were also positive for EWS, TAF15 and stress granule markers. The link between SG and FET proteins warrant further investigations. Refs: Brelstaff J et al 2011; Acta Neuropathol, 122(5):591-600. Lashley T et al 2011; Brain, 134(9):2548-2564.

Disclosures: R. Bandopadhyay: None. **J. Brelstaff:** None. **C. Persad:** None. **T. Lashley:** None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.25/R11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: MicroRNA-21 influences the progression of Cervical Spondylotic Myelopathy by altering astrocyte reactive state and onset of symptoms

Authors: *A. M. LALIBERTE[1], S. K. KARADIMAS[1], M. G. FEHLINGS[2];
[1]Genet. and Develop., Univ. of Toronto, TORONTO, ON, Canada; [2]Neurosurg., Krembil Neurosci. Centre, Toronto Western Res. Institute, Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Background: Cervical Spondylotic Myelopathy (CSM), the most common form of adult spinal cord dysfunction, is a progressive neurodegenerative disease that is caused by compression of the cervical spinal cord by spontaneously generated bony osteophytes. Progressive compression and loss of spinal cord microvessels is thought to cause regional hypoxia, leading to gray matter degeneration. Recently, our laboratory has developed a model of CSM that reproduces the compression and resulting neurological deficits of the human disease,

finally allowing in depth examinations of CSM molecular pathology. A screen of microRNAs expressed in CSM animals determined that microRNA-21 (miR-21) was highly expressed, particularly in the advanced stages of disease. MiR-21 has previously been identified as a molecule of interest in both spinal cord injury and hypoxia; however, relatively little is known about its role in these conditions. Recent findings in spinal cord injury have implicated miR-21 in suppression of astrocyte hypertrophy. Since astrocytes are important regulators of neurovascular interactions, inflammation, and synaptic integrity, we sought to investigate the effect of miR-21 in the progression of CSM symptoms and pathology. Methods: MiR-21 knockout (KO) CSM animals were acquired and compared to wild type CSM animals. Gradual compression of the spinal cord occurred over 2, 6, or 12 weeks (consistent with no, moderate, or severe behavioral deficits, respectively). Catwalk gait analysis system was used to periodically measure motor deficits. Immunohistochemistry and stereology were used to monitor progression of CSM pathology Results: Using CatWalk gait analysis, miR-21 KO appeared to have altered gait from wild type animals, displaying decreased stride length and swing speed in both forelimbs and hindlimbs as early as 2 weeks from the start of compression. As predicted, astrocyte morphology was much more hypertrophic in miR-21 KO animals. Surprisingly, lesion size was generally smaller in the miR-21 KO animals. Conclusions: Knockout of miR-21 appears to dramatically increase astrocyte hypertrophy in CSM animals, a finding consistent with other spinal cord pathologies. Interestingly, this phenomenon appeared to have contradictory effects on behavior and tissue integrity, possibly due to differing cell-specific mechanisms.

Disclosures: **A.M. Laliberte:** None. **S.K. Karadimas:** None. **M.G. Fehlings:** None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.26/R12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JSPS KAKENHI Grant Number 22592257

Title: Sentizaitation of trigeminal nervous system by infraorbital nerve injury exacerbates the pathophysiology of migraine

Authors: M. TOOYAMA[1], *C. KUDO[1], C. MUKAI[1], Y. MORIMOTO[2], H. NIWA[1]; [1]Dept. of Dent. Anesthesiol., Osaka Univ. Grad. Sch. of Dent., Suita, Osaka, Japan; [2]Kyusyu Univ. Hosp., Fukuoka、Fukuoka, Japan

Abstract: Background and Aims: Migraine is a chronic neurological disorder by recurrent of severe unilateral headache. It has recently been reported that migraineurs are often suffering from headache attacks accompanying with the cutaneous allodynia in the craniocervical region, and this allodynia is supposed to be a risk factor for migraine chronification. In addition, some migraineurs experience the non-odontogenic toothache instead of headache during the migraine attack period. These clinical findings suggest that branches of trigeminal nerve interact and sensitize each other. Here, we examined the influence of the sensitization of 2nd branch of trigeminal nerve on migraine attack by using the capsaisin-induced migraine animal model to demonstrate the correlation between the pathophysiology of migraine and the sensitization of the trigeminal nervous system. Methods: Male Sprague-Dawley rats (250-300g) were divided into three groups: (a) the chronic constriction injury of the infraorbital nerve (CCI-ION) and capsaicin administration (CCI-ION+cap), (b) CCI-ION and vehicle administration (CCI-ION+vehicle), (c) sham operation and capsaicin administration (sham+cap). The sensitization of ION was induced by CCI, and the allodynia in the region of the 2nd branch of the trigeminal nerve developed in 14 days. At day 14, rats were anesthetized, and 10 mM capsaicin was applied to the dura mater on the right transverse sinus to induce the migraine attack. Four minutes later, the brainstem and trigeminal ganglion (TG) were harvested. We performed the immunohistochemical staining in the trigeminal spinal subnucleus caudalis (Vc), upper cervical spinal cord (C1-C2) and TG to examine the expression of phosphorylated extracellular signal regulated kinase (pERK), as a marker of the excitability of nociceptive neurons after noxious stimulation of peripheral tissues. Results: The number of pERK immunoreactive cells (pERK-IR) in Vc and C1-C2 in CCI-ION+cap group significantly increased at 3600 and 4320 μm caudal from the obex compared to sham+cap group and CCI-ION+vehicle group. In the TG, pERK-IR neurons tended to increase in CCI-ION+cap group compared to sham+cap group. Conclusions: Our data showed that the sensitization of 2nd branch of the trigeminal nerve (ION) by CCI caused the sensitization of 1st branch, resulting in the enhancement of pERK expression in capsaicin-induced migraine rats peripherally and centrally in the trigeminal nociceptive pathway. From these results, it is suggested that the existence of the sensitization of a branch of trigeminal nerve can be the one of the exacerbation factors of the pathophysiology of migraine.

Disclosures: M. Tooyama: None. **C. Kudo:** None. **C. Mukai:** None. **Y. Morimoto:** None. **H. Niwa:** None.

Poster

797. Other Neurodegenerative Disorders II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 797.27/S1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIGMS IMSD 5R25GM055036

Title: Magnetic field and induced electrical field distributions of Figure-Eight TMS

Authors: *I. BASALDUA;
UMBC, BALTIMORE, MD

Abstract: Currently transcranial magnetic stimulation (TMS) has been widely accepted and used for laboratory experiments and clinical applications. Among different TMS structures the figure-8 type of stimulator has the advantage of having a better focused field as most people intuitively believed. However, even though it may be better focused than other types of stimulators the exact magnetic field distribution and the corresponding induced electrical field (or current) have not been revealed by vendors. To most users knowing the exact peak field location and the field distribution, which provides information about the desired and possibly unexpected excitation locations, is important for their applications. In this work we measured both the magnetic field and induced electrical distributions of a figure-8 stimulator. The magnetic field is measured by a 3-dimension magnetic meters from the vendor Trifield model VGM. Projection of magnetic field to al x-y, x-z, z-x planes and an overall 3-D plot of magnetic field vectors were generated and will be presented in the meeting. The distribution provides a clear view not only the strength but also direction of the magnetic field. To study the induced current distribution we use a twisted wire pair with negligible physical foot print to measure the electrical field instead. The measured voltage difference cross two spatial points with a fixed length provides initial recorded data of the induced electrical field. As we can theoretically prove that it doesn't matter what kind of resistance is used inbetween the two points, using a low resistance resistor to hold the two points will create higher induced current and potentially distorted TMS created field. So, it is important to minimize the induced current during the measurement of the field distribution. We have obtained a trustworthy measurement of the induced field distribution using transient magnetic field created by an electrical pulse generator and high current amplifier setup. The measured results match well with the 3-D magnetic field distribution driven by DC current. Besides learning from the measured results that the maximum induced field is indeed right at the center location of the figure 8 stimulator and immediately below the center on the surface, we also can see clearly how fast the field attenuated when the location is gradually away from the center.

Disclosures: I. Basaldua: Other; UMBC.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.01/S2

Topic: C.06. Developmental Disorders

Support: The Seaver Foundation

NIMH Grant (MH093725, JDB)

A gift from William G. Gibson and Paulina Rychenkova, PhD

Title: Behavioral, molecular and electrophysiological deficits in the Shank3-deficient rat model for autism

Authors: *H. HARONY-NICOLAS[1,2], O. BOZDAGI-GUNAL[1,2], M. KAY[3], N. DASKALAKIS[1], B. KLEI[4], M. BAXTER[5], K. ROEDER[4], S. WAGNER[3], J. D. BUXBAUM[1,5,6,2,7];
[1]Dept. of Psychiatry, Icahn Sch. of Med., New York, NY; [2]Seaver Autism Ctr. for Res. and Treatment, New York, NY; [3]Univ. of Haifa, Haifa, Israel; [4]Carnegie Mellon Univ., Pittsburgh, PA; [5]Dept. of Neurosci., [6]Dept. of Genet. and Genomics Sci., [7]Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Shank3 is a scaffolding protein that forms a key structural part of the postsynaptic density (PSD) of excitatory synapses. Haploinsufficiency of SHANK3 causes a monogenic form of autism spectrum disorders (ASD). Characterization of mouse models with Shank3 mutations provided evidence for impaired glutamatergic synaptic function in hippocampal and striatal brain slices and deficits in several behavioral measures related to ASD. Yet, our knowledge about the affected molecular pathways, the alterations in brain circuitries and their relation to the behavioral deficits observed in ASD is scanty. The aim of our study is to analyze the effect of Shank3 deficiency on ASD associated behaviors and to uncover the alterations in synaptic plasticity in brain regions and circuitries associated with the impaired behaviors. At the molecular level, we aim to identify the affected pathways that underlie the behavioral and synaptic deficits. For this purpose we have developed the Shank3-deficient rat, a genetically modified rat model for ASD. We are using electrophysiological, biochemical and genome wide transcriptional analyses to study the effect of the disruption in Shank3 on synaptic functioning and are applying behavioral analysis to relate changes to higher order processes. Our results reveal that Shank3 deficiency leads to attentional and social behavioral deficits, both of which are prevalent in subjects with ASD. These behaviors are highly dependent on the intact function

of brain regions associated with ASD; the hippocampus, the PFC or both. Our *in vitro* electrophysiological analysis demonstrated impairment in long-term potentiation (LTP) and depression (LTD) in the hippocampus while the *in vivo* analysis showed impaired mPFC LTP in response to hippocampal stimulation. Finally, our high-throughput RNA sequencing, unbiased proteomic analysis, and functional enrichment analysis, showed that decreased Shank3 levels affect distinct biological mechanisms, including actin remodeling and that Shank3 perturbed signatures map to frontal and fetal developmental periods, consistent with previous findings indicating that many ASD genes map to these time periods. By further characterizing this ASD rat model we will be able to investigate the perturbed pathways to define molecular and cellular components that could be targeted for developing therapies for SHANK3-haploinsufficiency syndromes and for ASD more broadly.

Disclosures: H. Harony-Nicolas: None. **O. Bozdagi-Gunal:** None. **M. Kay:** None. **N. Daskalakis:** None. **B. Klei:** None. **M. Baxter:** None. **K. Roeder:** None. **S. Wagner:** None. **J.D. Buxbaum:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.02/S3

Topic: C.06. Developmental Disorders

Support: The Alan Edwards Centre for Research on Pain

Canadian Pain Society

Title: The Tube Co-occupancy Test (TCOT): A novel way to measure affiliative behaviour among laboratory mice

Authors: *A. H. TUTTLE[1,2], K. DOSSETT[1], L. GERSTEIN[1], L. STEIN[1], R. PEARL[1], M. SUKOSD[1], P. LEGER[1], D. YACHNIN[1], J. S. AUSTIN[1], J. S. MOGIL[1,2];
[1]Dept. of Psychology, McGill Univ., Montreal, QC, Canada; [2]Alan Edwards Ctr. for Res. on Pain, Montreal, QC, Canada

Abstract: Empathy for another's physical pain has been previously demonstrated in humans and mice, and is shown to be dependent on familiarity between individuals. Our recent efforts indicate that social stress may be responsible for the absence of empathy for the pain of

strangers, as well as a contributing factor to prosocial behaviors in rodents. Previous attempts to explain prosocial rodent behaviour have implicated sex-specific hormones and candidate autism genes. However, the corresponding neurobiological mechanisms mediating rodent familiarity behaviour, or the initial social interactions between mice, are unknown. Here we propose a novel and fully automated behavioural test, the Tube Co-Occupancy Test (TCOT) as a way to study the effects of stress on rodent social behaviour. Our assay quantifies rodent willingness to co-occupy a "safe" PVC tube placed in a brightly lit open field. Prior to testing mice are either housed together (familiars) or separately (strangers). Preliminary results show that outbred strangers demonstrate significantly reduced co-occupancy behaviour when compared to familiar mice. Furthermore, we show that autistic-like rodent models (Fmr1 -/-, BTBR strain) show decreased co-occupancy behaviours when compared to C57BL/6 controls. Our assay features a new way to test social behaviours in laboratory rodents that we hope will be useful in understanding dysfunctional social affiliation in autism spectrum disorders.

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Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.03/S4

Topic: C.06. Developmental Disorders

Support: Canadian Institutes for Health Research

Ontario Brain Institute

Title: Consistency between the neuroanatomical and behavioural phenotype in the 15q11-13 duplication mouse model

Authors: *J. ELLEGOOD[1], N. NAKAI[2], J. NAKATANI[3], T. TAKUMI[2], J. P. LERCH[1]; [1]Mouse Imaging Ctr., Hosp. For Sick Children, Toronto, ON, Canada; [2]RIKEN Brain Sci. Inst. (BSI), Wako, Japan; [3]Shiga Med. Univ., Shiga, Japan

Abstract: Background - Copy number variations (CNVs) are thought to account for ~20% of autism cases (Beaudet et al. 2007). A very large CNV (6.3Mb) located at 15q11-13 is a common

chromosome abnormality (Takumi, 2010). The 15q11-13 region contains several maternal (Mat) and paternal (Pat) imprinting genes. 15q11-13 Mat and Pat duplications (Dp) have both been associated with Autism and occur in ~5% of autism patients (Takumi, 2010). The behavioural phenotypes for both the MatDp/+ and PatDp/+ have been thoroughly investigated (Nakatani et al. 2009), and several autism-like behaviours were found in the PatDp/+ but not in the MatDp/+. Objectives - The purpose of this study is to use magnetic resonance imaging (MRI) in both the PatDp/+ and MatDp/+ 15q11-13 mouse models to investigate the neuroanatomical phenotype and see how it relates to the behavioural phenotype. Methods - Twenty mice were included for the 15q11-13 PatDp/+ (10 PatDp/+ and 10 WT littermates) and 17 mice for the MatDp/+ (9 MatDp/+ and 8 WT littermates). Imaging was performed ex-vivo using a 7T MRI with a T2 weighted, 3D fast spin echo sequence which acquires data at an isotropic resolution of 56 μm (Lerch et al. 2011). Using image registration the brains were aligned, and the volumes of 62 different regions (Dorr et al. 2008) were calculated. Multiple comparisons were controlled using False Discovery Rate (FDR, Genovese et al. 2002). Results and Discussion - Mirroring the previously reported behavioural findings (Nakatani et al. 2009), multiple significant differences were found in the PatDp/+ mice, and no differences were found in the MatDp/+ mice. For the PatDp/+ mice, 15 of the 62 regions were found to be significantly smaller in absolute volume (mm3). The majority of differences were localized to subcortical structures. The hypothalamus, the inferior and superior colliculi, the granular layer of the hippocampus, and thalamus were all significantly decreased in volume (-5-6%). Contrary to previous reports on autism related mouse models involving neuroligin3 and integrinβ3 (Ellegood et al. 2010, 2012) no differences in the large white matter tracts were found in the 15q11-13 implying the structural integrity of the white matter is unaffected. Further, the reported behavioural findings highlighted a reversal learning impairment in the PatDp/+ mice, which could be related to the 6% smaller granular layer found in the hippocampus of the PatDp/+. Conclusions - This study highlights the complimentary benefit of examining the neuroanatomy in conjunction with behaviour, and can also provide a explanations for some of the reported behavioural findings.

Disclosures: J. Ellegood: None. **N. Nakai:** None. **J. Nakatani:** None. **T. Takumi:** None. **J.P. Lerch:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.04/S5

Topic: C.06. Developmental Disorders

Support: Simons Foundation

Title: Automated variant scoring for the prioritization of candidate genes in the autism genetic database AutDB

Authors: *E. LARSEN[1], M. ZIATS[2], S. BANERJEE-BASU[1];
[1]Mindspec Inc., McLean, VA; [2]Baylor Col. of Med., Houston, TX

Abstract: A major focus of research in the post-genomic era is to decipher the heterogeneous genetic landscape underlying the pathogenesis of complex human diseases such as Autism Spectrum Disorders (ASD). Hundreds of candidate genes containing ASD-associated rare and/or common genetic variants have been identified, and the advent of new techniques such as next generation sequencing (NGS) has significantly accelerated the discovery of candidate genes containing ASD-linked rare variants. With the rapid growth of genetic data obtained from ASD individuals adding to the already complex genetic landscape of this disease, there is a critical need for resources that not only specialize in the storage of this data, but that also provide tools for assessing the strength of evidence for a given gene in ASD and identifying high-priority candidate genes for further investigation. The Human Gene module of the autism genetic database AutDB (http://autism.mindspec.org/autdb/Welcome.do) was developed to serve as a publically available web-based modular database for the ongoing curation and visualization of ASD candidate genes, which are systemically extracted from peer-reviewed primary scientific literature and manually curated for inclusion. The number of ASD susceptibility genes in the Human Gene Module of AutDB has increased from 304 genes in December 2011 to 604 genes in March 2014, which demonstrates both the continued discovery of ASD candidate genes and the ongoing curation of these genes into AutDB. In addition, NGS techniques have contributed to a dramatic increase in the number of rare variants identified in ASD candidate genes (from 1202 in Dec 2011 to 3652 in March 2014) compared to common variants (from 534 to 878 over the same period). We have developed an automated gene scoring strategy that takes advantage of the richly-detailed annotation available in the Human Gene module to score candidate genes based on the strength of evidence of individual ASD-associated rare or common variants for a given gene. ASD-associated rare variants are scored based on the degree of variant specificity and segregation with ASD, whereas common variants are scored based on the degree of association with ASD and replication of association. Furthermore, the functional effect of a given variant (rare or common) is factored into determining the overall variant score. This strategy has allowed us to identify a number of high priority ASD candidate genes. Moreover, ongoing annotation of ASD-associated gene variants in the Human Gene module will allow candidate gene scores to be frequently updated, providing users with up-to-date gene prioritization information.

Disclosures: E. Larsen: None. **S. Banerjee-Basu:** None. **M. Ziats:** None.

Poster

798. Autism Behavioral Analysis II

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Program#/Poster#: 798.05/S6

Topic: C.06. Developmental Disorders

Support: NIMH (R00MH085946)

NIH (DP2MH10001101).

NIGMS (R25GM56847)

Title: Changes in prefrontal microcircuit organization increase repetitive network activity in two mouse models of autism

Authors: *F. LUONGO, C. ZIMMERMAN, A. BRUMBACK, M. HORN, V. SOHAL; UCSF, San Francisco, CA

Abstract: Pathological changes in neocortical microcircuits are believed to instigate neuropsychiatric disorders including autism, but measuring such circuit-level changes remains challenging. Deficits associated with disorders such as autism often extend across multiple cognitive, behavioral, sensory, and affective modalities, suggesting that these diseases may comprise common information processing phenotypes at the level of neuronal networks. Such network-level phenotypes might be missed by studies of individual cells and instead require studies of datasets that probe patterns of network activity. Here, we use single-photon GCaMP imaging to measure activity patterns within isolated prefrontal circuits from two etiologically distinct models of autism: FMR1 knockout mice and mice exposed to valproic acid *in utero*. Both models exhibit enhanced functional interactions between prefrontal neurons and an increase in repetitive patterns of network activity that could plausibly contribute to perseveration and stereotyped behavior. Notably, these changes occur despite the absence of increased network excitability. Furthermore, these changes do not occur in mice which model schizophrenia (DISC1 mutant mice), nor in mice that have undergone chronic treatment with Fluoxetine, an SSRI which may ameliorate symptoms of autism. This suggests that the changes observed in VPA-exposed and FMR1 KO mice may be specifically associated with prefrontal microcircuit dynamics in models of autism. We develop a novel computational method for generating surrogate datasets with matching activity statistics but arbitrary correlation matrices and show that the increased primary and higher order interactions drive the increased repetitive activity in these mouse models. Lastly, we show that cholinergic modulation normally decorrelates activity, possibly contributing to attention, while defects in this mechanism underlie the abnormalities we

observed and may thereby contribute to attentional deficits in autism. Studies of this sort could provide a foundation for understanding common phenotypes at the level of circuits and thus inform circuit-level remedies for ASD and other neuropsychiatric diseases.

Disclosures: F. Luongo: None. **C. Zimmerman:** None. **A. Brumback:** None. **M. Horn:** None. **V. Sohal:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.06/S7

Topic: C.06. Developmental Disorders

Title: Brain structure volumes as a means of measuring changes in neuron gene expression

Authors: *D. J. FERNANDES, J. P. LERCH, J. ELLEGOOD;
Mouse Imaging Ctr., Toronto, ON, Canada

Abstract: Autism Spectrum Disorders (ASD) are characterized by social deficits, communication difficulties, and repetitive behaviours. The genetics behind autism is heterogeneous; with over 250 genes implicated in susceptibility of autism. Genetic defects lead to changes in protein expression at the synapse, which in turn causes changes in the volumes of brain structure during development and to behaviours associated with autism. A better understanding of how mutations in genetics lead to changes in protein expression could help develop potential therapies for autism. We measured the volume of brain structures with single gene knockouts and found significant differences in the volumes of certain structures when compared to wild-type mice. We then used the Allen Brain Atlas to find which genes are preferentially expressed in these regions in wild-type mice. For example, in mice with Intersectin-1 knockout, we found significant differences in the volumes of certain structures: such as the cerebral peduncles and the dentate gyrus. As expected, the Allen Brain Atlas showed Intersectin-1 was found to be preferentially expressed in these regions. However, other genes expressing synaptic proteins -- such as adhesion regulating molecule 1, myotubularin related protein 2, synaptic vesicle glycoprotein, etc. -- were also found to be preferentially expressed. While only Intersectin-1 was knocked out, the expression of several proteins involved with synaptic function was altered. Further investigation is currently being undertaken to look at other gene knockouts and how they alter protein expression at the synapse. This investigation could help create a network of gene expression, where the knockout of one gene leads to changes in

expression of other genes and further our understanding of genetically diverse disorders, such as autism.

Disclosures: D.J. Fernandes: None. **J.P. Lerch:** None. **J. Ellegood:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.07/S8

Topic: C.06. Developmental Disorders

Support: NIH F31 MH098636-02

Title: Behavioral characterization of an ephrin-a knockout mouse model for autism spectrum disorders

Authors: *R. WURZMAN[1,2], P. FORCELLI[3], L. F. KROMER[2];
[1]Rachel Wurzman, Arlington, VA; [2]Neurosci., [3]Pharmacol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: EphA receptors and ephrin-A ligands play important roles for neural development and also synaptic plasticity in select cases where expression persists into adulthood. Recently, EPHA3 and EPHA7 gene mutations were linked with Autism Spectrum Disorders (ASDs) and developmental neurological delays, respectively. Ephrin-A2 and ephrin-A3 are two high affinity binding partners for EphA3 and EphA7, and which are expressed on astrocytic processes at glutamatergic synapses. As binding partners to EphA3, we propose that these ephrins also could be implicated in ASD. Supporting this hypothesis, deletions of ephrin-A2 or ephrin-A3 have each been associated with isolated deficits in learning and memory behavior in rodents. To better characterize a potential role for these ligands in ASDs, we performed a comprehensive behavioral characterization of sensorimotor, learning, social, and anxiety-like behaviors in ephrin-A2/-A3 double knockout (DKO) mice. These behaviors included the open field, elevated plus maze, morris water maze, and assays of prepulse inhibition of startle response, marble burying, social approach, and grooming. The predominant phenotype in ephrin-A2/A3 DKO mice was repetitive and self-injurious grooming behaviors, such as have been associated with corticostriatal circuit abnormalities in other rodent models of neuropsychiatric disorders. However, despite the self-injurious grooming phenotype, analysis of striatal dendritic spine morphology failed to detect abnormalities in spines at excitatory corticostriatal synapses.

Therefore, we are currently investigating dendritic spine changes in other brain regions known to be associated with the observed behavioral abnormalities as a possible alternative explanation for abnormal sensorimotor behaviors in these mice. Consistent with an ASD phenotype, ephrin-A2/-A3 DKO mice also exhibited decreased preference for social interaction, a shift towards self-directed activity (e.g., grooming) in novel environments, and increased prepulse inhibition of acoustic startle. Although there were no gross deficits in cognitive assays, subtle differences in performance on learning and memory tests resembled traits observed in humans and other rodent models of ASD. We therefore conclude that ephrin-A2/-A3 DKO mice have utility as a novel ASD model with an emphasis on sensory abnormalities and restricted, repetitive behavioral symptoms.

Disclosures: R. Wurzman: None. **L.F. Kromer:** None. **P. Forcelli:** None.

Poster

798. Autism Behavioral Analysis II

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Program#/Poster#: 798.08/S9

Topic: C.06. Developmental Disorders

Support: SFARI 206683

Title: Enhancing AMPA signaling improves sociability in an autism mouse model

Authors: *M. HAN[1], R. M. MEJIAS-ESTEVEZ[2], R. L. HUGANIR[3], T. WANG[2];
[2]McKusick-Nathans Inst. of Genet. Med., [3]The Solomon H. Snyder Dept. of Neurosci., [1]The Johns Hopkins Univ., Baltimore, MD

Abstract: Despite rapid advancement in the understanding of the causes to autism, little progress has been made toward developing effective therapies. No drugs are currently available to target social behavioral deficits in autism. Glutamate, which mediates the majority of excitatory neurotransmission through the ionotropic and metabotropic (mGluR) receptors, has been previously implicated in the pathophysiology of autism. More specifically, disturbances in mGluR signaling have been implicated in Fragile X syndrome and autism, but the role of AMPA glutamate signaling in this disorder is unknown. We recently investigated the role of AMPA signaling disturbance in autism and identified mutations in glutamate receptor interacting protein 1/2 (GRIP1/2) that contribute to social behavioral deficits in patients with autism. These mutations alter dynamic AMPA-receptor synaptic recycling and synaptic strength. We

hypothesized that drugs modulating AMPA receptor function could improve social deficits in autism. We tested S18986, a positive allosteric modulator of AMPA receptors, on social behaviors using adult male BTBR mice, a well-characterized mouse model of autism in a cross over study. Acute administration of S18986 at an optimized dose results in a significant improvement in sociability in BTBR mice as measured by the ratio of the time spent sniffing a novel mouse to the time spent sniffing an empty cage in a modified three chamber sociability test (n=25; carrier only: 2.50+/-0.41; S18986: 4.78+/-0.90, p =0.023). We observed similar improvement in the preference to social novelty using the same study cohort. Results from these studies indicate that AMPA signaling pathway should be further investigated as a potential drug target for the correction of social deficits in autism.

Disclosures: **M. Han:** None. **R.M. Mejias-Estevez:** None. **R.L. Huganir:** None. **T. Wang:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

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Program#/Poster#: 798.09/S10

Topic: C.06. Developmental Disorders

Support: NSF IGERT Grant 1144399

UConn Research Foundation

Title: Learning delays in a mouse model of autism spectrum disorder

Authors: ***A. R. RENDALL**, D. T. TRUONG, R. H. FITCH;
Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with core symptoms of atypical social interactions, language impairments, and repetitive behaviors. It has also been reported that individuals with ASD have difficulty with multisensory integration, and this may disrupt higher-order cognitive abilities such as learning and social communication. Impairments in the integration of sensory information could in turn reflect diminished cross-modal white matter connectivity, as reported in some DTI/MRI studies. To date, causal mechanisms in ASD remain poorly understood, but likely include a combination of genetic and environmental risk factors. Indeed, the genetic contribution in ASD appears to be

strong, with heritability estimates as high as 90%. However, no single gene has been identified, and over 100 risk genes have been reported. Most of these genes play a critical role in neurodevelopment. One of these genes -- contactin-associated-like-protein 2 (*CNTNAP2*) -- was first associated with Specific Language Impairment, and more recently has been linked to ASD. *CNTNAP2* is located on ch7, and encodes a cell adhesion protein regulating synaptic signal transmission. Human *CNTNAP2* variants have also been specifically associated with difficulties with non-word repetition, a measure of working memory. To better understand the behavioral and biological underlying mechanisms of ASD, a transgenic mouse model was created with a genetic knockout (KO) of the rodent homolog *Cntnap2*. Initial studies of this mouse (Peñagarikano et al., 2011) found poor social interactions, behavioral perseveration, and reduced vocalizations -- all strongly resembling the human symptoms. *Cntnap2* KO mice also show abnormalities in myelin formation, consistent with a hypo-connectivity model of ASD. Furthermore, these mice showed abnormal cortical neural synchrony, fewer inter-neurons, and atypical neuronal migration. The current study was designed to further assess the intermediate behavioral phenotype of this mouse model, with a focus on learning and memory. *Cntnap2* KO and wild-type mice were tested on a 4/8 radial arm water maze for 14 consecutive days. Results showed that *Cntnap2* KO mice exhibited significant deficits in working and reference memory during the acquisition period of the task. During the retention period (i.e., after an asymptote in errors), *Cntnap2* KO mice performed comparably to wild-type mice. These findings suggest that *CNTNAP2* may play an underlying role in the development of neural systems important to learning and cross-modal integration, and disruption of this function could be associated with delayed learning in ASD.

Disclosures: A.R. Rendall: None. **D.T. Truong:** None. **R.H. Fitch:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.10/S11

Topic: C.06. Developmental Disorders

Support: NSF IGERT Grant 1144399

UConn Research Foundation

Title: Morphological changes in the medial geniculate nucleus of Cntnap2 KO mice

Authors: *D. T. TRUONG, A. R. RENDALL, R. H. FITCH;
Behavioral Neurosci., Univ. of Connecticut, Storrs, CT

Abstract: Atypical cell morphology in the medial geniculate nucleus (MGN) of the thalamus has been reported in dyslexic brains (Galaburda et al., 1994), and it has been suggested that these anomalies could relate to impairments in rapid auditory processing seen in infants, children and adults with (or at risk for) language-based disorders. Associated research with genetic and injury-based rodent models of language-related neurodevelopmental disorders (e.g., developmental dyslexia) has confirmed evidence of simultaneous impairments in rapid auditory processing, and anomalies in MGN cell morphology, in several of these models. *CNTNAP2* is a gene that has been implicated in autism spectrum disorder (ASD) as well as specific language impairment -- two neurodevelopmental disorders associated with language deficits. Interestingly, behavioral examination of *Cntnap2* knock-out (KO) mice conducted in our lab showed atypical auditory processing behaviors, including impaired silent gap detection, as well as an unexpected enhancement frequency discrimination. These patterns are in fact consistent with behavioral findings from ASD populations, and may relate to the language deficits that often occur with ASD. To investigate whether differences in MGN morphology could be related to the abnormal pattern of auditory processing behaviors observed in *Cntnap2* KO mice, we examined the MGN from male KO and matched wildtype mice that were previously tested on our auditory battery. Numbers and sizes of neurons were estimated in the MGN using optical fractionator and nucleator probes, respectively. We found significantly fewer neurons in the MGN of *Cntnap2* KO mice. In addition, cumulative cell size distribution in the MGN indicated that *Cntnap2* KO mice had more small than large neurons in the MGN in comparison to congenic controls. These findings suggest that changes in MGN morphology may contribute to the atypical auditory processing behaviors observed in *Cntnap2* KO mice, and could have relevance for auditory and language anomalies in human language-disordered populations including ASD.

Disclosures: D.T. Truong: None. **A.R. Rendall:** None. **R.H. Fitch:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.11/S12

Topic: C.06. Developmental Disorders

Title: Functional characterization of Itgb3 promoter gene variants associated with elevated serotonin blood levels

Authors: *S. GABRIELE[1], C. LINTAS[1], R. SACCO[1], A. M. PERSICO[1,2];
[1]Univ. Campus Biomedico, Rome, Italy; [2]Mafalda Luce Ctr. for Pervasive Developmental Disorders, Milan, Italy

Abstract: Genetic factors contribute significantly to autism spectrum disorders (ASD). The ITGB3 gene, located on human chr. 17q21.32, encodes integrin beta 3, the beta subunit of the platelet membrane adhesive protein receptor complex GP IIb/IIIa. Integrin beta 3 interacts with the serotonin transporter SERT, coded by the SLC6A4 gene, regulating its trafficking on the plasma membrane of platelets and serotonin (5-HT) uptake in synapses. ITGB3 and SLC6A4 genes were both identified as quantitative trait loci (QTLs) for 5-HT blood levels, which are known to be elevated in a consistent subgroup of ASD patients. We have previously reported that the *rs2317385* G allele at the 5' end is significant associated with 5-HT blood levels in 293 Italian families with an autistic proband (Napolioni et al., Eur J Hum Genet. 2011). To identify functional ITGB3 variants contributing to elevated 5-HT blood levels, we sequenced the ITGB3 promoter region, exons and exon-intron junctions in twenty individuals selected on the basis of known genotypes, and we detected six SNPs in linkage disequilibrium with *rs2317385* and all located in the promoter region of ITGB3. Four different haplotypes involving these SNPs were selected, two associated with "high" and two with "low" 5-HT alleles at *rs2317385*. Each haplotype was cloned into pGL4.10, transfected into two hematopoietic cell lines, K-562 and HEL 92.1.7, and analysed by luciferase assay. ITGB3 promoter activity is strongly modulated in HEL 92.1.7 cells, where luciferase expression is significantly increased by the presence of a G allele at *rs55827077* ($P<0.05$), but not in K-562 cells. Functional experiments to explain this cell specificity are ongoing, as well as similar experiments in neuronal cell lines, Neuro2A and SH-SY5Y. These results support a cell type-specific modulation of ITGB3 promoter activity by genetic variants linked to high 5-HT blood levels in families with autistic probands.

Disclosures: **S. Gabriele:** None. **C. Lintas:** None. **R. Sacco:** None. **A.M. Persico:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 798.12/T1

Topic: C.06. Developmental Disorders

Title: Common functional genetic variants support Th2 and NK immune pathway contributions to the pathogenesis of Autism Spectrum Disorder

Authors: *I. PIRAS[1,2], S. GABRIELE[1], V. NAPOLIONI[1], R. SACCO[1], A. M. PERSICO[1,2]; [1]Unit of Child and Adolescent Neuropsychiatry & Lab., Rome, Italy; [2]Mafalda Luce Ctr. for Pervasive Developmental Disorders, Milan, Italy

Abstract: Converging evidence suggests that immune system dysregulation is involved in the pathophysiology of Autism Spectrum Disorder (ASD) both in children and adults. Altered immune processes include ongoing neuroinflammation in post-mortem brains, elevated pro-inflammatory cytokines in cerebrospinal fluid and blood, altered immune cell function, presence of brain-specific auto-antibodies, and dysregulated immune transcriptome. Previous studies have highlighted SNPs located in immune genes, such as *CD99L2*, *JARID2* and *TPO*, as well as genetic variation at *MHCII*, *C4B* and *MIF*, as risk factors for ASD. Another strategy to assess whether dysimmunity contributes to ASD pathogenesis or represents a collateral by-standing effect is to investigate the association of ASD with common SNPs known to influence immune gene expression or function (e.g cytokine levels) affecting several distinct immune cascades. To this aim, we genotyped 484 simplex and 18 multiplex Italian families with an ASD proband at 34 known functional SNPs located in 26 immune genes involved in all major immune pathways. SNPs yielding significant associations involved two immune pathways: [A] Statistically significant opposite transmission patterns between ASD and unaffected siblings were evident for rs2243250, located in the promoter of the *IL4* gene, and rs231775, a missense variant located in the *CTLA4* gene). The combination of high *IL4* expression and low *CTLA4* function alleles was associated with autism and the opposite alleles were overtransmitted to unaffected siblings. IL-4 is mainly produced by Th2 cells and at the same time is the key signal to induce Th2 differentiation from naïve T cells, whereas CTLA-4 is a critical and potent inhibitor of Th2 differentiation. [B] Similarly, the combination of high expression alleles at rs361525 and rs2430561*,* functional SNPs located in the promoter and in intron 3 regions of the *TNFA* and *IFNG* genes*,* respectively, was associated with autism, while the opposite alleles were protective. Among various functions, TNF-α and IFN-γ enhanced the cytolytic function of Natural Killer (NK) cells. These results indicate that common variants conferring hyper-responsive Th2 (*IL4* and *CTLA4*) and NK (*TNFA* and *IFNG*) activity also confer autism vulnerability. Hence previously-reported increases in plasma Th2 cytokine levels and NK cell activation do not appear to represent mere by-standing effects, but may be instead part of the pathophysiological cascade resulting in autistic disorder in a sizable subgroup of patients.

Disclosures: I. Piras: None. **S. Gabriele:** None. **V. Napolioni:** None. **R. Sacco:** None. **A.M. Persico:** None.

Poster

798. Autism Behavioral Analysis II

Location: Halls A-C

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Program#/Poster#: 798.13/T2

Topic: C.06. Developmental Disorders

Title: Linguistically deprived children: Meta-analysis of published research indicates mental synthesis disability - Implications for novel intervention strategies for children with language delay

Authors: *A. VYSHEDSKIY[1,2], R. DUNN[2];
[1]Boston Univ., Boston, MA; [2]ImagiRation LLC, Boston, MA

Abstract: We analyzed published reports of individuals not exposed to a syntactic language until puberty: two feral children, who grew up without hearing any language, and eight deaf linguistic isolates, who grew up communicating to their families using homesign, a system of gestures which allows them to communicate simple commands, but lacks much in the way of syntax. This is typical of families with deaf children, which are isolated from a sign language community; instead of learning a formal sign language, they normally spontaneously develop a homesign system. All tests performed on these individuals were sorted by their reliance on the subject's ability to actively create novel mental images (a function we are calling *mental synthesis*). For example, a subject's ability to follow a direction to "put the bowl *behind/in front of/on/under* the cup," has significant indication of their capacity towards *mental synthesis*. To correctly place a bowl *behind* or *in front* of a cup, one first needs to mentally synthesize the novel image of a bowl behind or in front of a cup. Someone who cannot simulate the process mentally would have no mental image of a bowl *behind* or *in front* of a cup and would therefore just use trial and error and probably place the cup and bowl into an incorrect arrangement. According to our analysis, feral children and deaf linguistic isolates performed poorly in all tests of *mental synthesis*. The consistent observation of *mental synthesis disability* within these individuals stands in stark contrast to their performance in memory as well as semantic tests: they could easily remember hundreds of newly learned words and recall previously seen images from memory but had real difficulty in any tasks requiring them to combine these images into novel configurations. Myelination and electrophysiological studies suggest that the likely problem of these individuals is the reduced connectivity between the PFC and the posterior cortex. The *PFC-posterior cortex* connection model indicates that syntactic language exercises in neurotypical children provide the necessary input for fine-tuning those connections. The model predicts that *mental synthesis* relies on synchronous networks, whereby fiber conduction velocity

is equilibrated by increased myelination of longer fibers. A lack of exposure to a syntactic language before the end of the critical period appears to result in reduced myelination and asynchronous connections between the PFC and the posterior cortex. Exposure to a syntactic language past the critical period can improve semantic content but not *mental synthesis* ability. Implications for novel intervention strategies for children with language delay are discussed.

Disclosures: **A. Vyshedskiy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ImagiRation LLC. **R. Dunn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ImagiRation LLC.

Poster

798. Autism Behavioral Analysis II

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Autism Speaks/NAAR

Nancy Lurie Marks Family Foundation

Title: Bidirectional changes in ube3a gene dosage reciprocally regulate aggression in models of angelman syndrome and idic15

Authors: *D. C. STOPPEL[1], J. TODOROVIC[2], M. P. ANDERSON[1];
[1]Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Univ. of Texas, Austin, TX

Abstract: Reciprocal changes in UBE3A gene dosage cause two neurodevelopmental disorders. Maternally inherited deletions of UBE3A cause Angelman syndrome, characterized by intellectual disability, motor defects, seizures, and a pathognomonic increased social motivation. Whereas maternally inherited triplications of UBE3A as in Idic15 Autism underlie decreased sociability and increased repetitive restrictive behaviors of this disorder. Aggression is a

common comorbidity in Autism but is not widely studied in mouse models. To study the regulation of aggression by Ube3a we compared mice with two extra copies of Ube3a (Ube3a-2x) that model Idic15 to maternal Ube3a knockout mice (Ube3a-mKO) in the resident intruder paradigm. Ube3a-2x mice showed increased aggression compared with wild-type animals while Ube3a-mKO mice had decreased aggression. These findings indicate that disturbances in Ube3a gene dosage reciprocally regulate aggression behavior in two mouse models of human developmental disorders.

Disclosures: D.C. Stoppel: None. **M.P. Anderson:** None. **J. Todorovic:** None.

Poster

798. Autism Behavioral Analysis II

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Topic: C.06. Developmental Disorders

Support: MEXT Grant KAKENHI 25640002

Title: Pax6 haploinsufficiency affects maternal separation-induced ultrasonic vocalization by advanced paternal age in the offspring

Authors: *N. OSUMI[1], R. KIMURA[1], K. YOSHIZAKI[1], K. KOIKE[1], V. TUCCI[2], H. INADA[1]; [1]Dept Devel Neurosci, Tohoku Univ. Grad Sch. Med., Sendai, Japan; [2]Dept. of Neurosci. and Brain Technologies, Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Human genetics and epidemiological studies have indicated that a neurodevelopmental gene PAX6 and advanced paternal age are genetical and environmental risk factors for autism. Here we examined a role of Pax6 in an advanced paternal age-related behavior impairment in mice. To obtain F1 offspring, young C57BL6/J (3-month-old) female mice were mated with male wild type (WT) or Sey/+ mice at three different ages, i.e., young, middle-aged (6-8-month-old) and advanced aged (>12-month old). F2 offspring were obtained from crossing young C57BL6/J female mice with young F1 WT offspring derived from young or advanced aged F0 WT father. Maternal separation-induced ultrasonic vocalization (USV) of both F1 and F2 offspring was measured at postnatal day 6. The number of USV in WT offspring derived from advanced-aged WT was dramatically decreased by 32.7% and 33.1% compared to that in WT offspring derived from young and middle-aged WT, respectively. In contrast, the number of USV was not different between the groups in F2 offspring, implicating that the USV

phenotype would be canceled in the next generation. Interestingly, the number of USV in Sey/+ offspring derived from middle-aged Sey/+ was significantly decreased by 58.8% compared to that in WT offspring. In contrast, the number of USV was not statistically altered between WT and Sey/+ offspring derived from young Sey/+, indicating that Pax6 haploinsufficiency accelerates behavioral abnormalities such as maternal separation-induced USV. To explore molecular mechanisms, histone methylation moieties were compared between young and advanced aged or middle aged WT and Sey/+ spermatocytes, in which Pax6 is predominantly expressed. We found significant changes in global methylation in advanced aged spermatocytes and middle-aged Sey/+ spermatocytes. Our results suggest an intriguing possibility that epigenetical changes in spermatogenesis may cause behavioral abnormalities such as maternal separation-induced USV in the offspring. We are testing whether these altered histone methylation remains in sperm and the developing brain.

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Poster

798. Autism Behavioral Analysis II

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Topic: C.06. Developmental Disorders

Support: Simons Foundation

Title: AutDB: Integrator of diverse data related to autism

Authors: ***S. B. BASU**[1], E. C. LARSEN[1], I. DAS[2], S. MUEND[1], R.-S. LIN[1], W. PEREANU[1]; [1]Mindspec Inc., McLean, VA; [2]MindSpec Inc., McLean, VA

Abstract: Although a genetic component underlying Autism Spectrum Disorders (ASD) has been firmly established from various lines of evidence, the identification of multitude of genes and chromosomal loci associated with ASD has made elucidation of pathophysiology difficult to resolve. A range of methodologies have identified both rare and common genetic variants in ASD candidate genes. Moreover, the recent development of high-throughput next generation sequencing technologies and the increasing usage of chromosomal microarray analysis has led to a significant expansion in the number of single nucleotide variants (SNVs) and copy number variants (CNVs) associated with ASD. To facilitate visualization and analysis of the vast genetic

heterogeneity underlying ASD, we have created the Autism Database (AutDB*; http://autism.mindspec.org/autdb/Welcome.do), a publicly available, manually curated, web-based, and searchable database for genes linked to ASD. Evidence regarding ASD candidate genes is systematically extracted from peer-reviewed, primary scientific literature and manually curated by our researchers for inclusion in AutDB. We have recently expanded AutDB utilizing a systems biology approach, implementing a modular framework that integrates diverse types of evidence about ASD candidate genes. Current modules within AutDB include: 1) Human Gene, which annotates both common and rare variants in ASD-linked genes; 2) Copy Number Variant (CNV), which catalogs deletions and duplications of chromosomal loci identified in ASD; 3) Animal Model, which catalogs behavioral, anatomical, and physiological data corresponding to genetic, induced, inbred and rescue models of ASD and 4) Protein Interaction (PIN), which builds interactomes of all known direct relationships for protein products of ASD-linked genes, documenting six major types of direct interactions: 1) protein binding, 2) DNA binding, 3), RNA binding, 4) protein modification, 5) direct regulation, and 6) autoregulation. To provide high-resolution view of diverse data linked to ASD, we have developed detailed annotation rules based on the biology of each data type and generated controlled vocabulary for data representation. In AutDB, we have seemlessly integrated ASD-specific genetic information to the corresponding data in Animal Model and Protein Interaction (PIN) modules. AutDB has grown rapidly; as of March 2014, it contains detailed annotations for 604 ASD associated genes with relevant evidence linking the gene to autism. AutDB is widely used by the autism research community as a reference resource for accelerating ASD research. * AutDB is licensed to Simons Foundation as SFARI Gene

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Poster

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Simons Foundation SFARI grant

Autism Science Foundation

Ellison Foundation of Boston

Rosanno Foundation

Title: Analysis of forebrain MEF2C function in autism-like behaviors

Authors: *A. J. HARRINGTON[1], J. KUMAR[1,2], J. RADUAZZO[1,3], C. W. COWAN[1];
[1]McLean Hosp., Belmont, MA; [2]Mstp, UT Southwestern, Dallas, TX; [3]Harvard Univ., Cambridge, MA

Abstract: Autism Spectrum Disorders (ASDs) are thought to result from brain synapse dysfunction. Emerging evidence indicates that rare, deleterious mutations increase the risk for developing autism, and many of these identified genes have known functions in the formation, pruning or plasticity of brain synapses. The myocyte enhancer factor 2 (MEF2) family of transcription factors regulates a gene expression program involved in activity-dependent synapse elimination in the developing brain. We found that MEF2 promotes synapse elimination through a process involving the RNA-binding protein, Fragile X Mental Retardation Protein (FMRP), the causal gene in the ASD, Fragile X Syndrome. More recently, mutations in MEF2C have been identified in patients with severe intellectual disability, epilepsy and autism, further supporting the critical link between MEF2 and normal brain development and function. To examine the role of MEF2 in brain development and autism-related phenotypes, we generated a conditional knockout (cKO) of the MEF2C gene within excitatory neurons of the forebrain in early brain development of mice. The MEF2C cKO mice are viable and healthy, but they display significant autism-like behavioral phenotypes, including reductions in social behaviors, restricted and repetitive motor behaviors, and deficits in social communication (ultrasonic vocalizations). Moreover, the mice are hyperactive, and they display robust deficits in reward-related behaviors. Ongoing studies are exploring the cellular, synaptic, and forebrain circuit abnormalities that accompany these behavioral abnormalities. Taken together, our findings reveal that genetic deletion of MEF2C in excitatory forebrain neurons in the mouse brain results in behaviors reminiscent of the three core diagnostic criteria for autism in humans, and as such, provide a new mouse model for studying potential brain dysfunctions that underlie autism-like behaviors.

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Poster

799. Autism Environment and Pathology

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.01/T7

Topic: C.06. Developmental Disorders

Support: Fond Nationale Suisse Grant

Title: Predictable enriched environments reduce hyper-emotionality in the VPA rat model of autism

Authors: *M. R. FAVRE, D. LA MENDOLA, J. MEYSTRE, D. CHRISTODOULOU, M. COCHRANE, H. MARKRAM, K. MARKRAM;
EPFL, Lausanne, Switzerland

Abstract: Autistic people present a spectrum of symptom severities and comorbidities, and are not equally responsive to treatment. Environmental stimulation and structured behavioral therapy are among the most effective treatments, but few studies have investigated the effects of predictability in the neurobiology autism, and individual differences. In rodents, environmental enrichment (EE) improves health, cognitive and emotion functions relevant for autism. In the present study, we investigate if EE predictability reduces hyper-emotionality, in the VPA rat model of autism, as proposed by the Intense World Theory of autism. We weaned male rats embryonically exposed either to VPA or to saline into a standard, or an unpredictable enriched, or predictable enriched home cage environment. Our univariate effects and multivariate Cronbach's alpha coefficients analyses corroborate to indicate that predictability is required for reversal of hyper-emotional behaviors in the VPA-exposed animals as a group. Correlations between a multivariate emotionality score and protein content support contributions from plasma corticosterone and glutamatergic-related proteins in the primary somatosensory cortex, dorsal and ventral hippocampus, and amygdala. Behavioral clustering of individual rats with Agglomerative Hierarchical Cluster Analysis further indicates that subgroups with lower emotionality form after predictable environment and also present particular levels of neurobiological proteins. Together, our results suggest that autistic-like individual rats are more sensitive to the environment than "neurotypical"-like rats and require a highly predictable type of enrichment for beneficial outcomes. These observations support autism as an intense world syndrome and have important implications for future research and personalized treatment.

Disclosures: M.R. Favre: None. **D. La Mendola:** None. **J. Meystre:** None. **D. Christodoulou:** None. **M. Cochrane:** None. **H. Markram:** None. **K. Markram:** None.

Poster

799. Autism Environment and Pathology

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Program#/Poster#: 799.02/T8

Topic: C.06. Developmental Disorders

Title: Individually specially targeted supplement and dietary intervention shows promising results in a cohort of children with autism spectrum disorder (ASD)

Authors: *H. B. WALKER;
Biomed Clin., Oslo, Norway

Abstract: The most important developmental phase in any person's life is the 1000 first days from conception.1, when basic genetic and epigenetic programming take place. Influences from different factors during this phase may change the way genes are expressed, even in generations. For most biochemical pathways there are different "security pathways", which in an exposed first generation may impose a shut-down or activation of genes with other consequences than in second generation. Current scientific data suggests that many illnesses may stem from the similar, underlying patho-physiological process as autism. Since the 1950's infants have been exposed to more xenobiotics before the age of 2 than before (new xenobiotics -vaccines, additives, paracetamol, antibiotics, etc.). An exposure for second generations like for the first - can influence epigenetics more. Feeding of infants has changed since the 1950's. Infant formula may differ in ingredients from mothers' milk. Children diagnosed with ASD have been rising in the past 20 years. It may indicate that people with a certain genetic DNA profile are more vulnerable to xenobiotics, and may develop an unbalance in their biochemistry manifesting itself as autism. We are born germfree. Microbiota will develop in specific order-in a window of establishment - during the first two years of life. If a window is missed, it may give lifelong consequences. Based upon principals summarised at the meeting at Nobel Forum, Karolinska Institute, May7th 2012.2,3,4. Biomed Clinic, Oslo, Norway has followed a cohort of ASD children age 2-10 years and tried successfully to regulate relevant factors. We use questionnaires going through a child's history, information from parents and observe the child. Urine tests showing 94 different markers and blood tests following 60 markers are evaluated. Thereafter, we design a specific plan for each child with follow-ups. Most start with a change in diet. Acetyl-L-carnitine and taurine are most commonly used for a year, thereafter in intervals. Essential minerals are supplemented depending on diet and/or test results. Vitamins- particularly B and D are evaluated. Other factors may be used. Improvement are found in all children and relevant cases will be presented. Autism can be treated. 1.Shenderov BA, Midtvedt T. Epigenomic programming. A future way to health? Microb Ecol Health Dis (MEHD). In press. 2.Walker HB. A true case story.MEHD. 2012, 3. Hugdah K et al.Autism spectrum disorders, functional MRI an MR spectroscopy- possibilities and challenges. MEHD, 2012, 4. Macfabe DF. Short-chain fatty acid fermentation products of the gut microbiota: implications in autism spectrum disorders MEDH 2012.

Disclosures: H.B. Walker: None.

Poster

799. Autism Environment and Pathology

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Topic: C.06. Developmental Disorders

Support: NIEHS 1R01ES021707

NIEHS 2P01ES011269

USEPA 8354320

Title: Methylome and synaptic gene impacts of an organic pollutant in Dup15q syndrome

Authors: *M. S. ISLAM[1], K. DUNAWAY[1], D. SCHROEDER[1], I. PESSAH[2], M. HORIKE[3], S.-I. HORIKE[3], J. LASALLE[1];
[1]Med. Microbiology and Immunol., [2]Univ. of California, Davis, Davis, CA; [3]Kanazawa Univ., Kanazawa, Japan

Abstract: Chromosome 15q11-13 duplication syndrome (Dup15q) is one of the most common copy number variations observed in autism-spectrum disorders. Unexpectedly, Dup15q human brain samples showed both DNA hypomethylation measured by LINE-1 pyrosequencing and significantly higher levels of the persistent organic pollutant PCB 95 than controls or idiopathic autism cases (Mitchell et al, 2012). Based on these prior observations, we investigated whether PCB 95 plays a causal or compounding role in DNA methylation differences observed in human Dup15q neurons using epigenomic approaches. Human SH-SY5Y neuroblastoma cells containing an additional maternal chromosome 15 (SH-15M, Meguro-Horike et al, 2011) were used to probe Dup15q and PCB 95 interactions. Whole genome bisulfite sequencing (MethylC-seq) revealed that SH-15M cells exhibited 2-14% higher frequency of large genomic domains of partial methylation (PMDs, Schroeder et al, 2011) compared to parental SH-SY5Y cells across all autosomes. Genes within SH-15M-gained PMDs were enriched for functions at the postsynaptic cell membrane, but were more specifically enriched for ion channels, neurotransmitter, and synaptic functions with PCB 95 exposure. Transcript levels of 48 autism candidate genes hypomethylated in SH-15M exposed to PCB 95 were compared across 48 different cell lines, single-cell clones, and postmortem brain samples in the Fluidigm Biomark.

Aberrant transcript levels were observed for multiple glutamate, serotonin, and GABAA receptor genes, with a significant compounding effect of SH-15M + PCB 95 for GRIA1, encoding ionotropic glutamate receptor AMPA1. The combination of a large chromosomal duplication (Dup15q) and exposure to a persisting organic pollutant (PCB 95) resulted large-scale genomic changes to the neuronal DNA methylome and synaptic gene transcript levels. These results have implications for understanding and treating complex gene x environment interactions in autism-spectrum disorders.

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Poster

799. Autism Environment and Pathology

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NCATS 9U54TR000021

FRAXA Research Foundation

Title: A hypothesis regarding the molecular mechanism underlying soy-induced seizures

Authors: *C. J. WESTMARK;
Univ. Wisconsin, MADISON, WI

Abstract: Numerous neurological disorders including fragile X syndrome, Down syndrome, autism and Alzheimer's disease are comorbid with epilepsy. We have observed elevated seizure propensity in mouse models of these disorders dependent on diet. Specifically, soy-based diets exacerbate audiogenic-induced seizures. We have also found associations between the consumption of soy-based infant formula and seizure incidence, epilepsy comorbidity and autism diagnostic scores in autistic children by retrospective analyses of the Simons Foundation Autism Research Initiative (SFARI) medical record database. There were seizure and infant formula data available for 1,949 autistic subjects (87% males) in the SFARI database. We found a 2.6-fold increase in the incidence of febrile seizures and a 4.8-fold increase in the incidence of simple partial seizures in autistic children fed soy formula. The soy-based formula was not associated

with statistically higher rates of infantile spasms, atonic (drop attack), grand mal (generalized tonic clonic), petit mal (absence) or complex partial seizures. There was a 2.1-fold increased incidence of epilepsy. In aggregate, these data demonstrate that soy-based diets are associated with increased seizure incidence in both mouse models of neurological disease and in autistic children. These data raise important questions regarding the neurological side effects of a soy-based diet during postnatal development. We evaluated autism testing scores in the SFARI autism population dependent on soy-based infant formula. We found exploratory associations between the consumption of soy-based infant formula and several autistic behaviors as assessed by sub-score and line-item analysis of the Aberrant Behavior Checklist (ABC), Autism Diagnostic Interview-Revised (ADIR) and Autism Diagnostic Observation Schedule (ADOS). Of note, subscale 5 scores (inappropriate speech) increased one grade from 3.4 ± 2.9(SD) in the non-soy female cohort to 4.4 ± 3.2(SD) in the soy cohort ($P \leq 0.05$). Likewise, subscale 1 scores (irritability) increased from 11 ± 8.7(SD) to 12 ± 9.0(SD) in males, which approached statistical significance ($P \leq 0.07$). In total, these data suggest that consumption of high levels of soy protein during postnatal development may affect neuronal excitability. We now present our theory regarding the molecular mechanism underlying soy-induced effects on seizure propensity. We hypothesize that soy phytoestrogens interfere with metabotropic glutamate receptor signaling through an estrogen receptor-dependent mechanism, which results in elevated production of key synaptic proteins and decreased seizure threshold.

Disclosures: C.J. Westmark: None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.05/T11

Topic: C.06. Developmental Disorders

Support: Johnson Center for Child Health and Development

SafeMinds

Title: Influence of pediatric vaccines on CNS development and behavior in the rhesus macaque: Relevance to autism

Authors: *B. GADAD[1], W. LI[1], U. YAZDANI[1], B. CURTIS[2], V. YUTUC[2], C. FERRIER[2], G. SACKETT[2], K. A. YOUNG[3], J. A. DARDEN[3], L. HEWITSON[4], D. C. GERMAN[1];

[1]Psychiatry, UT Southwestern Med. Ctr., Dallas, TX; [2]Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA; [3]Psychiatry and Behavioral Sci., Texas A&M Hlth. Sci. Ctr., Temple, TX; [4]Johnson Ctr. for Child Hlth. and Develop., Austin, TX

Abstract: Autism spectrum disorders (ASDs) are a heterogeneous group of neuro-developmental disorders characterized by impairments in social interaction and communication, and restricted stereotyped behaviors that manifest in early childhood. The etiology of ASDs is unknown but involves interactions between genetic and environmental factors. Some reports suggest that exposure to ethyl mercury, in the form of thimerosal, in childhood vaccines play a causative role in ASDs (*Austin et al., 2008*). In ASD post-mortem brains, reductions in the number of cerebellar Purkinje cells (*Skefos et al., 2010*) and amygdala lateral nucleus cells (*Schumann & Amaral*, 2006), and reductions in the cell size of CA1 hippocampus cell (*Kemper & Bauman*, 1993) have been reported. Using male rhesus macaques, receiving thimerosal-containing vaccines (TCVs) following the pediatric schedule from the 1990's (e.g., Hep B, DTaP, Hib, MMR) and from 2008 (with fewer TCVs) administered at 6 different time points (from birth to 12 months of age, and animals sacrificed at ~18 months), we examined cerebellar cells, amygdala cells and hippocampus cells (n=8-16/group). In the same animals we also examined learning, cognition and social behavior in vaccinated and control animals. Social behaviors from 8 to 18 months of age are currently being analyzed. There were no significant changes in Purkinje cell number or cell size, CA1 cell size, dentate gyrus volume, hippocampal neurogenesis, or lateral nucleus of the amygdala volume/cell number in animals in the 1990's vaccination group vs. saline controls. These data do not support a role for TCVs in the neuropathology of ASDs

Disclosures: B. Gadad: None. **W. Li:** None. **U. Yazdani:** None. **B. Curtis:** None. **V. Yutuc:** None. **C. Ferrier:** None. **G. Sackett:** None. **K.A. Young:** None. **J.A. Darden:** None. **L. Hewitson:** None. **D.C. German:** None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.06/T12

Topic: C.06. Developmental Disorders

Title: Prenatal fluoxetine exposure does not increase autism-related behaviors in adult mice

Authors: *F. MALARKEY[1,2], E. I. MESHREKY[3], N. J. MCGOVERN[3], J. P. HEAD[3], M. P. LEUSSIS[3];
[1]Emmanuel Col., Fullerton, ; [2]Psychology, [3]Emmanuel Col., Boston, MA

Abstract: SFN 2014 – Prenatal fluoxetine permanent effects Prenatal fluoxetine exposure does not increase autism-related behaviors in adult mice. Francis J. Malarkey, Elaria I. Meshreky, Nathan J. McGovern, Joshua P. Head, Melanie P. Leussis Although autism is a highly heritable disorder, the role of environmental factors in autism spectrum disorder has recently received increased attention. Relatively little is known about which environmental factors contribute to the ontogeny of autism. The current study examined the role of prenatal exposure to fluoxetine on subsequent behaviors relevant to autism in adulthood. C57BL/6J dams were administered the selective serotonin reuptake inhibitor (SSRI) fluoxetine (0.6mg/kg/day, IP) or vehicle from embryonic days 8 to 18. Adult male progeny were subsequently tested in a behavioral test battery designed to examine changes in social/communicative behaviors and repetitive behaviors. Mice were also tested for anxiety-related behaviors, as previous studies indicate that prenatal fluoxetine exposure increases anxiety. The test battery included tests such as social preference, scent marking in response to an estrus female stimulus, self-grooming, marble burying, elevated plus maze, and novelty-suppressed feeding. The results indicate that mice treated with fluoxetine prenatally were significantly more anxious in the elevated plus maze, but not in a novelty-suppressed feeding paradigm. Fluoxetine-treated mice did bury significantly more marbles than controls, but this increase in repetitive behavior did not extend to the self-grooming task as control and fluoxetine-treated mice did not differ on this measure. Further, prenatal fluoxetine treatment did not appear to alter social behavior in either the social preference task or a scent-marking task. These findings suggest that, at least in mice, prenatal exposure to fluoxetine does not significantly increase the risk of developing autistic-relevant behaviors in adulthood.
Keywords: behavior, development, SSRI **Support:** Emmanuel College

Disclosures: F. Malarkey: None. **E.I. Meshreky:** None. **N.J. McGovern:** None. **J.P. Head:** None. **M.P. Leussis:** None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.07/U1

Topic: C.06. Developmental Disorders

Support: Johnson Center for Child Health and Development

Simons Foundation

DOD AR110172

Title: Blood biomarkers for Autism: Peptoids and proteins

Authors: *D. C. GERMAN[1], U. YAZDANI[1], S. SINGH[1], Y. DENG[1], S. ZAMAN[1], L. HEWITSON[2];
[1]Psychiatry, U Texas Southwestern Med. Cntr., DALLAS, TX; [2]Johnson Ctr. for Child Hlth. and Develop., Austin, TX

Abstract: Autism spectrum disorders (ASDs) are a heterogeneous group of neuro-developmental disorders characterized by impairments in social interaction and communication, and restricted stereotyped behaviors that manifest in early childhood. Research findings have identified widespread changes in the immune system of children with autism, at both systemic and cellular levels. In order to identify individuals with ASDs and treat them at the earliest possible age, biomarkers for the disorder are desirable. A blood biomarker would be ideal because it is non-invasive and inexpensive. To identify blood biomarkers of ASDs we have used two approaches - peptoids and proteins. We have used on-bead displays of thousands of different peptoids (N-substituted oligoglycines) to screen for candidate IgG antibody biomarkers for ASDs. This approach represents a *high-throughput screening* approach with a *combinatorial peptoid library* to identify antibodies in the serum that are sensitive and specific for the identification of ASD. We have identified several peptoids (ASD1, ASD19-22) that uniformly bind lower levels of IgG in serum from ASD boys compared to typically developing (TD) boys (n=50/group; p=0.06). These peptoids also binds low levels of IgG in unaffected siblings from ASD families (pooled serum from n=10). Currently we are working to identify the antibody/antibodies that are recognized by the ASD1 peptoid. We have also used the Myriad Rules Based Medicine Luminex platform to determine whether there are differences in the levels of inflammatory proteins in the blood of ASD individuals (DiscoveryMAP 175+). Comparing serum from 30 ASD boys and 30 TD boys (ages 2-8 yrs.) we found 8 proteins that were significantly different between the two groups (<0.05). These proteins include alpha-fetoprotein, ferritin, interleukin-8 and thyroid stimulating hormone. We are cross-validating these findings on the Meso Scale Discovery platform to determine whether this panel of 8 protein biomarkers will have high sensitivity/specificity for the identification of ASDs.

Disclosures: D.C. German: None. **U. Yazdani:** None. **S. Singh:** None. **Y. Deng:** None. **S. Zaman:** None. **L. Hewitson:** None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.08/U2

Topic: C.06. Developmental Disorders

Support: Dwoskin Family Fdn

Katlyn Fox Fdn

Luther Allyn Dean Shouds estate

Dept. of Defense (US)

Title: Aluminum-induced gene expression alterations in mice brains

Authors: *D. LI, Y. LI, C. SHAW;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The consensus regarding the mode of inheritance of autism is that it is a polygenic model with multiple gene-gene and gene-environment interactions. However, how genes interact with environmental factors remains to be discovered. In young children, a highly significant correlation exists between the number of pediatric aluminum-adjuvanted vaccines administered and the rate of autism spectrum disorder (ASD). Furthermore, increased anxiety and reduced exploratory behavior and locomotor activities have been observed in aluminum-injected mice in our previous study. Aluminum, as a commonly known toxin, could possibly drive the immune response by being carried into the central nervous system by circulating macrophages and further disturb prenatal or postnatal neural system development. In this study, we propose a "two-hit model" to explain the potential interaction between aluminum and ASD-related genes, in which genetic predisposition (first insult) can sensitize children's neural system to toxic aluminum (secondary insult), and together they trigger downstream immune dysfunction and eventually result in developmental delay. To test this hypothesis, we selected 17 candidate genes which have implied function in both ASD and innate immune response. By measuring the expression level of these 17 genes in mice brains after repetitive subcutaneous administration of aluminum in amounts comparable to those children receive via routine vaccinations, a spectrum of expression change was detected in comparison with control mice. Some of the activators and effectors of macrophage, such as CCL2, IFNG and TNF, were significantly up-regulated, while the inhibitor of NF-κB, NFKBIB, and the excitatory neurotransmitters, ACHE, were remarkably down-regulated in aluminum-injected male mice. The down-regulation of NFKBIB led to

activation of NF-κB. Therefore, the immune system was activated and the synaptic function was decreased in male brains as a result of aluminum injection. Unlike male, female mice were less responsive to environmental insult. Fewer genes with altered expression level were discovered in females. These results strongly suggest that aluminum could impair brain function by penetrating brain and further interacting with key players of immune and neural system in males, while females may be protected from the aluminum toxicity by their more robust neurobiological system. Our results shed new lights on the etiology of ASD, gene-toxin synergy and vaccine safety.

Disclosures: **D. Li:** None. **Y. Li:** None. **C. Shaw:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Dwoskin Family Foundation (DFF), Kaitlyn Fox Foundation, Luther Allyn Shourds Dean estate. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Corp. Inc..

Poster

799. Autism Environment and Pathology

Location: Halls A-C

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Program#/Poster#: 799.09/U3

Topic: C.06. Developmental Disorders

Support: NIH Grant R01HD067135

NIH Training Grant T32GM008181

Title: Epigenetic modulation of BDNF transcription in the valproic acid model of autism

Authors: M. KONOPKO[1], A. DENSMORE[2], C. D. ROBY[3], *B. K. KRUEGER[2];
[1]Program in Neurosci., [2]Dept Physiol., [3]Dept Pharmacol., Univ. Maryland Sch. of Med., BALTIMORE, MD

Abstract: Valproic acid (VPA) is an anti-epileptic and mood-elevating drug that increases the risk of autism in the offspring of mothers who use it during pregnancy. Fetal exposure to VPA has been extensively used as an animal model of autism. Previously, we showed that administration of VPA to pregnant mice at E12.5 transiently increases BDNF expression in the fetal brain by activating promoters 1, 4 and 6 (P1, P4 and P6). Based on the activity of VPA as a histone-deacetylase inhibitor, we utilized chromatin immunoprecipitation to determine whether

histone-3 acetylation at one or more of these promoters was affected by fetal exposure to VPA. VPA administered to the dam at E12.5 increased acetylation of lysine 9 and/or 14 (H3K9/14ac) at P1, P4 and P6 2−4-fold in the fetal brain. We also examined the CpG methylation patterns in P1, P4 and P6 in response to VPA. Only 4 of the 106 CpGs in or near P1, P4 and P6, analyzed by bisulfite sequencing, were significantly methylated in the control fetal brain (35−60% methylated). No methylation of non-CpG Cs was observed. The four methylated sites were located in P4, in a cluster consisting of two individual CpGs and one CpG doublet within a 96 bp sequence. Overall methylation of this cluster was reduced by about 20% (11−31% at individual CpGs) in fetal brains exposed to VPA. This methylated CpG cluster is located just upstream from previously-described calcium-dependent regulatory elements and contains CpGs implicated in MeCP2 silencing of BDNF transcription in cultured neurons. Additionally, one of the individual CpGs and the doublet are immediately followed by an identical 7 bp sequence, conserved in humans, which was identified as a potential Elk-1/ETS regulatory element not previously implicated in BDNF transcription, raising the possibility that CpG methylation may directly suppress binding of a transcription factor to these sites. These data suggest that the stimulation of BDNF expression induced by VPA in the fetal brain may be mediated in part by increased H3K9/14ac at P1, P4 and P6 and by reduced methylation of a cluster of four CpGs in P4.

Disclosures: M. Konopko: None. **B.K. Krueger:** None. **A. Densmore:** None. **C.D. Roby:** None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.10/U4

Topic: C.06. Developmental Disorders

Title: Prenatal fluoxetine exposure does not alter ultrasonic vocalizations but does improve performance on some neuromotor coordination tasks in 3-11 day-old mouse pups

Authors: *J. M. THANOS, E. M. WEBER, N. J. MCGOVERN, M. P. LEUSSIS; Emmanuel Col., Boston, MA

Abstract: The present study examined the effects of prenatal fluoxetine exposure on isolation-induced ultrasonic vocalizations and neuromotor coordination in 3-11 day old mouse pups. C57BL/6J dams were administered the selective serotonin reuptake inhibitor (SSRI) fluoxetine

(0.6mg/kg/day, IP) or vehicle from embryonic days 8 to 18. Pups were then tested on postnatal days (P) 3,5,7,9, and 11. Neuromotor developmental paradigms included surface righting, negative geotaxis, and forelimb grip. Ultrasonic vocalizations were recorded in pups on P8, P10, and P12 after a five-minute separation from the dam. Mice were also weighed every other day to examine growth from P3-P13 and at P21. There was no significant effect of prenatal fluoxetine treatment on ultrasonic vocalizations during development in either male or female pups. Prenatal fluoxetine treatment did alter some measures of neuromotor development, including decreasing surface righting time and increasing forelimb grip strength. Negative geotaxis was not affected by prenatal fluoxetine treatment. It is possible these effects reflect a change in weight in the fluoxetine-exposed mice as weight increased from P3-P13 for all, but males were not significantly different across drug conditions while females exposed to fluoxetine were heavier than saline-treated females. This still held true at weaning (P21) when females treated with fluoxetine were heavier than controls, while in males there was no difference in weight. The results suggest there are no significant effects of prenatal fluoxetine treatment on communication and anxiety during development, as measured in the isolation-induced ultrasonic vocalization paradigm. This suggests prenatal fluoxetine exposure does not increase autism-related behaviors during early postnatal development.

Disclosures: J.M. Thanos: None. **E.M. Weber:** None. **N.J. McGovern:** None. **M.P. Leussis:** None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.11/U5

Topic: C.06. Developmental Disorders

Support: Canadian Foundation of Innovation (CFI)

Natural Sciences and Engineering Research Council of Canada (NSERC)

Ontario Graduate Scholarship Program (OGS)

Title: Prostaglandin E2 promotes earlier differentiation of neuroectodermal stem cells: A link to Autism

Authors: *C. T. WONG[1,2], N. USSYSHKIN[3], H. LI[1], D. A. CRAWFORD[1,2,3];
[1]Kinesiology and Hlth. Sci., [2]Neurosci. Grad. Diploma Program, [3]Dept. of Biol., York Univ., Toronto, ON, Canada

Abstract: Background: Prostaglandin E_2 (PGE_2) is a metabolite of membrane phospholipids and serves as a bioactive signalling molecule. It binds to four EP receptors (EP1-4) to regulate physiological functions in early development. Existing literature provides evidence that the prostaglandin pathway is an autism candidate signalling pathway[1]. The levels of PGE_2 can be altered through genetic changes along the pathway or exogenous factors such as dietary intake, oxidative stress, and exposure to infections or chemical agents, which have all been associated with Autism Spectrum Disorders (ASD) [1]. Our previous work in undifferentiated neuroectodermal (NE-4C) stem cells showed that PGE_2 interacts with Wnt signalling[2]. Addition of PGE_2 increased Wnt-dependent cell migration, speed and distance travelled. PGE_2 also modified the expression of Wnt-target genes including *Ctnnb1*, *Ptgs2*, *Ccnd1* and *Mmp9*. These genes have been previously linked to ASD. This study examines the effect of PGE_2 on NE-4C cell differentiation. **Objective**: To elucidate the mechanisms of how increases in PGE_2 may elicit functional changes in prenatal development of the nervous system and to study how PGE_2 may affect the differentiation behaviour and gene expression of NE-4C stem cells. **Methods**: Mouse NE-4C cells were differentiated into neurons through a continuous serum-free media protocol *in vitro*. Time-lapse microscopy was used to collect images over an 8 day differentiation period. Cell behaviour such as neurosphere (NSp) formation (area, perimeter and roundness) was quantified. Real time PCR was used to measure the expression of target genes. **Results**: We showed that NE-4C cells undergo distinct stages of differentiation: proliferation, inward migration, aggregation, and NSp formation. We found that 1μM PGE_2 treatment accelerates differentiation through earlier expression of neuronal marker (*Tau*) and formation of NSp. Moreover, during the stage of NSp formation, PGE_2 treatment resulted in changes to the expression level of adhesion molecules: higher levels of *Ncad* and lower levels of *Ncam*. Wnt-target gene expression was also modified by PGE_2: *Wnt8a*, *Wnt3*, *Tcf4*, and *Ccnd1* increased while *Wnt2* and *Mmp9* decreased. Interestingly, *Ncam*, *Tcf4*, *Ccnd1*, *Wnt2* and *Mmp9* have been linked to ASD. **Conclusions**: Our results show that elevated levels of PGE_2 alters NE-4C cell differentiation *in vitro* such as progression and gene expression. We also demonstrate that PGE_2 and Wnt signalling pathways likely collaborate with each other in early development. Our data suggests that prenatal increase of PGE_2 could affect developmental processes and result in pathologies of the nervous system that lead to ASD.

Disclosures: C.T. Wong: None. **N. Ussyshkin:** None. **H. Li:** None. **D.A. Crawford:** None.

Poster

799. Autism Environment and Pathology

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 799.12/U6

Topic: C.06. Developmental Disorders

Support: SafeMinds

Title: Effects of thimerosal on the expression levels of ghrelin and its receptor genes in immature neuronal cells

Authors: *E. M. SAJDEL-SULKOWSKA;
Psychiatry, Harvard Med. Sch/BWH, Boston, MA

Abstract: Ghrelin is an important orexigenic peptide synthesized by gastric mucosa and involved in gut-brain communication. A number of recent studies have reported ghrelin synthesis in specific neuronal populations in the brain; its biological effects in CNS include modulation of membrane excitability, neurotransmitter release, neuronal gene expression and neuronal survival and excitability. These actions are mediated by an interaction between ghrelin and a growth hormone secretagogue receptor (GHSR1). The present study was undertaken to: (1) confirm the expression of ghrelin and GHSR1 genes in the neuronal stem cells and primary brain culture of immature neurons; and (2) examine the effects of thimerosal (TM) on the expression of these genes. TM, an ethyl mercury containing preservatives, is used in a variety of biological products, including some vaccines, and reported as cytotoxic to neural tissue. We have shown previously that *in vivo* perinatal TM exposure in rats resulted in neurobehavioral abnormalities that were associated with changes in expression of TH-dependent genes in several regions of rat brain. In the present study we examined the effect of TM (17 nM) in neuronal stem cells prepared from Wistar rats, and wild type and p53 knockout mice. We also examined the chronic effect of TM on the expression of ghrelin/GHSR1 in the primary cerebellar cultures prepared from P1 rats. Gene expression was determined by qRT-PCR and the relative gene expression values were calculated using the $2(-\Delta\Delta Ct)$ method. We report that the addition of TM resulted in growth inhibition in rat (72.5%) and mice stem cells (57%), and a smaller inhibition (38%) in p53 knockout mouse cells, suggesting that the TM effect is mediated by the p53 protein. The addition of TM to primary cerebellar cultures, resulted in altered expression of several genes. Notably, expression of ghrelin was increased twofold and expression of GHSR1 gene was increased fourfold in TM-treated cultures. The results of the present study demonstrate that ghrelin and its receptor GHSR1 are expressed in immature cerebellar neurons. Furthermore, TM affects neuronal cell growth and the expression of ghrelin and GHSR1 genes at equivalent concentrations. These results further support developmental effects of TM. Future studies will establish effect of TM on ghrelin and GHSR1 expression in the gastric mucosa. Altered

expression of ghrelin/receptors in the gut as well as the brain may contribute to the disturbance of the brain-gut axis observed in various pathologies, including autism.

Disclosures: E.M. Sajdel-Sulkowska: None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.01/U7

Topic: C.08. Ischemia

Support: NIH Grant NS050730

Title: Brain-derived estrogen exerts anti-inflammatory and neuroprotective actions in the rat hippocampus

Authors: *Q.-G. ZHANG[1,2], R. WANG[3,2], H. TANG[3], Y. DONG[1], A. CHAN[1], D. BRANN[1,2]; [1]Georgia Regents Univ., Augusta, GA; [2]Charlie Norwood Dept. of Veteran Affairs Med. Ctr., Augusta, GA; [3]Neurobio. Inst. of Med. Res. Center,Hebei United Univ., Tangshan, China

Abstract: The ovarian steroid hormone 17β-estradiol (E2) functions as a critical mediator of neuroprotection in several neurodegenerative diseases. Besides acting as a neuroprotective agent, E2 plays a role in synaptic plasticity and cognition. Much of this work has focused on the role of gonadal-derived E2, but recent studies have demonstrated a potential neuroprotective role for E2 produced in the brain by the cytochrome p450 aromatase, which catalyzes the conversion of androgens to E2. Results revealed aromatase is highly expressed in hippocampus of the brain, particularly in the CA1 region, an area critical for learning and memory. Aromatase co-localized primarily in neurons in non-ischemic animals. However, following global cerebral ischemia (GCI), aromatase became highly expressed in GFAP-positive astrocytes in the hippocampal CA1 region at 2-3 days post GCI reperfusion. An ELISA for E2 and IHC for E2 confirmed the GCI-induced elevation of local E2 in the CA1 region and that the increase in local E2 occurred in astrocytes. A role for brain-derived E2 in neuroprotection in the hippocampus was implicated by the finding that central inhibition of aromatase by administration of an aromatase inhibitor (letrozole, 30 μg/10μl) resulted in a significant decrease of E2 levels in CA1 region, and increase in hippocampal neuronal cell death following GCI in ovariectomized rats. Furthermore, central administration of aromatase antisense (AS) oligonucleotides, but not missense (MS) oligonucleotides, blocked the increase in aromatase and local E2 in astrocytes after GCI, and

resulted in a significant increase in GCI-induced hippocampal CA1 region neuronal cell death and neuroinflammation. As a whole, these results suggest that brain-derived E2 exerts important neuroprotective and anti-inflammatory actions in the hippocampal CA1 region following GCI.

Disclosures: Q. Zhang: None. **R. Wang:** None. **H. Tang:** None. **Y. Dong:** None. **A. Chan:** None. **D. Brann:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.02/U8

Topic: C.08. Ischemia

Support: the Grants in Aid from the Ministry of Science, Education, Culture and Sports of Japan (Grant Number 23592253)

Title: Prolonged exposure to ketamine but not thiopental sodium might potentiate glutamate-induced neurotoxicity in rat primary cultured cortical neurons

Authors: *S. SHIBUTA, T. MORITA, T. KAMIBAYASHI, Y. FUJINO;
Osaka Univ. Grad Sch. Med., Osaka 565, Japan

Abstract: A number of the studies have shown that anesthetics administration during perinatal period exhibits a neurotoxicity afterwards. Especially, an NMDA receptor antagonist, ketamine-induced neurotoxicity in the developing brain is thought to associate with the upregulation of NMDA receptors. In the present study, we examined whether a long time exposure to the most widely-used intravenous anesthetics, ketamine and thiopental, during perinatal development periods would intensify glutamate-induced neurotoxicity which occurs later using rat primary cultured neurons *in vitro*. Primary cultures of cortical neurons from E16 Wistar rats were prepared. During the first 72 hr in the primary culture, the neurons were exposed to 1, 10 or 100 μM ketamine or thiopental, respectively. As a control, sister culture dishes were exposed to the same amount of PBS or distilled water without anesthetics. Cultured neurons were exposed to glutamate at the concentrations of 3, 30 or 300 μM 13-14 days later. The survival rate of neurons was analyzed after a 24-hr period of glutamate exposure. Survival rates were calculated using the following formula; the amount of unstained cells at the end of the experiment divided by the amount of whole cells shortly before the experiment. Survival ratios (SR) were calculated as follows; a survival rate of a certain dish divided by a survival rate of the control sister culture

dish. Therefore, SR of control neurons was defined as 1. Without exposure to glutamate, the SR of the neurons, which was exposed to 100μM ketamine in the first 72 hr of the culture, was not significantly attenuated (0.923). When neurons were exposed to 3μM glutamate, the SR of the neurons, which were exposed to ketamine 100μM in the first 72 hr of the primary culture, was 0.839. This SR was significantly lower than control (ket=0, glu=0), however, was not significantly lower than the SR of the neurons of ket=0, glu=3 (SR=0.9033). When neurons were exposed to 30 or 300μM glutamate, the SR of the neurons were significantly lower compared to control without regard for the exposure to ketamine in the first 72 hr of the primary culture. Moreover, the concentration of ketamine in the first 72 hr of the primary culture did not affect the SR of the neurons significantly. In contrast to ketamine, thiopental, exposed in the first 72 hr of primary culture, did not show any significant difference in the SR at any dose. In E16 rat primary cortical cultured neurons, prolonged exposure to high concentration of ketamine but not thiopental for the first 72 hr might alter the sensitivity against glutamate-induced neurotoxicity marginally two weeks later.

Disclosures: **S. Shibuta:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); receipt of a drug. **T. Morita:** None. **T. Kamibayashi:** None. **Y. Fujino:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.03/U9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Università Cattolica S. Cuore

Title: Estrogen administration modulates parvalbumin expression during trimethyltin-induced hippocampal neurodegeneration

Authors: *F. MICHETTI[1], V. CORVINO[2], E. MARCHESE[2], V. DI MARIA[2], F. BIAMONTE[3], M. GELOSO[2];
[2]Anat. and Cell Biol., [3]Histology and Embryology, [1]Univ. Cattolica S. Cuore, Roma I-00168, Italy

Abstract: Parvalbumin (PV) interneurons are known to give a crucial contribute to cognitive processes, being also responsible for a peculiar form of experience-related plasticity, involved in

learning and memory processes. They are modulated in several conditions of neural injury, and their dysfunction severely impairs brain functions, thus representing a potential target for neuroprotective strategies (1-3).The neurotoxicant trimethyltin (TMT) induces severe hippocampal neurodegeneration, selectively sparing PV interneurons, accompanied by behavioural alterations including cognitive deficit and seizures, and is regarded to be an useful model for the study of neurodegenerative processes and neuroprotective strategies (4-6). Since beta-estradiol (E2) is known to play a role in neuroprotection and to modulate PV expression in different models of brain damage (7,8), we explored the role of E2 on neuronal death and PV expression in the hippocampus of TMT-treated rats. Animals (n=6/group) received i.p. E2 (100 microg/rat) for 2 consecutive days starting from the day of TMT or saline administration, and were sacrificed on post-treatment day 7. Unbiased stereological analysis of Fluoro-Jade- or PV-stained hippocampal sections evidenced that E2 administration induces in TMT-treated rats a significant increase in the number of PV interneurons in CA1, which correlates with the degree of neuronal degeneration ($p<0.05$),while the extent of neuronal death in CA1 and CA3 did not appear to be significantly influenced. The present data add information to depict a possible role for *in vivo* E2 administration in the modulation of mechanisms of cell plasticity and neuroprotection in the adult brain. -1 Andrioli A,Alonso-Nanclares L, Arellano JI, DeFelipe J Neuroscience 2007,149:131-43 -2 Donato F,Rompani SB, Caroni P Nature 2013, 504:272-6 -3 Cabungcal JH, Steullet P, Kraftsik K, Cuenod M, Do KQ Biol Psychiatry 2013, 73:574-82 -4 Geloso MC,Corvino V, Michetti F Neurochem Int 2011, 58:729-38 -5 Corvino V,Marchese E,Giannetti S,Lattanzi W,Bonvissuto D,Biamonte F,Mongiovì AM,Michetti F,Geloso MC J Neurochem 2012, 122:415-26 -6 Corvino V,Marchese E,Podda MV,Lattanzi W,Giannetti S,Di Maria V,Cocco S,Grassi C,Michetti F,Geloso MC PloSOne 2014DOI:10.1371/journal.pone.0088294 -7 Rewal M, Wen Y, Wilson A, Simpkins JW, Jung ME Alcohol Clin Exp Res 2005,29:1837-44 -8 Macrì S,Biamonte F,Romano E,Marino R,Keller F,Laviola G Psyconeuroendocrinology 2010,35:1374-87

Disclosures: **F. Michetti:** None. **V. Corvino:** None. **E. Marchese:** None. **V. Di Maria:** None. **F. Biamonte:** None. **M. Geloso:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.04/U10

Topic: C.08. Ischemia

Support: Medical Reseach Council

Title: Characterisation of corticospinal tract termination patterns originating from the intact hemisphere following recovery from ischaemic stroke in the rat

Authors: *E. J. MITCHELL, D. DEWAR, D. J. MAXWELL;
Inst. of Neurosci. and Psychology, Glasgow, United Kingdom

Abstract: A limited degree of functional recovery commonly occurs in the first few weeks following stroke but the neural basis of this is poorly understood. Previous reports have implicated plasticity of the corticospinal tract (CST) in this process where motor recovery was correlated with crossing of intact (contralesional) CST fibres into the contralateral denervated spinal grey matter. The present study was conducted to determine if new CST axonal terminals arising from the non-ischaemic hemisphere form in the impaired half of the spinal cord replace synaptic connections lost after stroke. We also investigated whether new or enhanced connections are formed between the contralesional CST and specific spinal interneuron populations with known commissural projections. Sprague-Dawley rats underwent occlusion of the left middle cerebral artery (MCAo) or sham occlusion surgery. Behavioural testing was conducted prior to MCAo and postoperatively for 28 days to monitor functional deficit and recovery. CST terminals from the non-ischaemic hemisphere were visualised by injecting cholera toxin B (CTb) subunit into the right forelimb motor cortex at day 28 and quantified in the impaired (right) half of the cervical spinal cord. Termination patterns to spinal interneurons were investigated using immunofluorescence: antibodies against CTb, choline acetyltransferase (ChAT), calbindin and calretinin were used to identify terminals and spinal neuron populations. All MCAo rats exhibited a sensorimotor deficit that spontaneously recovered over 28 days. MCAo did not alter the number of labelled terminals in the impaired half of the spinal cord. ChAT, calbindin and calretinin expressing neurons were rarely contacted by labelled CST terminals in both sham and MCAo rats. The results suggest that terminal remodelling from the CST originating in the non-ischaemic hemisphere does not contribute to spontaneous recovery following MCAo.

Disclosures: E.J. Mitchell: None. **D. Dewar:** None. **D.J. Maxwell:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Topic: C.08. Ischemia

Support: TSGH-C98-11-S01-04

TSGH-C99-11-S01-04

TSGHC100-11-S01-3

TSGH-C102-076

the Intramural Research Program, National Institutes of Health (IRP-NIH)

Teh-Tzer study group for the Human Medical Research Foundation (A1001028-2)

Title: An open-label treatment with valproate for patients with acute middle cerebral artery infarction: evidence for improved functional recovery

Authors: *G.-S. PENG[1], J.-T. LEE[2], C.-H. CHOU[3], Y.-F. SUNG[3], C.-M. CHU[4], J.-S. HONG[5], D.-M. CHUANG[6];
[1]Neurol., Tri-Serv Gen Hosp, Taipei, Taiwan; [2]Neurol., [3]Tri-Service Gen. Hosp., Taipei, Taiwan; [4]Natl. Def. Med. Ctr., Taipei, Taiwan; [5]Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC; [6]NIH, Bethesda, MD

Abstract: Introduction: Increasing evidence from studies using animal stroke models suggests that valproate comprises multiple neuroprotective mechanisms against ischemic brain damage and functional deficits. This study tested whether valproate could improve functional recovery in patients suffered from acute middle cerebral artery (MCA) infarction. Methods: An open-label trial was performed by treating patients with valproate 3 to 24 hours after acute MCA infarction, and with confirmation by brain MR imaging. A total of 34 eligible patients were evenly distributed into the valproate and non-valproate groups. All patients were examined for neurological function, laboratory data and brain MR imaging at stroke onset, as well as 2 weeks and 3 months of follow-up. Results: There was no significant difference in demographics and baseline characteristics between the valproate and non-valproate groups. All patients were elderly having high score of pretreatment National Institutes of Health stroke scale (NIHSS) and slow stroke lesion growth with a final large infarct volume at the 2nd week of follow-up (71.5±12.3, years;17.7±8.2; 110.7±137.1 cm3 in the valproate group; 72.7±9.1, years; 17.4±8.6; 106.5±84.5 cm3 in the non-valproate group). The infarct volume and function outcome showed no difference when compared between the valproate and non-valproate groups at 3 months of follow-up. Notably, comparison of functional outcome between pre-treatment and post-treatment at 3 months of follow up showed that the valproate group had highly significant improvement in all the modalities, including NIHSS (17.0±2.3 vs. 9.6±2.3, p=0.004), modified Rankin scale (mRS;4.3±0.3 vs. 3.1±0.5, p=0.007), and Barthel index (BI;11.1±4.0 vs. 48.9±10.7,p=0.001), while the non-valproate group showed no significant improvement in NIHSS and mRS, and had only mild improvement in the BI scale (9.2±3.5 vs. 37.1±12.3,p=0.022). Conclusions: This open-

label trial demonstrated for the first time that patients with acute MCA infarction receiving valproate treatment had marked improvements in functional outcome at 3 months of follow-up compared to those at pre-treatment. This study suggests the potential use of valproate in the treatment of patients suffered from acute ischemic stroke.

Disclosures: **G. Peng:** None. **J. Lee:** None. **C. Chou:** None. **Y. Sung:** None. **C. Chu:** None. **J. Hong:** None. **D. Chuang:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.06/U12

Topic: C.08. Ischemia

Support: KAKENHI:24890181

Title: Bilateral cortical hyperactivity detected by functional MRI associates with improved motor function following intravenous administration of mesenchymal stem cells in a rat stroke model

Authors: ***S. OKA**[1], M. SASAKI[1], J. SUZUKI[2], J. D. KOCSIS[3], O. HONMOU[1];
[1]Neural Regenerative Med., [2]Radiology, Sapporo Med. Univ., Sapporo, Japan; [3]Yale Univ., New Haven, CT

Abstract: Intravenous administration of mesenchymal stem cells (MSCs) derived from bone marrow improved motor function in rat cerebral infarction models. In this study, MSCs were intravenously infused 6 hours after right middle cerebral artery occlusion (MCAO) induction in rat. Functional MRI (fMRI) during electrical stimulation of the left forepaw and behavioral testing (treadmill stress test) were performed at day 1, 4, 7, 14, 21, 28 and 42 following MCAO. In medium infused group (n=20) electrical stimulation of the left forepaw elicited a unilateral (right cortex) activated signal detected by fMRI in the infarcted somatosensory cortex. In the MSC infused animals two fMRI patterns were observed: unilateral (n=17) and bilateral (n=19) activation of sensorimotor cortex. In the MSC group both unilateral and bilateral cortical activated animals displayed significantly improved motor function compared to the medium infused group. The bilateral activated pattern in the MSC group, however, demonstrated the greatest functional recovery. Lesion volume as evaluated from high intensity signals using T2WI was less in the MSC groups as compared to the medium group, but the lesion volume for the

unilateral and bilateral signals in the MSC group was the same. These results suggest that the presence of a bilateral signal in sensorimotor cortex as detected by fMRI was more predictive of improved functional outcome than lesion volume alone.

Disclosures: S. Oka: None. **M. Sasaki:** None. **J. Suzuki:** None. **J.D. Kocsis:** None. **O. Honmou:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Topic: C.08. Ischemia

Support: Swiss National Science Foundation Grant 3100A0-149315

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Forschungskredit UZH Grant 54150602

Title: Nogo-A neutralization improves visual recovery after partial excitotoxic damage of the retina

Authors: *N. JORDI, V. PERNET, S. JOLY, M. E. SCHWAB;
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Abstract: Ischemic insults like those occurring in glaucoma or after artery or vein occlusions cause irreversible vision deficits. At the molecular level, ischemic injuries induce retinal neuronal cell death in a manner thought to depend on excessive N-methyl-D-aspartate (NMDA) receptor activation, a process called excitotoxicity. In the present study, we hypothesised that the inactivation of the neuronal plasticity inhibitor Nogo-A could improve visual recovery at the functional and anatomical level after NMDA-induced excitotoxicity of retinal ganglion cells (RGCs) in adult mice, a model mimicking ischemic damage. WT and Nogo-A KO mice were intraocularly injected with increasing doses of NMDA. The visual function was longitudinally assessed by measuring the spatial frequency threshold using the optokinetic response (OKR). The cell death of RGCs was examined three weeks after NMDA injection in WT and Nogo-A KO retina flat-mounts by immunohistochemistry for β3-tubulin, a specific marker for RGCs. After injection of 0.25mM NMDA into WT eyes, the OKR was rapidly abolished; recovery started at 2 wks to reach 80% at 3 wks.. In contrast, Nogo-A KO mice only showed a transient

loss of OKR at 1 day and a rapid recovery starting as early as two days post injection. At the end of the experiment (3 weeks), the recovery was complete (95%) in the Nogo-A KO mice. At higher concentration of NMDA (2.5mM) the OKR was absent and neither WT nor Nogo-A KO mice showed significant behavioural improvements over the course of the experiment. The density of surviving β3-tubulin-labelled RGCs did not differ between the two genotypes after administration of NMDA, suggesting that neuronal cell death was not affected by the ablation of Nogo-A and cannot explain the difference of OKR improvement in Nogo-A KO mice. However, neuronal cell death is likely to prevent OKR recovery at 2.5 mM NMDA for the two groups of animals: at 0.25 mM NMDA, 25% of RGCs died in the two genotypes while at 2.5mM NMDA, the RGC population was reduced by 80%. Using the OKR behaviour test, our data revealed that the effect of NMDA-induced excitotoxicity on the visual function can be strikingly improved by inactivating the neuronal plasticity repressor Nogo-A in adult mice, provided that cell death is limited. The cellular and molecular mechanisms underlying the visual recovery in Nogo-A KO mice are currently under investigation and could be relevant to treat a large spectrum of visual affections.

Disclosures: **N. Jordi:** None. **V. Pernet:** None. **S. Joly:** None. **M.E. Schwab:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Program#/Poster#: 800.08/U14

Topic: C.08. Ischemia

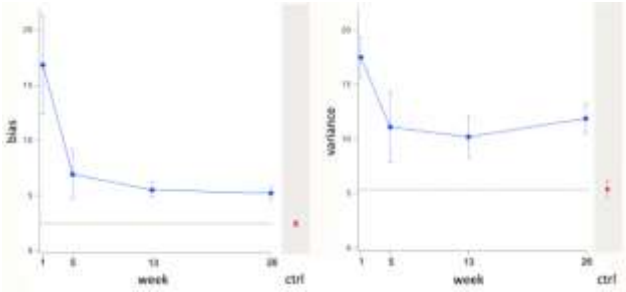
Support: James S. McDonnell Foundation Grant 220020220

Title: A limited time window for arm motor control recovery after stroke

Authors: J. C. CORTES[1], J. GOLDSMITH[2], M. D. HARRAN[1], J. XU[4], N. KIM[4], J. STEIN[3], A. LUFT[6], P. CELNIK[5], J. W. KRAKAUER[4], *T. KITAGO[1];
[1]Motor Performance Lab, Dept. of Neurol., [2]Mailman Sch. of Publ. Hlth., [3]Rehabil. and Regenerative Med., Columbia Univ., New York, NY; [4]Brain, Learning, Animation, and Movement Lab., [5]Human Brain Physiol. and Stimulation Lab., Johns Hopkins Univ., Baltimore, MD; [6]Neurosci. Ctr., UZH ETH Zurich, Zurich, Switzerland

Abstract: Animal studies have demonstrated that most recovery occurs within a limited time window after stroke that lasts about 4 weeks. Human studies, which have largely relied on

clinical measures of recovery like the Fugl-Meyer score, also show that most recovery occurs in the first 4 weeks after stroke. In this study, we sought to characterize the time window for recovery of arm motor control after stroke using kinematic measures. Our kinematic planar reaching task was designed to be an assay of basic motor control that minimizes both the effects of strength and the possibility of compensatory strategies. Using functional principal components analysis (FPCA), reaching deficits can be quantified in terms of increased trajectory bias (differences in mean trajectories) and variance (differences in variability around those means), compared to healthy age-matched controls. We studied patients with newly diagnosed ischemic stroke (n=12, mean age=58.3 yrs, 6M, 4 dominant side affected) with residual unilateral arm impairment. Patients were evaluated at four time points during the first six months after the stroke (within 2 weeks of stroke, at week 5, week 13, and week 26 post-stroke). Subjects performed visually-guided, center-out, gravity-supported planar reaching movements to eight radially-arrayed targets (8 cm distance) for each arm. Initial trajectories with both the affected and unaffected arms showed greater bias and variance compared to healthy controls. From the first time point to week 5, we found significant improvements in both bias and variance for the affected arm (p=0.00065 and 0.0345, respectively, Fig.1). For the unaffected arm, there was improvement in trajectory variance (p=0.003) but not in bias (p=0.0065). After week 5, we saw no further improvements in our kinematic measures for either arm. We conclude that there is a limited, approximately five-week time window for spontaneous recovery of proximal arm motor control.



Disclosures: **J.C. Cortes:** None. **T. Kitago:** None. **J. Goldsmith:** None. **M.D. Harran:** None. **J. Xu:** None. **N. Kim:** None. **A. Luft:** None. **P. Celnik:** None. **J.W. Krakauer:** None. **J. Stein:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.09/U15

Topic: C.08. Ischemia

Support: NINDS T32 NS069562

Title: The effects of targeted intracerebral saporin injection on recovery from stroke

Authors: *A. BECKER[1], M. GOLDBERG[2];
[1]UT Southwestern, Dept. of Neurol. and Neurotherapeutics, Dallas, TX; [2]Dept. of Neurol. and Neurotherapeutics, UT Southwestern, Dallas, TX

Abstract: It is well known that the diffuse neuromodulatory systems of the brain play a role in cortical plasticity that may extend to cortical reorganization after brain injury. The basal forebrain cholinergic system in particular is necessary for both cortical plasticity and behavioral recovery from cortical electrolytic lesions. The role of the cholinergic system in recovery from stroke has never been directly investigated. In this experiment, we asked the question: is the basal forebrain cholinergic system in the mouse necessary for behavioral recovery from stroke? To answer this question, we administered intracerebral injections of the selective immunotoxin mu p75-saporin bilaterally to the cholinergic nucleus basalis in young adult mice. Using choline acetyltransferase immunohisochemistry, cresyl violet staining, and fluoro-jade B staining we discovered a dose at which these injections eliminate local cholinergic neurons while leaving other cell types and cholinergic cells outside the nucleus basalis unharmed. We report the effects of these injections on behavioral recovery from a subsequently induced photothrombotic cortical ischemic stroke.

Disclosures: A. Becker: None. **M. Goldberg:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Topic: C.08. Ischemia

Support: NIH Grant 1U54NS083924-01

NIH Grant 8G12MD007583

Title: Neuroprotection of the cembranoid 4R compound against stroke

Authors: *A. H. MARTINS[1], P. A. CORDOLIANI[1], L. A. LLOPART[1], D. PEREZ[1], Y. LI[2], B. D. FORD[2], V. A. ETEROVIC[1], P. A. FERCHMIN[1];
[1]Univ. Central Del Caribe, Bayamon, Puerto Rico; [2]Morehouse Sch. of Med., Atlanta, GA

Abstract: Ischemic stroke is an important cause of death and disabilities. There are none neuroprotective agents available and just one drug named tPA approved by FDA, leaving the search for new drugs an important segment of research. Here we present a new compound named 4R-cembranoid, which has been shown neuroprotective characteristics in different *in vitro* and *in vivo* models of neuronal injury. In the present work, 6mg/Kg of 4R injected subcutaneously 2 hours after transient middle cerebral artery occlusion (MCAO) decreased the infarct volume in male rats, as measured by 2,3,5-triphenyltetrazolium chloride 24 hours after ischemia. The average of infarct area in vehicle treated rats was 31.67% ± 4.19 and 13.99% ± 9.2 ($n=5$, $p<0.01$) in 4R treated rats. On the other hand we analyzed the 4R-induced neuroprotection in hippocampus since significant memory impairment has been observed with delayed neuronal hippocampal death after MCAO. We analyzed the 4R-induced neuroprotection using the recovery of population spikes as measurement of functional neurons. Hippocampal slices submitted to 10 min of OGD were superfused with 10 μM of 4R immediately or 30 minutes after the initiation of deprivation. We observed a significant recovery of population spikes in both treatments; 27.024± 5.2% (OGD), 56.7 ± 7.2 (immediately after) and 65 ± 7.1 (30 minute after). On the other hand, a possible mechanism of action was evaluated. Although, in experiments using NMDA as a model of injury in hippocampal slices, the PI3K-Akt kinase were used as neuroprotective pathway, 4R-induced neuroprotection does not was abolished when 10 μM of LY294002, an PI3K-Akt, inhibitor was added, showing possible independence of PI3K in OGD model. In summary, experiments have shown that 4R decreased the neuronal death using different models of action.

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Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Topic: C.08. Ischemia

Support: National Institute on Aging RO1 AG031811 (JC)

National institute of neurological disorder and stroke (NINDS) R01NS083078-01A1 (JC)

National Institute on Aging RO1 AG 037506 (MC)

R41NS080329-01A1

Title: Neurorestorative therapy of stroke in type two diabetes rats treated with human umbilical Cord blood cells

Authors: *P. VENKAT[1,2], T. YAN[1,3], M. CHOPP[1,2], A. ZACHAREK[1], R. NING[1], Y. CUI[1], C. ROBERTS[1], N. KUZMIN-NICHOLS[4], C. SANBERG[4], J. CHEN[1];
[1]Neurol. Res., Henry Ford Hosp., Detroit, MI; [2]Oakland Univ., Rochester, MI; [3]Neurol. of Tianjin Med. Univ. Gen. Hosp., Tianjin Neurolog. Inst., Tianjin, China; [4]Saneron CCEL Therapeutics, Inc., Tampa, FL

Abstract: Background and Purpose: Diabetes mellitus (DM) is a high risk factor for ischemic stroke. Patients with DM stroke have worse outcomes, poor long term recovery, risk of recurrent strokes and extensive vascular damage. We investigated the neurorestorative effects and the underlying mechanisms of stroke treatment with human umbilical cord blood cells (HUCBCs) in Type two diabetes mellitus (T2DM) rats. Methods: Adult male T2DM rats were subjected to 2h of middle cerebral artery occlusion (MCAo). Three days after MCAo they were treated via tail-vein injection with: 1) phosphate-buffered-saline (PBS); or 2) HUCBCs (5x10^6); n=10/group. Results: HUCBC treatment of stroke initiated at 3 days after MCAo in T2DM rats significantly improved long term functional outcome ($p<0.05$), but did not significantly decrease brain hemorrhage ($p=0.22$), blood glucose level and body weight, marginally decreased lesion volume ($p=0.078$) and BBB leakage ($p=0.1$) in the ischemic brain when compared to the non-treatment T2DM-MCAo control group. HUCBC treatment significantly promoted white matter remodeling as indicated by increased expression of Bielschowsky silver (axons marker), Luxol fast blue (myelin marker), SMI-31 (neurofilament) and Synaptophysin in the ischemic border zone (IBZ). HUCBC treatment of stroke in T2DM rats significantly increased M2 macrophage polarization (increased M2 macrophage CD163, decreased M1 macrophage ED1 expression) in the ischemic brain compared to non-treatment T2DM-MCAo controls ($p<0.05$). HUCBC promoted vascular remodeling, and significantly increased arterial density and diameter (αSMA expression). HUCBC treatment significantly increased Angiopoietin-1 expression and decreased pro-inflammatory factors i.e., Angiogenin, matrix metalloproteinase 9 (MMP9), receptor for advanced glycation end-products (RAGE) and TLR-4. However, HUCBC treatment of T2DM rats also significantly increased arteriosclerosis-like changes, identified by increased internal carotid artery thickness and intima thickness (Trichrome staining analysis). Conclusion: HUCBC treatment initiated 3 days after stroke significantly increased WM and vascular remodeling in the ischemic brain as well as decreased neuro-inflammatory factor expression in the ischemic brain in T2DM rats and promoted M2 macrophage polarization. HUCBC reduction of neuroinflammation and increased vascular and WM-axonal remodeling may contribute to the

HUCBC induced neurorestorative effects in T2DM rats. However, HUCBC treatment in T2DM rats also induces potential adverse effects, e.g. atherosclerosis.

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Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Program#/Poster#: 800.12/U18

Topic: C.08. Ischemia

Support: AHA 10GRNT4570012

Title: Gender influences early gene expression after subarachnoid hemorrhage

Authors: F. A. SEHBA[1], D. KIM[2], *V. L. FRIEDRICH JR[2];
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Abstract: Early mortality after aneurysmal subarachnoid hemorrhage (SAH) is significant, with 45% deaths occurring within 48 hrs. More women than men suffer from SAH and their age at SAH on average is greater by 5 years than males, yet prognosis is similar. We previously hypothesized that gender affects prognosis, and have studied the influence of gender on acute physiology and early brain injury after experimental SAH. We found that increases in intracranial pressure and in systemic blood pressure at SAH, anatomical damage to small cerebral vessel, and neuronal apoptosis all are greater in males than in females. We are currently pursuing this effect at on levels of particular gene transcripts and of proteins. The genes studied include two groups. Genes in group-1 encode products essential for maintaining blood vessel integrity and function: thrombomodulin (TM), endothelial nitric oxide Synthase (eNOS), intracellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor (VEGF), and vasopressin (VSP). Genes in group-2 encode cytokines involved in cerebral inflammation;

interleukin-1beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α). SAH is induced in three month old male and female rats by endovascular puncture and animals are sacrificed at 3 hrs later. Gender and age matched sham surgery animals are controls. We have found significant gender differences in the expression of group-1 but not of group-2 genes. In group-1 expression of TM, eNOS, ICAM-1 and VSP increased in SAH males and only of VSP increased in SAH females; in group-2 expression of IL-1 β but not of TNF- α increased in males and females after SAH. Our results, though preliminary, suggest that gender influences expression after SAH of genes whose products are essential for maintaining blood vessel integrity.

Disclosures: F.A. Sehba: None. **D. Kim:** None. **V.L. Friedrich Jr:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Topic: C.08. Ischemia

Support: R25 NS070697 (NINDS)

R01 NS076628 (NINDS)

R01 NS063226 (NINDS)

UL1 RR024156 (NCATS)

NSF 0954796

Title: Assessment of cortical vascular responsivity to calcium channel blockers in the context of vasospasm after subarachnoid hemorrhage

Authors: *D. Y. CHUNG[1], M. A. SHAIK[2], S. H. KIM[2], M. G. KOZBERG[2], E. M. C. HILLMAN[2];
[1]Columbia Univ., New York, ; [2]Columbia Univ., New York, NY

Abstract: Delayed cerebral ischemia (DCI) and cerebral vasospasm after subarachnoid hemorrhage (SAH) is an important problem in survivors of aneurysm rupture. It was previously thought that DCI resulted exclusively from large vessel spasm, and the calcium channel blocker nimodipine was developed in order reverse this spasm. Nimodipine was found to be effective in

preventing DCI and remains the standard of care today. However, it has been noted that nimodipine does not need to reverse radiographic large vessel vasospasm in order to exert its effect. Conversely, other calcium channel blockers have been found to improve radiographic large vessel vasospasm, yet fail to improve outcomes in patients with DCI secondary to SAH. The mechanism of action of these drugs on the brain's microvasculature is still unknown. Here, we present work in an exposed-cortex in-vivo rodent model using simultaneous high-speed, high-resolution multispectral optical intrinsic signal imaging (MS-OISI) and laser speckle flowmetry. These methods allow us to assess the acute vascular response to administered vasoactive drugs in-vivo, including careful characterization of the relative dilation of large and small vessels, as well as hemoglobin oxygenation and flow rates in cortical vessels. 2-photon microscopy permits examination of diving arterioles and capillary dynamics. Mean arterial blood pressure and arterial blood oxygenation levels are simultaneously monitored over the course of 2-3 hours post-administration, along with successive assessment of the brain's hemodynamic response to somatosensory stimulation. Our initial results suggest differential action of the two different calcium channel blocker drugs tested of the different vascular compartments of the cortex, providing a basis for clinical improvement in the nimodipine case. Our latest results will be presented.

Disclosures: D.Y. Chung: None. **M.A. Shaik:** None. **S.H. Kim:** None. **M.G. Kozberg:** None. **E.M.C. Hillman:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

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Support: NIH Grant P20GM103554

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Title: PTEN-induced kinase 1 (PINK1) and protein kinase A (PKA) form a new neuroprotective signaling axis

Authors: *R. K. DAGDA[1], T. DAS BANERJEE[1], R. Y. DAGDA[1], G. L. CRAVISO[2], A. PHAM[2];
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Abstract: Background and Objective: PINK1, a kinase associated with familial Parkinson's disease (PD), and PKA are two cytoprotective kinases that are localized to cytosolic and mitochondrial compartments to regulate critical physiological functions in neurons. In developing neurons, PINK1 amplifies downstream PKA signaling to enhance dendritic complexity, mitochondrial dynamics and survival. We hypothesize that PINK1 activates distinct subcellular pools of PKA to confer neuroprotection against chemical models of PD. Methods/Results: Morphometric analyses of neurites (axons and dendrites) suggests that conditions of pharmacological activation of PKA by cyclic AMP (cAMP) and forskolin, transient overexpression of wild-type PINK1, untargeted PKA, mitochondrially-targeted catalytic subunit of PKA or the mitochondrial targeted protein scaffold of PKA (D-AKAP1) but not a PKA binding deficient mutant of D-AKAP1 significantly prevent the loss of neurites in primary neurons induced by the Parkinsonian toxin 6-hydroxydopamine. Treating primary neurons with cAMP, transient overexpression of untargeted or mitochondrial PKA in SH-SY5Y cells increases the basal oxygen consumption rates and partially protects cells against toxin-induced decreases in mitochondrial respiration as measured by a XFE24 Analyzer. Conversely, transient expression of PD-associated mutations in PINK1 show impaired neurite outgrowth in primary neurons and in bovine primary chromaffin cells. PD-associated mutations in PINK1 show impaired PKA signaling in primary neurons. Mechanistically, PINK1 amplifies downstream PKA signaling by elevating the catalytic subunits of PKA, increasing the levels of cytoskeletal associated PKA, and enhancing transcriptional activation of PKA-regulated promoters as demonstrated by Western blot analyses and luciferase reporter assays of transfected cells. Discussion and Conclusions: Overall, PINK1 and PKA form a signaling axis that confers protection of neurites against chemical models of PD, elevates mitochondrial function, and promotes neurite outgrowth by relocating and activating PKA holoenzymes. Therefore, enhancing PKA signaling may be a therapeutic strategy for treating PD.

Disclosures: R.K. Dagda: None. **T. Das Banerjee:** None. **R.Y. Dagda:** None. **G.L. Craviso:** None. **A. Pham:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.15/U21

Topic: C.08. Ischemia

Support: NIH grant R01AG033720

Rose Mary Kubik Donation

Title: Isoform-specific NOS inhibition preserves axon function against ischemia in an age-dependent manner by enhancing free radical scavenging and preserving mitochondrial structure and function

Authors: *C. BASTIAN, J. ZALESKI, A. RUNKLE, S. BRUNET, S. BALTAN;
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Abstract: White matter (WM), which comprises half of the human brain, is frequently damaged during stroke. In young WM, injury mechanisms follow a temporal pattern starting with loss of ionic homeostasis followed by excitotoxicity and oxidative stress. The incidence of stroke increases with aging. The susceptibility of aging WM to ischemia increases because of enhanced excitotoxicity, oxidative stress and mitochondrial dysfunction. The role of oxidative stress mechanisms in WM ischemic injury remains unexplored. We hypothesized that nitric oxide synthase (NOS)-mediated oxidative stress contributes to ischemic injury by generating free radicals, which impair mitochondrial structure and function in an age-dependent manner. We confirmed that mouse optic nerves (MON) express all three isoforms of NOS (nNOS (neuronal), eNOS (endothelial) and iNOS (inducible)) in an age- and cell-specific manner. We recorded evoked compound action potentials (CAPs) from MONs treated with L-NAME (L-NG-nitroarginine methyl ester, pan-NOS blocker) before or after oxygen glucose deprivation (OGD) and observed improved axon recovery in young MONs. L-NAME promoted axon function only when applied before OGD in aging MONs. SMT (S-methyl-isothiourea, iNOS blocker) and 3-Br7NI (3-bromo-7-nitroindazole, nNOS blocker) before OGD protected aging MONs at lower doses of drugs compared to young MONs. In addition, 3-Br7NI administered after OGD protected only aging MONs. SMT administered after OGD failed to protect either age group. Unlike other isoform-specific inhibitors, L-NIO (N5-(1-iminoethyl)-L-ornithine, eNOS inhibitor) applied before or after OGD improved axon function recovery in both young and aging MONs. L-NAME treatment before OGD preserved levels of reduced glutathione, a free radical scavenger. Moreover, fluorescent imaging and 3-dimensional electron microscopy studies of axonal mitochondria revealed that L-NAME treatment preserved mitochondrial structure and preserved ATP levels against OGD. Our studies demonstrate that NOS inhibition promotes axon recovery by increasing free radical scavenging and preserving mitochondrial structure and function. Age- and isoform-specific NOS inhibition is therefore a potential therapeutic target for stroke victims.

Disclosures: C. Bastian: None. **J. Zaleski:** None. **A. Runkle:** None. **S. Brunet:** None. **S. Baltan:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.16/U22

Topic: C.08. Ischemia

Title: mitoNEET agonist NL-1 promotes cell proliferation in hippocampal dentate gyrus

Authors: *A. MDZINARISHVILI, R. T. CARROLL, W. J. GELDENHUYS;
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Abstract: The discovery of neurogenesis in the adult brain and the regenerative potential of neural stem cells hold the promise for restoration and regeneration of neural population and regeneration of neural circuits necessary for cerebral functions. Pharmacological compounds that enhance hippocampal neurogenesis could provide new therapeutic approaches for many neurodegenerative diseases (Alzheimer's, Parkinson's, MS, stroke and etc.). mitoNEET is a novel mitochondrial protein which belongs to a zinc-finger group of proteins that is conserved throughout evolution. mitoNEET is an iron-sulfur containing protein located on the outer mitochondrial membrane of mitochondria which regulates mitochondrial bioenergetics. Recently, the anti-diabetic drug pioglitazone (a thiazolidinedione) was found to be a ligand for mitoNEET, and has been also shown *in vivo* to be neuroprotective against ischemia-reperfusion injury after brain stroke. In this study, our goal was to evaluate a mitoNEET ligand NL-1, a derivative of pioglitazone, with its pro-neurogenic activities in the adult mice hippocampal dentate gyrus (DG). In our prior studies we have shown that NL-1 has neuroprotective effects in MCAO murine stroke models. In this study, CD-1 mice (25-27g) were treated with NL-1 (10 mg/kg) or vehicle control; 30-40 min later 5-bromo-2-deoxyuridine (BrdU; 300 mg/kg, i.p.) a marker of cell division was injected. Mice were sacrificed after 5 h of BrdU injection. Immunohistochemical assay was used to determine the number of BrdU-labeled progenitor cells in the hippocampal DG. After a single injection of NL-1 the number of BrdU-labeled neural progenitor cells had increased significantly by 39% compare to vehicle control. These results clearly demonstrated that mitoNEET ligand NL-1 increases and promotes proliferation of neural progenitor cells in hippocampal DG. We did not find any significant difference in cell proliferation process in subventricular zone (SVZ) the other neurogenic zone, after drug administration. These data suggest that a positive neurogenic effect of NL-1 in hippocampal DG is probably implemented through the mitoNEET. Positive effects of NL-1 on neural progenitor cells proliferation in hippocampal DG also suggests that mitoNEET ligand NL-1 is a new

candidate for developing of novel neuroprotective and neurorestorative therapies in neurodegenerative diseases.

Disclosures: A. Mdzinarishvili: None. **R.T. Carroll:** None. **W.J. Geldenhuys:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.17/U23

Topic: C.08. Ischemia

Support: BIOL Research Fund from SHU

Title: Investigation of desferrioxamine-induced adaptive responses in human neuronal cells

Authors: J. CANDELORA, F, L. RASMUSSEN, A. HERRERA, *J. L. KO;
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Abstract: Cancer, diseases, surgery, or traumatic injury can trigger hypoxia condition, which can further provoke pain sensation mediated by the mu-opioid receptors (MOR). Effect of hypoxia on MOR expression was therefore examined using human neuronal cells treated with desferrioxamine (DFO) to create a hypoxic-mimic condition. The transcription regulator PCBP-1 is known to modulate the MOR gene expression, and its interacting protein RACK-1, identified by two hybrid system recently, can negatively regulate MOR gene expression. RT-PCR analysis revealed a decrease of MOR expression under DFO treatment, whereas the RACK-1 expression was increased. Results from DFO-induced hypoxia supported the notion that RACK-1 participates in the regulation of MOR expression. Neuronal cells surviving under DFO challenge also displayed an activation of JAK/STAT pathway via Western blot analysis. An increase of the expression of suppressors of cytokine signaling proteins, negative regulators of the JAK/STAT pathway, was also detected using RT-PCR, suggesting that an up-regulation of suppressors of cytokine signaling proteins under hypoxia may play a role in a form of neuroprotection by aiding in the decrease of inflammation associated with JAK/STAT pathway.

Disclosures: J. Candelora: None. **J.L. Ko:** None. **L. Rasmussen:** None. **A. Herrera:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

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Program#/Poster#: 800.18/U24

Topic: C.08. Ischemia

Support: Canadian Institute of Health Research

Manitoba Health Research Council

Manitoba Institute of Child Health

Canadian Stroke Network

Title: BNIP3 interacting with LC3 triggers excessive mitophagy

Authors: *R. SHI[1], S. ZHU[2], V. LI[4], S. B. GIBSON[3], X. XU[5], J. KONG[1];
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Abstract: A basal level of mitophagy is essential in mitochondrial quality control in physiological conditions while excessive mitophagy contributes to cell death in a number of diseases including ischemic stroke. Signals regulating this process remain unknown. BNIP3, a pro-apoptotic BH3-only protein, has been implicated as a regulator of mitophagy. Here, using *in vivo* and *in vitro* models of stroke, we show that BNIP3 and its homologue BNIP3L (NIX) are highly expressed in a 'delayed' manner and contribute to delayed neuronal loss following stroke. Deficiency in BNIP3 significantly decreases both neuronal mitophagy and apoptosis but increases non-selective autophagy following ischemic/hypoxic insults. The mitochondria-localized BNIP3 interacts with the autophagosome-localized LC3, suggesting that BNIP3, similar to NIX, functions as a LC3-binding receptor on mitochondria. Although NIX expression is upregulated when BNIP3 is silenced, upregulation of NIX cannot functionally compensate for the loss of BNIP3 in activating excessive mitophagy. Therefore, NIX primarily regulates basal level of mitophagy in physiological conditions, whereas BNIP3 exclusively activates excessive mitophagy leading to cell death.

Disclosures: R. Shi: None. **S. Zhu:** None. **V. Li:** None. **S.B. Gibson:** None. **X. Xu:** None. **J. Kong:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.19/U25

Topic: C.08. Ischemia

Support: CIHR

Heart and Stroke

University of Ottawa

Title: The impact of metabolic syndrome on cortical microvasculature - form and function

Authors: *M. GOMEZ-SMITH[1,3], C. NGUEMENI[2,3], M. JEFFERS[2,3], A. DORR[4], B. STEFANOVIC[4,5,3], D. CORBETT[3,2,7,6,1];
[1]Neurosci., [2]Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; [3]Canadian Partnership for Stroke Recovery, Ottawa, ON, Canada; [4]Sunnybrook Res. Inst., Toronto, ON, Canada; [5]Med. Biophysics, [6]Med., Univ. of Toronto, Toronto, ON, Canada; [7]Med., Mem. Univ., St. John's, NL, Canada

Abstract: Metabolic syndrome (MetS) is a leading risk factor for ischemic stroke. Given that nearly 25% of the world population has MetS, the number of stroke patients needing treatment will rise considerably in the coming years. As it stands, the only post-stroke therapy available to patients is rehabilitation. Animal models are extensively used to optimize human rehabilitation by developing therapies aimed at enhancing neuroplastic mechanisms. Nonetheless, the majority of animals used in stroke rehabilitation studies are young and healthy - they lack the co-morbidities of stroke patients. To better approximate the clinical condition, our lab is using a model of MetS, the Cafeteria (CAF) diet. Before initiating rehabilitation studies with this model, we will confirm that this diet exerts effects on the cerebral vasculature at the structural and, even more importantly, at the functional level. CAF rats were provided with free access to standard rodent pellets and water as well as a varied daily supply of highly processed human junk food items (i.e. chips, cookies, bacon, etc.) and a 12% sucrose solution. Several histological analyses were performed to determine whether intact CAF animals have an impaired blood brain barrier or exhibit structural vascular abnormalities. In others, strokes were surgically induced using endothelin-1 injected into forelimb motor cortex. Infarct size was evaluated in both CAF and standard diet (SD) animals. After 3 months of treatment, CAF animals exhibit 4/5 criteria for MetS. CAF animals are overweight (CAF=767±16g, SD=676±13g), have increased circulating triglycerides (CAF=183±21mg/dl, SD=95±9mg/dl), reduced HDL cholesterol (CAF=70±4mg/dl,

SD=94±3mg/dl) and impaired glucose tolerance (insulin: CAF=7.1±1ng/ml, SD=3.3±0.5ng/ml; blood glucose two hours after i.p. GTT test: CAF=8.5±0.7mmol/L, SD=6.7±0.3mmol/L). Preliminary histological analysis indicates that CAF animals trend towards larger infarcts and increased blood brain barrier leakage. There is also some indication that CAF animals have increased erythrocyte aggregation. We have succeeded in generating a rat model of MetS that closely approximates the clinical condition. Preliminary results indicate that the vasculature of CAF animals may be compromised. Further analysis is being performed to determine whether or not structural damage to the vessels is accompanied by functional deficits, as assessed via *in vivo* two-photon fluorescence microscopy. Future directions involve testing our animals on cognitive tasks to determine whether they exhibit signs of emerging vascular cognitive impairment.

Disclosures: M. Gomez-Smith: None. **C. Nguemeni:** None. **M. Jeffers:** None. **A. Dorr:** None. **B. Stefanovic:** None. **D. Corbett:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.20/U26

Topic: C.08. Ischemia

Support: Academy of Finland

Protea ERA-NET Neuron Program

Title: Degradation of collagen XV by tissue plasminogen activator may be protective after stroke

Authors: H. DHUNGANA[1], M. HUUSKONEN[1], T. PIHLAJANIEMI[2], R. HELJASVAARA[2], J. PARCQ[3], Y. POMESHCHIK[1], P. KORHONEN[1], T. MALM[1], K. KANNINEN[1], V. KEKSA-GOLDSTEINE[1], M. PRUVOST[3], D. VIVIEN[3], *J. KOISTINAHO[1], S. LEMARCHANT[1]; [1]Univ. of Eastern Finland, Kuopio, Finland; [2]Univ. of Oulu, Oulu, Finland; [3]Univ. of Caen Basse-Normandie, Caen, France

Abstract: Recombinant tissue plasminogen activator (rtPA) is the only acute treatment for ischemic stroke in patients eligible for thrombolysis. The ability of rtPA to dissolve the stable fibrin meshwork of blood clot formed at the site of the injury depends on its ability to convert the fibrin-bound plasminogen to active plasmin. Chondroitin sulfate proteoglycans (CSPGs) are

another substrate of tPA. In this study, we aim to study weather collagen XV, a type of CSPGs, could represent a putative substrate for rtPA, thereby having impact on the outcome of stroke. In order to determine the effect of tPA on collagen XV *ex vivo*, protein extract isolated from perfused cortex of C57BL/6J mice was incubated at 37°C for 2 h with or without increasing doses of rtPA. The degradation of collagen XV was then monitored by Western blotting. We further carried out *in vivo* thromboembolic ischemic study on 8-month-old female wild-type (WT) and collagen XV knock-out (KO) mice where the representative groups received intravenous infusion of either rtPA (10 mg/kg) or saline for 40 minutes. Infarct volumes were determined with MRI 48 h after the surgery. Protein levels of vascular endothelial growth factor A (VEGF-A) were determined by western blot. *Ex vivo* analyses demonstrated a significant ($P < 0.05$) dose-dependent degradation of collagen XV in the presence of rtPA when compared to controls without rtPA. Following stroke, Collagen XV KO mice exhibited significantly smaller lesion ($P = 0.0179$) compared to WT mice. Even though, early thrombolysis was beneficial in rtPA treated WT mice compared to saline treated WT counterparts ($P = 0.0161$), no added beneficial effect was observed in rtPA treated collagen KO mice compared to saline treated counterparts. In addition, we also observed 2-fold increase in VEGF-A in ipsilateral hemisphere of rtPA treated ischemic WT mice compared to vehicle treated ischemic and sham counterparts. Similar increase of VEGF-A was also evident in both rtPA and vehicle treated ischemic collagen KO mice compared to sham KO mice. This study indicates that collagen XV is a novel substrate for rtPA and that the lack of collagen XV is protective after stroke. The neuroprotection observed in collagen XV KO animals might be attributed to the increased VEGF-A production following stroke in the ischemic territory. The findings also suggest a possible beneficial effect of rtPA-induced collagen XV degradation after stroke. Further studies will be carried out to determine the mechanism involved in neuroprotection following collagen XV degradation using several molecular approaches.

Disclosures: **H. Dhungana:** None. **J. Koistinaho:** None. **M. Huuskonen:** None. **T. Pihlajaniemi:** None. **R. Heljasvaara:** None. **J. Parcq:** None. **Y. Pomeshchik:** None. **P. Korhonen:** None. **T. Malm:** None. **K. Kanninen:** None. **V. Keksa-Goldsteine:** None. **M. Pruvost:** None. **D. Vivien:** None. **S. Lemarchant:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.21/U27

Topic: C.08. Ischemia

Title: Characterization of the glycoprotein dickkopf-3 in cerebral blood vessels of spontaneously hypertensive rats

Authors: *C. L. BUSCETI[1], S. MARCHITTI[1], M. COTUGNO[1], P. DI PIETRO[1], F. BIANCHI[1], B. RIOZZI[1], S. SCARPINO[2], R. STANZIONE[1], M. MADONNA[1], S. DI CASTRO[1], G. BATTAGLIA[1], V. BRUNO[1,3,5], S. RUBATTU[1,4], M. VOLPE[4,1], F. NICOLETTI[1,3,5];
[1]Neurosci., I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]Hystopathology and Pathological Anat., [3]Physiol. and Pharmacol., [4]Cardiol., Univ. Sapienza, Roma, Italy; [5]I.R.C.C.S. Neuromed, Sapienza University, Italy, and Univ. of Lille, France, LIA (International Associalted Laboratories), Roma, Italy

Abstract: Dickkopf-3 (Dkk-3) is a member of the dickkopf protein family acting as a pro-apoptotic factor on cancer cells and as a differentiation factor in remodelling the tumor vasculature in endothelial cells. Remarkably, dkk-3 gene maps within the chromosome 1 region linked to stroke phenotype in the model of stroke-prone spontaneously hypertensive rat (SHRsp), which develops stroke after high-salt/low potassium diet (Japanese diet, JD). This raises the intriguing possibility that Dkk-3 gene may be considered as a candidate gene for stroke susceptibility in this strain. Our preliminary data showed that: (i) Dkk-3 is upregulated in cerebral blood vessels of SHR stroke resistant (SHRsr) but not in SHRsp rats after one month of JD, (ii) the Dkk-3 upregulation in cerebral blood vessels of SHRsr rats is associated to a vascular endothelial growth factor (VEGF) upregulation, and (iii) VEGF is upregulated in human endothelial cells cultures following incubation with human recombinant Dkk-3 peptide (hrDkk-3). Here, we performed double fluorescence immunoistochemical analysis to described the cellular localization of Dkk-3 in cerebral blood vessels of striatal, hippocampal and cortical brain regions of SHRsr rats. Our results showed that Dkk-3 expression is localised at endothelial level, as suggested by the co-localization with the endothelial marker reca-1. In order to establish a causal relationship between Dkk-3 blood vessel expression and occurrence of stroke exists, we used SHRsr rats on JD subjected to cerebral lentiviral dkk-3 silencing which were monitored for motor disability by the rotarod test. Results showed that Dkk-3 silencing in cerebral blood vessels is associated with a greater motor disability in SHRsr rats maintained for 7 weeks on JD. Taken together these data suggest that in response to hypertension the glycoprotein Dkk-3 is upregulated in endothelial cells of cerebral blood vessels. This glycoprotein may acts by enhancing the VEGF expression which could play a protective role against the hypertension mediated endothelial damage. Lack of vascular expression of Dkk-3 in spontaneous hypertensive stroke-prone rats may represent a novel mechanism favoring stroke occurrence.

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Madonna: None. **S. Di Castro:** None. **G. Battaglia:** None. **V. Bruno:** None. **S. Rubattu:** None. **M. Volpe:** None. **F. Nicoletti:** None.

Poster

800. Ischemia: Cellular Mechanisms and Neuroprotection V

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 800.22/U28

Topic: C.08. Ischemia

Title: Ethyl pyruvate inhibits HMGB1 phosphorylation and release by chelating calcium

Authors: *J.-K. LEE, H.-K. LEE, I.-D. KIM, L. LUO, H.-B. LEE, S.-W. KIM;
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Abstract: Ethyl pyruvate (EP), a simple aliphatic ester of pyruvic acid, has been shown to have anti-inflammatory effects and to confer protective effects in various pathological conditions. Recently, a number of studies have reported EP inhibits highmobility group box 1 (HMGB1) secretion and suggested this might contribute to its anti-inflammatory effect. Since, EP is used in a calcium-containing balanced salt solution (Ringer's solution), we wondered if EP directly chelates $Ca^{2+}$and it is related with the EP- mediated suppression of HMGB1 release. Calcium imaging assays revealed that EP significantly and dose-dependently suppressed high K^{+}-induced transient $[Ca^{2+}]_i$surges in primary cortical neurons and similarly,fluorometric assays showed that EP directly scavenges Ca^{2+}, as the peak of fluorescence emission intensities of Mag-Fura-2 (a low-affinity Ca^{2+}indicator) was shifted in the presence of EP at concentrations of $\geq$13 mM. Furthermore, EP markedly suppressed the A23187 (a calcium ionophore)-induced intracellular Ca^{2+}surge in BV2 cells (a microglia cell line) and under this condition, A23187-induced activations of Ca^{2+}-mediated kinases (protein kinase C alpha and calcium/calmodulin-dependent protein kinase IV), HMGB1 phosphorylation, and subsequent secretion of HMGB1 were also suppressed. Moreover, the above-mentioned EP-mediated effects were independent of cell death or survival, which suggests that they are not indirect outcomes of the protective effect of EP, but rather direct effects. Together these results indicate that EP directly chelates Ca^{2+}, and that it is, at least in part, responsible for the suppression of HMGB1 release by EP.

Disclosures: J. Lee: None. **H. Lee:** None. **I. Kim:** None. **L. Luo:** None. **H. Lee:** None. **S. Kim:** None.

Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 801.01/U29

Topic: C.08. Ischemia

Title: Human cerebral microvascular endothelium: Linoleoyl ethanoliamide, a novel natural lipid substance, is a potential vasodilator

Authors: P. CASTRI[1], T. KINO[2], Y. CHEN[2], R. ABUTARBOUSH[2], F. LENZ[3], E. SHOHAMI[4], R. MECHOULAM[4], R. MCCARRON[2], *M. SPATZ[1];
[1]Stroke Br., NIH-NINDS, BETHESDA, MD; [2]Neuro Trauma Dept., Naval Med. Res. Ctr., Silver Spring, MD; [3]Johns Hopkins Univ., Baltimore, MD; [4]Dept. of Surgery, Hebrew Univ., Jerusalem, Israel

Abstract: INTRODUCTION Linoleoyl Ethanoliamide (LEA) is one of the endogenous lipids structurally related to endocannabinoids, specifically to arachidonoyl ethanoliamide (also known as anandamide). This agent was found in plant and animal tissues as well as in human plasma. Reports indicate that it is a biologically active substance which has anti-inflammatory properties. This study examines whether LEA is able to induce cellular activities involved in vasorelaxation. It will evaluate if LEA reorganizes cytoskeleton in control and endothelin-1 (ET-1) stimulated endothelial cells derived from human brain microvessels (HBEC). It will also assess LEA induction of phosphorylation of various kinases (MAPK, Akt, JNK, c-Jun, and VASP) and the possible mediation by CB1, CB2 and TRPV1 receptors. METHODS Cultured HBEC which were >95% Factor VIII+ were exposed to LEA for 15 min alone or pre-treated with selective antagonists for CB-1, CB-2 and TRPV1 receptors (SR141716A, SR141728A and capsazepine, respectively). The levels of phosphorylation of p44/42 MAPK, Akt, JNK, c-Jun and VASP were determined by Western blot analysis. RESULTS 1.LEA activates CB1 and CB2 receptors in HBEC. 2.LEA induces reorganization of cytoplasmic actin fibers in control and ET-1 treated HBEC. 3.LEA stimulated stress-linked kinases (MAPK, Akt, JNK, c-Jun, and VASP) up to 3 fold. 4.All the above mentioned HBEC responses to LEA were mediated by CB1, CB2, TRPV1 receptors and involved the Rho/PI3/Akt pathway. 5.LEA inhibited Rho kinase activity (40%). CONCLUSION The results indicate that LEA is able to induce the reorganization of cellular-actin fibers and the phosphorylation of stress-linked kinases. All these HBEC responses were mediated by cannabinoid (CB1, CB2) and vanilloid (TRPV1) receptors, since they were inhibited by their respective antagonists. The reactions all involved the Rho/PI3/Akt signal

transduction pathway. The findings indicate that LEA may act as a Rho kinase inhibitor and strongly suggest that LEA may act as a relaxing agent *in vivo*.

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Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.08. Ischemia

Support: NIH/NINDS R21NS064185

NIH/NINDS R01NS082225

UNM Behavioral Health BBI/CoBRE Research Award

Title: *In vivo* mir-155 inhibition supports recovery after mouse stroke

Authors: *E. CABALLERO-GARRIDO[1], J. PENA-PHILIPPIDES[1], Y. YANG[2], D. BRAGIN[1], T. ROITBAK[1];
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Abstract: Recently identified small molecules microRNAs (miRNAs) regulate the expression of genes and proteins in many tissues, including brain and vasculature. Based on our experiments involving inhibition and overexpression of specific miRNAs, in combination with *in vitro* morphogenesis assay and cell signaling pathway analysis, the miRNA miR-155 was identified as the potential primary regulator of endothelial morphogenesis. We found that the specific inhibition of miR-155 subsequently altered the expression of major components of the mTOR and TGF-β signaling pathways in the endothelial cells (ECs). Overexpression of this miRNA in ECs suppressed, while inhibition activated, the *in vitro* endothelial morphogenesis. Our animal studies suggest that miR-155 could also mediate vascular remodeling after stroke: *in vivo* injections of specific miR-155 inhibitor after distal/direct middle cerebral artery occlusion (dMCAO) in mice, supported post-ischemic revascularization. Using two-photon laser scanning microscopy technique, we detected increased vascular density and blood flow velocity in the peri-infarct area of the injured mouse brain. Similar to miR-155 inhibition in the ECs, the *in vivo*

injections of anti-miR-155 resulted in the activation of mTOR and TGF- β signaling pathways in the mouse brain tissue, detected by the increase in Smad-2 protein expression and phosphorylation, as well as mTOR phosphorylation at Ser-2448. In addition, intravenous anti-miR-155 injections resulted in a significant increase of anti-inflammatory (Interleukin 1 receptor antagonist Il1rn) and decrease of pro-inflammatory (macrophage colony stimulating factor Csf1) cytokine gene expression. Our preliminary studies including MRI measurements and behavioral assessment ("adhesive removal" and "cat walk" tests) indicate that these effects of miR-155 inhibition also correlated with decreased infarct size and improvement of the animal functional recovery after stroke. Based on our findings and preliminary results, we hypothesize that miR-155 inhibition following ischemia supports cerebral vasculature, as well as improves the animal post-stroke recovery process. In addition, we propose that *in vivo* inhibition of specific miRNAs could be utilized to manipulate the revascularization process following stroke in order to improve post-ischemic recovery in the brain.

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Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Topic: C.08. Ischemia

Support: NIH Grant 4R00NR013593-03

Title: Scanning and transmission electron microscopy of the glial scar after stroke

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Abstract: "It is the pervading law of all things organic and inorganic, of all things physical and metaphysical, of all things human and all things superhuman, of all true manifestations of the head, of the heart, of the soul, that the life is recognizable in its expression, that form ever follows function. This is the law." The American Architect Louis Sullivan coined the phrase "form ever follows function" to encapsulate the principle that in architecture the shape of an object should reflect it's intended function or purpose. In nature form also "ever follows

function", and so to extend our knowledge about the function of the astroglial scar that forms in the brain after stroke we used electron microscopy to reveal its ultrastructure. BALB/cJ mice underwent distal middle cerebral artery occlusion and the structure of the glial scar was evaluated one and four weeks later. We discovered that hypertrophied astocytic processes form a dense mesh that is effective at keeping large phagocytic immune cells away from adjacent healthy tissue, but is porous to smaller immune cells that by electron microscopy strongly resemble lymphocytes. To uncover the identity of these cells we used immunohistochemistry, and discovered that for weeks after stroke CD3+ T lymphocytes leak out of the lesion and home to areas of degenerating white matter. Furthermore, immunohistochemistry revealed that immunoglobulin also leaks out of the glial scar into adjacent non-infarcted tissue, where its presence correlates with progressive dysfunction in the hippocampus and delayed loss of neurons between weeks one and seven after stroke. Together, these findings indicate that the astroglial scar is an effective border at containing larger inflammatory cells, but is porous to smaller immune cells such as T lymphocytes, and to immunoglobulin.

Disclosures: T.V. Nguyen: None. **J. Beischel Frye:** None. **O.M. Hussein:** None. **K.P. Doyle:** None.

Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 801.04/U32

Topic: C.08. Ischemia

Support: NIH grants R01 NS070835 and R01 NS0721267.

Title: Myeloperoxidase inhibition improved neuroprotective and beneficial effects after ischemic stroke

Authors: *H. KIM[1], J. CHEN[1], G. WOJTKIEWICZ[2], L. BURE[3], W. YING[4], M. MOSKOWITZ[4], J. LEE[5];
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Abstract: Myeloperoxidase, a key inflammatory enzyme, is markedly elevated after stroke. We had reported that 4-aminobenzoic acid hydrazide (ABAH), a specific irreversible MPO inhibitor, increased neurogenesis after stroke. Here, we investigated whether post-insult ABAH treatment in wildtype (WT) and MPO-/- mice can exert neuroprotective effects after transient middle cerebral artery occlusion (tMCAO) in mice. Additionally, we asked whether ABAH treatment only during subacute stroke can reduce brain infarct volume and improve behavioral deficits. We found that post-insult ABAH treatment significantly reduced brain infarct volume ratio (D21/D1) on MRI imaging compared with saline-treated control mice ($P<0.05$). ABAH treatment in WT mice and MPO-/- mice robustly reduced cell loss and Fluoro-Jade B (FJ-B) immunostaining in the ischemic brains compared with saline-treated WT mice on day 7 after stroke. ABAH treatment in WT mice and MPO-/- mice both showed decreased loss of oligodendrocytes as detected by myelin basic protein (MBP) immunostaining in the ipsilateral white matter at 7 days after stroke. Stroke-induced activation of microglia (CD11b+, Iba-1+) and macrophages/monocytes (ED1+) and matrix metalloproteinase-9 (MMP-9+) cells, were decreased by ABAH treatment. ABAH treatment also increased cytoprotective proteins such as heat shock protein 70 (HSP70), HSP27, and p-AKT (Ser 473) in the ipsilateral striatum and cortex on day 3 after tMCAO. HSP70 has multiple roles including anti-apoptotic, neuroprotective, and anti-inflammatory effect after stroke, and ABAH treatment in WT mice and in MPO -/- mice on day 7 after stroke increased the number of HSP 70+/NeuN-positive cells in the brain. Notably, ABAH treatment starting in the subacute stage of stroke still significantly reduced brain infarct size ratio (D21/D1) compared with acute ABAH treatment on day 21 after stroke ($P<0.05$). While treatment during the acute phase mildly improved functional outcome, treatment during the subacute phase exhibited marked improvement in behavioral outcome ($P<0.05$). Taken together, our results show that inhibition or lack of MPO contributes to the prevention of cell death, oligodendrocyte loss, and upregulation of anti-apoptotic and anti-inflammatory proteins that lead to improved outcome. Therefore, post-insult MPO inhibition may provide a potential therapeutic target for subacute stroke therapy. Key words: Stroke; neuroprotection; myeloperoxidase; MRI; 4-aminobenzoic acid hydrazide (ABAH)

Disclosures: H. Kim: None. **J. Chen:** None. **G. Wojtkiewicz:** None. **L. bure:** None. **W. Ying:** None. **M. Moskowitz:** None. **J. Lee:** None.

Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Program#/Poster#: 801.05/U33

Topic: C.08. Ischemia

Support: NIH Grant NS46742

Title: The gene silencing transcription factor REST represses miR-132 expression in hippocampal neurons destined to die

Authors: *J.-Y. HWANG, N. KANEKO, K.-M. NOH, F. PONTARELLI, R. S. ZUKIN; Dept Neurosci, Albert Einstein Col. Med., New York, NY

Abstract: The gene silencing transcription factor REST/NRSF (Repressor Element-1 (RE1) Silencing Transcription Factor/Neuron-Restrictive Silencer Factor) actively represses a large array of coding and noncoding neuron-specific genes important to synaptic plasticity including miR-132. miR-132 is a neuron-specific microRNA and plays a pivotal role in synaptogenesis, synaptic plasticity and structural remodeling. However, a role for miR-132 in neuronal death is not, as yet, well-delineated. Here we show that ischemic insults promote REST binding and epigenetic remodeling at the miR-132 promoter and silencing of miR-132 expression in selectively-vulnerable hippocampal CA1 neurons. REST occupancy was not altered at the miR-9 or miR-124a promoters despite the presence of RE1 sites, indicating REST target specificity. Ischemia induced a substantial decrease in two marks of active gene transcription, dimethylation of lysine 4 on core histone 3 (H3K4me2) and acetylation of lysine 9 on H3 (H3K9ac) at the miR-132 promoter. RNAi-mediated depletion of REST *in vivo* blocked ischemia-induced loss of miR-132 in insulted hippocampal neurons, consistent with a causal relation between activation of REST and silencing of miR-132. Overexpression of miR-132 in primary cultures of hippocampal neurons or delivered directly into the CA1 of living rats by means of the lentiviral expression system prior to induction of ischemia afforded robust protection against ischemia-induced neuronal death. These findings document a previously unappreciated role for REST-dependent repression of miR-132 in the neuronal death associated with global ischemia and identify a novel therapeutic target for amelioration of the neurodegeneration and cognitive deficits associated with ischemic stroke.

Disclosures: J. Hwang: None. **N. Kaneko:** None. **K. Noh:** None. **F. Pontarelli:** None. **R.S. Zukin:** None.

Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Support: CEHD in MSM

NIH R01NS066027

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AHA 0840132N

ALZ IIRG-10-173350

Title: Role of serum- and glucocorticoid-regulated kinases in stroke

Authors: *K. INOUE, T. YANG, Z. ZENG, Z.-G. XIONG;
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Abstract: Stroke is one of the most common causes of disability and death in US. Despite the long history of effort in the search of effective treatment, tissue plasminogen activator is still the only FDA approved agent for stroke. However, it has limited therapeutic time window and potential side effect of intracranial hemorrhage. Serum- and glucocorticoid-inducible kinases (SGKs) encode serine/threonine kinases and regulate the function of many proteins including ion channels and transporters. One of the important roles of SGKs is associated with blood pressure control, mediated through its regulation of renal epithelial Na+ channels. Regulation of most channels/transporters by SGKs acts through E3 ubiquitin ligase Nedd4-2, and subsequently surface expression of the targets are influenced. SGKs are also expressed in the brain. Recent studies have revealed the contribution of SGKs to physiological functions such as fear retention, learning and memory. However, the role of SGKs in neurological disorders such as stroke remains elusive. We therefore examined the effect of SGK inhibition on the outcome of stroke. SGK inhibitors, gsk650394 and EMD638683, were applied through intracerebroventricular injection 30 min prior to 1h middle cerebral artery occlusion (MCAO). Volumes of brain lesion were evaluated 24h after MCAO. We showed that both SGK inhibitors significantly reduced the infarct volumes. Because excessive intracellular Ca2+ increase via N-methyl-D-aspartate (NMDA) receptors is a main cause of neuronal injury under oxygen/glucose-deprivation condition and because SGKs are known to facilitate response of NMDA receptors, we examined the effect of SGKs on NMDA toxicity in cultured mouse cortical neurons. We demonstrated that SGK inhibitors ameliorate NMDA-induced neurotoxicity, suggesting that the beneficial effect of the SGK inhibitors on ischemic brain injury is, at least in part, mediated by the inhibition of NMDA channels. Indeed, we found that SGK inhibitors significantly decrease the amplitude of NMDA current. In summary, our studies suggest that SGKs play an important role in stroke and that the effects of SGKs are mediated in part through NMDA receptors.

Disclosures: K. Inoue: None. **T. Yang:** None. **Z. Zeng:** None. **Z. Xiong:** None.

Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

Location: Halls A-C

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Program#/Poster#: 801.07/U35

Topic: C.08. Ischemia

Support: KAKEN Grant 25560257

Title: Contribution of the contralesional sensorimotor cortex to the motor recovery in the rat brain after focal ischemia

Authors: *S. MOMMA[1], M. GOTO[1], S. TOMOKO[1], T. MIKAMI[2], A. MITANI[1];
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Abstract: Functional recovery following stroke is significantly improved with rehabilitation. It has been reported that the rehabilitative training enhances neuronal reorganization and alters the cortical maps in the affected hemisphere(Nudo et al., 1996). However, little is known about the contribution of contralesional hemisphere to motor recovery. In the present study, we examined the functional changes induced in the rat contralesional sensorimotor cortex following training of affected forelimb using intracortical microstimulation (ICMS), multi-unit recording and injection of biotynilated dextran amine (BDA). Following pre-infarct training of skilled reaching, rats were randomly divided into three groups: training, non-training and sham. Stroke was produced on the sensorimotor cortex of the training and non-training groups by photothrombosis method to affect trained forelimb, and only the training group received the skilled reaching training in which rats were forced to use their affected hand for 6 weeks. Skilled reaching was used as a behavioral test to evaluate their motor function. As a result, the training group scored significantly higher than the non-training group. ICMS applied to the contralesional hemisphere showed that the threshold for ICMS-evoked affected hand movements decreased in the training group. In addition, we recorded multi-unit activities in the contralesional sensorimotor cortex with a telemetry system during a series of reaching movement. The firing rate in the training group significantly increased as they reached their affected hand to a pellet. We finally injected BDA into the contralesional sensorimotor cortex to investigate the morphological changes that would underlie the reorganization. These results suggested that the contralesional sensorimotor cortex might contribute motor recovery of affected hand following rehabilitative training.

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Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Program#/Poster#: 801.08/U36

Topic: C.08. Ischemia

Support: NSFC81371305

Title: Optogenetic inhibition of striatum neurons promotes neurogenesis after ischemic stroke through GABA-A receptor

Authors: *Y. WANG[1], X. HE[2], Y. LU[2], L. JIANG[2], Z. ZHANG[2], G.-Y. YANG[2];
[1]Med-X Res. Inst., [2]Med-X Res. Institute, Sch. of Biomed. Engin., Shanghai Jiao Tong Univ., Shanghai, China

Abstract: Introduction: Endogenous neurogenesis occurs after cerebral ischemia in response to hypoxia-induced stress. However, how this process is regulated remains unclear. We use suture-induced middle cerebral artery occlusion (MCAO) model as stroke model, use optogenetic tool to regulate the activity of striatum neurons after stroke and investigated their roles in neurogenesis and neural functional recovery after ischemic stroke. **Methods:** Lentivirus carrying ChR2-YFP or NpHR-YFP fusion protein under CaMKII promoter control were injected into the striatum of experimental mice. Optical fiber or optrode was implanted in the same position at 7 days after gene transfection. MCAO surgery was performed at 14 days after gene transduction. Four days after MCAO surgery, mice were treated with 473nm or 594nm laser twice a day for 15 minutes each session for 4 consecutive days. Electrophysiology recording confirmed the validity of ChR2 and NpHR channels *in vivo*. Neurological behavior tests were performed at 3, 7 and 35 days after MCAO. BrdU/NeuN and BrdU/DCX double staining were used to quantify neurogenesis. Bicuculline was used as a specific GABA-A receptor antagonist. Cresyl violet staining of brain sections was used to evaluate brain atrophy at 35 days after MCAO. **Results:** When stimulated by 473nm laser, the firing rate of striatum neuron was enhanced in ChR2 transduced mice. When stimulated by 594nm laser, the firing rate of striatum neuron was reduced in NpHR transduced mice. The number of BrdU/DCX double positive cells significantly increased in the NpHR group comparing to GFP control group and ChR2 group in the sub-ventricular zone. Nestin positive cells were increased both in SVZ and peri-infarct area 7 days after MCAO in the NpHR group. The number of BrdU/NeuN double positive cells also increased at 35 days after MCAO. Brain atrophy was reduced in the NpHR group compared with ChR2 or GFP group. Neural behavior tests showed that inhibition of striatum neuron activity promotes motor function at 7 days and 35 days after MCAO. ChR2 transduced mice showed less

neurogenesis and worsened neurological function compared to GFP control group. Administration of bicuculline to ChR2-transduced mice rescued the inhibition of neurogenesis by ChR2, suggesting that the regulation of neurogenesis by striatum neurons is likely through GABA-A receptor. **Conclusion:** Activation of striatum neurons inhibits neurogenesis after ischemic stroke, while inhibition of these neurons promotes neurogenesis and recovery. Such effects on neurogenesis by striatum neurons are likely regulated through GABA-A receptor.

Disclosures: Y. Wang: None. **X. He:** None. **Y. Lu:** None. **L. Jiang:** None. **Z. Zhang:** None. **G. Yang:** None.

Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Topic: C.08. Ischemia

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Swedish Medical Research Council

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Title: CLIC4 translocation is implicated in the development of Perinatal Brain Injury

Authors: *C. THORNTON[1], U. RUETSCHI[2], R. VONTELL[1], P. GRESSENS[1,3], H. ZETTERBERG[2], H. HAGBERG[1,2];
[1]Ctr. For the Developing Brain, KCL, London, United Kingdom; [2]Sahlgrenska Academy, Univ. of Gothenburg, Gothenburg, Sweden; [3]INSERM, U676, Paris, France

Abstract: Perinatal cerebral hypoxic-ischaemic injury (HI) is a major cause of neurological damage and underlies the development of human disorders such as epilepsy and cerebral palsy. HI results in an initial cellular energy depletion, followed by a recovery phase and a secondary energy failure during which the majority of brain injury occurs. The mitochondrion may act as a convergence point for mechanisms underlying the pathological damage; mitochondrial outer membrane permeabilisation (MOMP) may represent the switch between reversible and irreversible injury. Previously, we showed that Bax-mediated apoptosis leading to MOMP is a critical pathway for the development of injury in neonatal brain. In the current study, we carried out an unbiased proteomics screen using Stable Isotope Labeling by Amino acids in Culture

(SILAC) to identify mitochondrial proteins differentially regulated in response to a Bax-mediated apoptotic stimuli. In addition to alterations in both individual proteins and pathways (e.g. ubiquitination proteins, voltage dependent anion channels) we identified the downregulation (0.37 fold) of chloride intracellular channel protein 4 (CLIC4) at the mitochondria. Interestingly, we found that in a cell line exposed to oxygen-glucose deprivation (OGD) and in mouse brain exposed to HI there was a time-dependent increase in CLIC4 mRNA expression, in contrast with the SILAC data. However, protein fractionation studies and immunofluorescence in the same models revealed that CLIC4 was rapidly translocated from the mitochondria, accumulating in the nucleus. In experiments modulating CLIC4 expression by siRNA knockdown and plasmid upregulation we find that CLIC4 alters the ability of cells to survive OGD and apoptotic insult. Finally we are currently examining the interaction between histamine H3 receptor and CLIC4 as in addition to apoptosis, a recent publication suggests that this pathway may regulate autophagy. Our study provides a new target implicated in HI-mediated apoptosis and may yet contribute to the emerging field of autophagy/mitophagy in the development of perinatal brain injury.

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Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF Grant (No.2012M3A9C6049935)

DGIST Convergence Science Center Program (14-BD-04)

Title: Negative regulation of autophagic cell death by calpain upon proteasome inhibition in adult hippocampal neural stem cells

Authors: *K. CHUNG, H. PARK, S. JUNG, S. HA, S. YOO, H. WOO, E.-K. KIM, C. MOON, S.-W. YU;
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Abstract: The mechanism by which autophagy interrelates with apoptosis remains one of the key unanswered questions in programmed cell death. We have previously reported that adult

hippocampal neural stem (HCN) cells undergo autophagic cell death (ACD) following insulin withdrawal. Autophagy is the central cell death mode of insulin-deficient HCN cells in spite of their intact apoptotic capability. Autophagy and ubiquitin-proteasomal system (UPS) are two major cellular degradation systems and their interplay is critical in regulation of cellular homeostasis and demise. Proteasome inhibition by lactacystin or MG132 induced apoptosis in insulin-deficient HCN cells defaultly undergoing ACD, but with different levels of autophagy. Lactacystin dampened the insulin withdrawal-mediated autophagy whereas MG132 further enhanced the level of autophagy. Genetic silencing of calpain 2 or ectopic expression of calpastatin increased ACD. Conversely, calpain over-expression decreased autophagy level. The expression level of calpain 2 and its protease activity were significantly reduced by MG132 while intracellular calcium concentration was significantly elevated to similar levels upon both lactacystin and MG132 treatment, suggesting that balance of calpain-calpastatin system is more important for regulation of autophagy than overall intracellular calcium level. These findings demonstrate the concurrent, yet uncomplementary regulation of UPS and autophagy; and unveil a molecular mechanism governing an interconversion between apoptosis and autophagic cell death through calpain.

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Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Topic: C.08. Ischemia

Support: Swiss National Science Foundation, NCCR Neural Plasticity and Repair

The Koetser Foundation

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Title: Reduced COX expression and metabolic adjustments in 3- nitropropionic acid-induced ischemia tolerance in the rat brain

Authors: O. BRACKO[1], V. DI PIETRO[3], G. LAZZARINO[4], A. M. AMORINI[4], B. TAVAZZI[4], J. ARTMANN[2], E. C. WONG[5], R. B. BUXTON[6], M. WELLER[1], A. LUFT[1], *S. WEGENER[1];
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Abstract: Ischemia tolerance can be achieved by the oxidative chain inhibitor 3-nitropropionic acid (NPA). We have previously shown that rats pre-treated with a sub-lethal dose of NPA 72 h before transient middle cerebral artery occlusion (MCAO) have smaller infarct lesions and less severe functional deficits compared to saline-treated controls. In order to characterize mechanisms of NPA induced ischemia tolerance, our goal was to find out if NPA preconditioning induces vascular remodeling or long-term changes in energy metabolism. We induced ischemia tolerance by a single 20mg/kg NPA i.p. injection in adult male Wistar rats and performed quantitative cerebral blood flow (CBF) measurements using arterial spin labeling (ASL)-MRI along with structural (T1- and T2w) imaging 72 hours later. After MRI analyses, animals were sacrificed and brains subjected to histological analysis including the expression of the neuronal marker NeuN, the endothelial cell marker RECA-1, and the mitochondrial respiratory chain marker cytochrome c-oxidase (COX). High-Performance liquid chromatography (HPLC) analyses were performed in brains of another subset of animals 72h after NPA vs. saline injection. NPA preconditioning did not result in any structural damage. HPLC analysis revealed a shift in the proportion of adenosine nucleotides towards a lower energy charge potential with a tendency to decrease the rate of ATP synthesis with a concomitant increase in its dephosphorylated products (AMP). There was a global CBF reduction of approximately 30% in NPA preconditioned animals compared to controls. Blood vessel density was not altered upon NPA treatment. However, the COX immunofluorescence signal was reduced in cortical and subcortical regions in NPA preconditioned animals. Our data suggest a novel link between NPA induced reduction in oxidative phosphorylation indicated by the reduced COX expression, a lower energy charge potential, and a global reduction in CBF as a potential mechanism underlying ischemia tolerance.

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Poster

801. Ischemia: Cellular Mechanisms and Neuroprotection VI

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 801.12/V4

Topic: C.08. Ischemia

Support: NIH NS082363

Title: Myeloperoxidase increases risk of formation and rupture of cerebral aneurysms

Authors: *Y. CHU[1], G. L. PIERCE[1], G. CHENG[2], L. WEGMAN-POINTS[1], H. GU[1], K. WILSON[1], R. A. PENA SILVA[1,3], F. M. FARACI[1], D. D. HEISTAD[1], D. HASAN[1]; [1]Univ. of Iowa, Iowa City, IA; [2]Univ. of Alabama Sch. of Med., Birmingham, AL; [3]Univ. de los Andes Sch. of Med., Bogota, Colombia

Abstract: Myeloperoxidase (MPO) is increased in many inflammatory diseases. MPO is associated with elevated risk for vascular disease in humans. We hypothesized that MPO is increased locally in human intracranial aneurysms (IA), and is critical for aneurysm formation and progression to rupture in a mouse model of IA. Blood, from the lumen of IA and femoral artery, was obtained from 17 patients who underwent endovascular coiling of IA. Tissues were collected from the wall of IA and from a superficial temporal artery in 12 patients who underwent microsurgical clipping of IA. We utilized a mouse model of IA in which aneurysmal rupture with subarachnoid hemorrhage (SAH) occurs spontaneously and causes neurological signs. Two groups of mice (12 WT and 12 MPO KO) underwent IA induction. Cerebral arteries from the mice were analyzed for inflammatory molecules and MMPs using qPCR. Plasma concentrations of MPO were 3-fold higher in aneurysms (100±15 ng/ml) (mean±SE) than in femoral blood (33±10; p=0.0007), whereas vascular peroxidase 1 (VPO1), a homolog of MPO, was not increased in aneurysm (384±51 μg/ml) vs. femoral blood (513±65; p=0.12). mRNA expression of MPO and VPO1 was similar in leukocytes recovered from aneurysmal blood and femoral blood. There were significantly more MPO-positive cells in aneurysm tissue (69±8 positive cells/field) than superficial temporal artery (6±3 positive cells/field, $p<0.001$). Incidence of aneurysms was significantly lower in MPO KO mice (50%) than WT control (92%, $p<0.05$). SAH was significantly lower in KO (8%) than WT (83%, $p<0.001$) mice. In cerebral arteries, neutrophil elastase, TNFα, CXCL1, COX2, MCP1, CD68 and arginase 1, MMP3, MMP13, and MMP9 were significantly lower, and both SM22 alpha and alpha smooth muscle actin significantly greater, in MPO KO mice. These findings suggest that MPO plays a critical role in the formation and rupture of IA in a mouse model of IA through inflammation/MMP/SMC mechanisms. A localized increase of MPO may contribute to IA in humans.

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Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Program#/Poster#: 802.01/V5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS052741

NMSS PP2009

CA1060A11

Title: Protease activated receptor-mediated mechanisms of axon injury and neuron degeneration

Authors: *H. YOON[1], M. RADULOVIC[2], I. A. SCARISBRICK[1];
[1]Physical Med. & Rehabilitation, Rehabil. Med. Res. Ctr., [2]Neurobio. of Dis. Program, Mayo Grad. Sch., Mayo Clin., Rochester, MN

Abstract: Serine protease-driven cascades are recognized to play important roles in CNS physiology during development and in adulthood, although knowledge regarding the scope of their physiologic actions and the consequences of their deregulation with injury or disease remains limited. We discovered that an important subset of serine proteases expressed endogenously in the CNS or upregulated therein after injury, such as thrombin, plasmin or select kallikreins, exert their cellular effects by selective activation of a family of G-protein coupled receptors referred to as Protease Activated Receptors (PARs). All 4 identified PARs were shown to be expressed in the adult brain and spinal cord and are therefore positioned to rapidly translate the dynamic changes that can occur in the proteolytic microenvironment with CNS injury or disease into rapid cellular responses. PARs are uniquely activated by specific serine proteases that selectively hydrolyze the extracellular N-terminus of the receptor resulting in differential recruitment of G-proteins and activation of discrete signaling cascades that have multiple downstream targets including gene transcription, cell motility, cytoskeletal rearrangement and secretion. For example, thrombin is the canonical activator of PAR1, whereas a more recently identified serine protease expressed at high levels in the CNS, neurosin (kallikrein 6), activates both PAR1 and PAR2. We hypothesize that serine protease-PAR signaling occurring at sites of CNS pathogenesis promotes axonopathy and neuron degeneration. Supporting this, we show that thrombin and neurosin, in addition to their cognate receptors, are elevated at sites of CNS injury, including murine spinal cord trauma and inflammatory demyelinating disease. Recombinant neurosin activated the ERK1/2 signaling cascade in primary neurons in a PI_3K and MEK-dependent fashion. Novel lipopeptide inhibitors specific for the intracellular loops of PAR1 or

PAR2, in addition to neurons derived from PAR deficient mice were utilized to demonstrate both receptors play a role in neurosin-elicited signaling, including activation of ERK1/2 and the pro-apoptotic protein Bim. Moreover, both neurosin and thrombin promoted axon injury in dissociated neurons and in organotypic spinal cord slice cultures with the effects in each case potentiated by excess glutamate. Importantly, protease-mediated neurotoxicity was significantly reduced in neurons and organotypic slices derived from PAR deficient mice. Together, these data point to a novel neurosin-PAR1 and -PAR2 mediated signaling axis in CNS neurons that is positioned to mediate neurotoxicity in cases of CNS injury and disease.

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Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Topic: C.08. Ischemia

Support: NIH grant NS066001

Title: Dysfunction, recovery, and hyperexcitability in the acute phase of ischemic stroke

Authors: *E. G. WANN, N. S. JACOBS, R. D. FROSTIG;
Neurobio. and Behavior, Univ. California Irvine, Irvine, CA

Abstract: We monitored spontaneous neuronal activity during the acute phase of ischemic stroke to map the time course of spontaneous activity patterns in the minutes and hours after an ischemic event. Spontaneous activity within the middle cerebral artery (MCA) region was measured using multi-site recordings across multiple cortical depths following permanent MCA occlusion (pMCAO). Infarct was assessed 24 hours after pMCAO using Nissl staining, and electrodes were then categorized based on their relative location to the infarct. Within the infarcted region in all cortical layers, spontaneous activity decreased directly after pMCAO and remained diminished for the five hour period sampled. In contrast, spontaneous neuronal activity at all cortical depths in regions adjacent to the area of subsequent infarct decreased initially but became hyperexcitable within two to three hours after pMCAO. An initial reduction in activity occurred even in electrodes distant to the infarcted region where activity recovered. Previous studies have shown that sensory stimulation delivered within two hours after pMCAO completely protects the cortex from impending stroke damage; however, the same sensory

stimulation results in additional damage if delivered three hours after pMCAO. Interestingly, the time window of sensory stimulation efficacy is consistent with the timing of hyperexcitability seen in the peri-infarct region. We are currently investigating whether sensory stimulation affects spontaneous activity directly after pMCAO and if hyperexcitability is blunted when sensory stimulation is delivered early. Supported by NIH grant NS066001.

Disclosures: E.G. Wann: None. **N.S. Jacobs:** None. **R.D. Frostig:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 802.03/V7

Topic: C.08. Ischemia

Support: Florida Department of Health Grant # 2KN09

Title: Intra-arterial delivery of mesenchymal stem cell protects brain from stroke injury via brain-derived growth factor (BDNF) signaling

Authors: *D. R. YAVAGAL[1], P. BHATTACHARYA[2], R. LOPEZ[2], A. KHAN[3], M. PEREZ-PINZON[2], J. M. HARE[2], A. P. RAVAL[2];
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Abstract: Stroke remains a leading cause of disability worldwide and ischemic stroke (occlusive clot-induced) accounts for almost 85% of total stroke cases. Although ischemic stroke patients are treated more often with thrombolytic agents, all neuroprotection trials for stroke have been unsuccessful and therefore, new pharmacological interventions are greatly needed. In last decade, laboratory studies suggest that stem cell therapy is a prospective treatment for stroke. There are multiple routes of stem cell delivery to the brain in ischemic stroke. Among these, the intra-arterial (IA) route of stem cell delivery has a high potential for clinical translation, in view of the increasing clinical application of endovascular therapy in the treatment of ischemic stroke. Recently, our study demonstrated that the intra-arterial delivery of mesenchymal stem cell (IA MSCs) at 24h after a reversible middle-cerebral artery occlusion (MCAo; for 90 min) reduces the infarct volume and improves neurological score in female rats at one month post-treatment. However, our study did not identify the mechanism by which IA MSCs protect female brain from ischemic damage. Identifying the mechanism by which IA MSCs protect the brain could be

of future implication in extending the period of beneficial effects of MSCs in recovery of stroke. Studies from various laboratory demonstrated that the growth factors play important role in preserving brain function after ischemia. Therefore, we propose to test the hypothesis that the IA MSCs treatment after MCAo increase brain-derived neurotrophic factor (BDNF) release and tyrosine kinase receptor sub-type B (TrkB) signaling in the female brain. To test proposed hypothesizes, ovariectomized rats were exposed to MCAo and treated intra-arterially either MSCs or phosphate buffered saline (PBS; vehicle control) a day later. Mesenchymal stem cells or PBS treated rats were sacrificed at 2, 4 8 or 30 days. The core, penumbra and contralateral regions of the brain were harvested to check protein levels of BDNF, TrkB and phosphorylated TrkB by Western blot analysis. We observed significant increase in the protein levels of BDNF and pTrKB in the penumbra region. IA MSCs increased protein levels of BDNF by two folds ($p < 0.05$) on days 2 and remain high until day 8, as compared to PBS treated groups. Similarly, we observed increased protein levels of pTrKB by one fold ($p <0.05$) on days 4 and 8 respectively, as compared to PBS treated groups, suggesting activation of BDNF-TrkB signaling after IA MSCs treatment. BDNF signaling is known to promote neuronal survival and stimulate neurogenesis in ischemic brain and direction of our future research.

Disclosures: D.R. Yavagal: None. **P. Bhattacharya:** None. **R. Lopez:** None. **A. Khan:** None. **M. Perez-Pinzon:** None. **J.M. Hare:** None. **A.P. Raval:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 802.04/V8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC

Title: The role of the NOD-like receptor, Nlrx1 in neuroprotection and neuronal cell death

Authors: *E. IMBEAULT[1], T. M. MAHVELATI[2], D. GRIS[2];
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Abstract: Neuronal cell death is a phenomenon that occurs during brain development as well as in pathological diseases. Depending on the environment in which the cells are; apoptosis, necrosis or autophagic cell death can contribute to neuronal cell death. Basal autophagy plays a

key role in homeostasis by degrading damaged organelles, proteins, and even pathogens. Thus, an unbalanced process in autophagy can lead to neuronal cell death by a mechanism called autophagic cell death. It is crucial for the brain to have a basal level of autophagy to maintain neurons intact and prevent neurodegenerative diseases. A recently discovered NOD receptor, Nlrx1, is thought to play a role in autophagy and in innate immunity by sensing viral RNA at the mitochondria. Therefore, we hypothesize that Nlrx1 plays a neuroprotective role in attenuating inflammation by inhibiting apoptosis in neurons. To determine the protective mechanism of Nlrx1 *in vitro*, a Knock-Down, a Knock-In and a Scrambled control of Nlrx1 in N2a cells have been generated. LDH assays for cell death detection with hypoxic conditions or treatments with rotenone and staurosporine have been done. After 24h treatment of rotenone or hypoxia, N2a Knock-In cells showed higher cell death than N2a Knock-Down. When cells are pre-treated with Z-VAD for 1h prior to staurosporine or rotenone treatments, there was a decrease in cell death in all cells, regardless of the treatment. Pre-treatment with Z-VAD, followed by rotenone treatment demonstrated that N2a Knock-In cells undergo less cell death compared to N2a Knock-Down. Z-VAD pre-treatment decreases cell death in N2a Knock-In cells compared to N2a Knock-Down cells and can be explained by the increase in the number of mitochondria seen in N2a Knock-In cells by electron microscopy. Thus, having more mitochondria promotes the apoptotic pathway mediated by mitochondria in N2a Knock-In cells. Finally, *in vitro* results show that Nlrx1 Knock-In cells are more sensitive to oxidative stress and more prone to apoptosis and N2a Knock-Down cells are more resistant to cell death. Further investigations will be done to determine the exact mechanism of apoptosis within the type of cell.

Disclosures: E. Imbeault: None. **T.M. Mahvelati:** None. **D. Gris:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 802.05/V9

Topic: C.08. Ischemia

Title: Roles of oxygen treatment in the development of ischemic brain injury, signal transduction and brain microcirculation after focal cerebral ischemia

Authors: *M. C. BEKER, A. B. CAGLAYAN, T. KELESTEMUR, E. YALCIN, G. OZTURK, E. KILIC;
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Abstract: Brain injury following ischemic stroke develops from a complex series of pathophysiological events, including over-activation of glutamate receptors, disruption of cellular calcium homeostasis, generation of reactive oxygen species (ROS) and spreading depression, which causes vasoconstriction in the penumbra. It is a matter of debate and an important issue in clinical aspect whether normobaric oxygen (NBO) treatment worsens ischemic stroke outcome. Here, we were interested whether different concentration of NBO treatment combined with free radical scavenger melatonin promotes neuronal survival, DNA fragmentation, brain oedema, blood-brain barrier (BBB) permeability, neurological outcome, broad spectrum of protein analysis, including Bcl-XL and eNOS activities. Furthermore, we have analyzed real time cerebral microcirculation by using laser speckle flowmetry before, during and after ischemia, including NBO treatment. For this aim, male 9 weeks old balb-c mice were anesthetized with 1% isoflurane (30% O2; rest N2O), and subjected to 30 min or 90 min of intraluminal middle cerebral artery occlusion (MCAo) and 72 hours or 24 hours reperfusion, respectively. Animals were treated with 21-, 70-, or 100% NBO alone or combined with melatonin (4mg/kg) just after reperfusion. NBO treatment was continued for the first 90 min of reperfusion. As compared with animals treated with 21% NBO treatment, 70% but particularly 100% NBO treatment. Seventy- but especially 100% NBO treatment decreased infarct volume, brain swelling, neurological outcome, neuronal survival and DNA fragmentation significantly, which was associated with improved BBB permeability, cerebral microcirculation and increased significant expressions of anti-apoptotic Bcl-XL and eNOS. Furthermore, melatonin treatment further improved neuroprotective effects of NBO treatment. Here, we provide evidence that NBO treatment is beneficial after ischemic stroke which was associated with improved brain microcirculation, BBB permeability and Bcl-XL and eNOS activities. Furthermore, free radical scavenger melatonin potentiates the effects of NBO treatment.

Disclosures: M.C. Beker: None. **A.B. Caglayan:** None. **T. Kelestemur:** None. **E. Yalcin:** None. **G. Ozturk:** None. **E. Kilic:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 802.06/V10

Topic: C.08. Ischemia

Title: Aerobic exercise is as effective as skilled reach training in improving functional recovery following ischemic insult in C57BL/6 mice

Authors: *A. L. KERR[1], T. MUELLER[2], M. DOMINGUEZ[1], R. HOLDEN[1], M. CURTIS[1], B. WALL[1];
[1]Psychology, [2]Illinois Wesleyan Univ., Bloomington, IL

Abstract: Stroke is a leading cause of serious, long-term disability with upper limb impairment among the most common, chronic deficits reported in survivors. Current rehabilitative strategies employed in humans are often ineffective in restoring pre-stroke behavioral performance. It has been found that focused rehabilitative training of the impaired limb effectively improves functional outcome in rodent models of ischemic stroke. However, the benefits reported with this training require intensive therapy with relatively long sessions implemented daily. Additional rehabilitative strategies that may be easier to implement in other populations (including humans) need to be established to promote better behavioral recovery following injury. Aerobic exercise has been found to have beneficial effects for both the brain and behavior in humans and rodents, with exercise improving learning and memory in aged populations. The current study investigates the role of aerobic exercise in functional recovery following focal ischemic insult in C57BL/6 mice. A total of 40 mice were trained to criterion on a skilled reaching task, the Pasta Matrix Reaching Task (PMRT), prior to receiving a unilateral ischemic stroke. Following four days of recovery, mice were divided evenly into one of four groups: skilled reach training of the impaired limb (on the PMRT), aerobic exercise, skilled reach training with aerobic exercise, or control procedures. Animals in aerobic exercise conditions had free access to running wheels, while control animals received no direct training of any limb. All mice were in their respective conditions for a total of two weeks and received weekly probe trials of their impaired limb. Our results indicate that aerobic exercise is as effective as skilled reach training in improving functional recovery of the impaired limb. The combined therapy of skilled reach training and exercise was no more beneficial than either condition alone. These findings suggest that aerobic exercise may be a feasible rehabilitative strategy for individuals with persistent upper extremity impairment following stroke.

Disclosures: A.L. Kerr: None. **T. Mueller:** None. **M. Dominguez:** None. **R. Holden:** None. **M. Curtis:** None. **B. Wall:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Topic: C.08. Ischemia

Support: NIH Grant HL093056 (BAH)

Tartar Trust Fellowship (DCP)

OHSU Graduate Research Fellowship (DCP)

OHSU Steinberg Trust (DCP)

Title: Mechanisms of p75NTR-dependent peri-infarct denervation

Authors: D. C. PARRISH[1], B. L. HEMPSTEAD[2], A. NYKJAER[3], *B. A. HABECKER[1]; [1]Physiol. & Pharmacol., OHSU, Portland, OR; [2]Weill Cornell Med. Col., New York, NY; [3]Aarhus Universitet, Aarhus, Denmark

Abstract: Several recent clinical trials revealed that the amount of sympathetic denervation of the heart after myocardial infarction (MI) predicts the risk for arrhythmias and sudden cardiac death. We previously showed that the p75 neurotrophin receptor (p75NTR) is required for denervation of the peri-infarct zone after cardiac ischemia-reperfusion injury. Both mature brain-derived neurotrophic factor (BDNF) and proneurotrophins, such as pro-nerve growth factor (proNGF) and proBDNF, can signal through p75NTR to initiate axon degeneration. We have shown that proNGF is elevated in the heart following MI. We quantified proBDNF protein in the left ventricle of BDNF-HA/HA mice 24 hours following ischemia-reperfusion or sham surgery by western blotting. ProBDNF was elevated in the infarcted left ventricle compared to sham-operated controls. We asked if the denervation of viable myocardium was caused by proneurotrophins. Because proneurotrophin signaling through p75NTR requires a sortilin family co-receptor, we quantified sympathetic innervation density by tyrosine hydroxylase immunohistochemistry in 12-18 week old sortilin -/- and wild-type control mice (C57Bl6/J) that were subjected to sham or ischemia-reperfusion surgery. The infarct and peri-infarct zones were largely denervated in wild-type mice 3 days after MI, and denervation of both the infarct and peri-infarct regions in sortilin -/- mice was identical to wild-type. BDNF signals through p75NTR to cause axon degeneration in sympathetic neurons, in part by increasing expression of tumor necrosis factor-alpha converting enzyme (TACE), an alpha-secretase that cleaves p75NTR. We examined expression of TACE in the stellate ganglia of wild type mice subjected to sham or ischemia-reperfusion surgery and found that TACE mRNA was elevated 3 days post-MI. We treated mice with either vehicle or the secretase inhibitor Marimastat (25mg/kg/day) for 3 days after MI and examined the innervation density of the peri-infarct area. Marimastat-treated mice lacked peri-infarct denervation compared to vehicle-treated controls, suggesting that TACE may play a role in peri-infarct axon degeneration.

Disclosures: D.C. Parrish: None. **B.A. Habecker:** None. **B.L. Hempstead:** None. **A. Nykjaer:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 802.08/V12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Hydrogen peroxide impairs GABA-dependent motor behavior in Caenorhabditis elegans

Authors: *L. HERNANDEZ[1], G. CAMARGO-HERNANDEZ[2], A. LOMELI-SILVA[3], M. A. RAMIREZ-HERRERA[1], A. HERNANDEZ-CHAVEZ[1];
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Abstract: Background: Oxidative stress (OS) is an imbalance between production of free radical (FR), reactive oxygen species (ROS) and endogenous antioxidant defense mechanisms where is favored the oxidant environment. In these conditions, could produce adverse modifications to cell component, such as lipids, proteins, and DNA. Thus, OS have been associated to many pathological conditions and diseases, including atherosclerosis, cancer, diabetes mellitus, neurodegenerative and psychological diseases, some of them, related to GABAergic activity. GABAergic activity is focused in synthesis and release of gamma-aminobutyric acid (GABA) and its postsynaptic receptors, which set up the primary inhibitory neurotransmission system in vertebrate and invertebrate animals. In this sense, the main aim of this work was to evaluate the effect of oxidative damage induced by hydrogen peroxide on GABAergic system, using *Caenorhabditis elegans (C. elegans)* as an *in vivo* model organism. *C. elegans* is a saprophyte nematode that recently it has been employed in oxidative stress studies. In *C. elegans*, GABA acts on neuromuscular junction by inducing relaxing of locomotion and foraging muscles, and contracting muscles implied in defecation. Particularly, GABAergic system is essential for stereotyped behaviors, such nose touch response (NTR). In this study, we examined this behavior after oxidative damage induced by hydrogen peroxide (H_2O_2), in presence or not of GABA or Curcumin (CUR). **Methods:** WT (Bristol N2) strain of *C. elegans*, age synchronized and cultured in NMG-agar plates seeded with E. coli OP50, were used in this study. They were transferred to 12-well plates and were exposed to different concentrations of H_2O_2 (0.1, 0.35 and 0.5 mM) for 30 min. Then, worms were relocated to Agar-NMG plates with no food, and individually were stimulated by touching their noses with an eyebrow hair. A reversal sinusoidal movement was considered as a normal motor behavior and a shrinking movement was scored as defective. This protocol was repeated by adding GABA as $GABA_A$

receptor agonist or CUR as antioxidant. **Results and Conclusions:** Concentrations of H_2O_2 0.35 mM and above induced an impairment of normal NTR in 39%, a statically significant change in relation to non-exposed animal, consequently we inferred that oxidation produces damages in GABAergic system. We confirm this when this effect was partially reversed in the co-exposure of H_2O_2 and GABA. On the other hand, the impairment of NTR could not be completely prevented with co-exposure of CUR and H_2O_2.

Disclosures: L. Hernandez: None. **G. Camargo-Hernandez:** None. **A. Lomeli-Silva:** None. **M.A. Ramirez-Herrera:** None. **A. Hernandez-Chavez:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 802.09/V13

Topic: C.08. Ischemia

Title: An introduction of photothrombotic focal cerebral ischemia model in the adult zebrafish

Authors: *X. YU, Y. V. LI;
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Abstract: As the more commonly used models (suture model and distal middle cerebral artery occlusion) for focal cerebral ischemia are often technically challenging, using simpler models to induce a predictable focal ischemic lesion have been developed as an alternative. One of these methods consists of the photothrombosis, which is induced by the illumination of the brain after the systemic delivery of a photosensitive dye, Rose Bengal. Rose Bengal releases singlet oxygen, which breaks the endothelial cells of the blood vessel under light exposure, and triggers the coagulation pathway in the location of the irradiated tissue. The zebrafish (Danio rerio) has shown a good correlation between zebrafish and rodent data and has demonstrated similarity to mammalian models in tests of toxicity. In this study, we developed a method to use adult zebrafish as a model of focal cerebral ischemia. During anesthesia, Rose Bengal was intraperitoneally delivered to zebrafish according to the body weight (50 μg/g, 100 μg/g, 200 μg/g and 500 μg/g) with different doses of light exposure. The recovery and behavioral changes after photothrombosis were recorded daily for 3 days. The mortality rate of each group was also measured. The results showed the photothrombosis effect is dose-dependent with Rose Bengal and light exposure. High dose of Rose Bengal (500 μg/g) was lethal for zebrafish without light exposure. However, smaller doses of Rose Bengal (50 μg/g, 100 μg/g) and light exposure

introduce brain damage on zebrafish. The optimal dose of Rose Bengal (100 μg/g) and light exposure (intensity of 800 μW/cm2 for 30 minutes) yielded significant brain damage. Quantified 2,3,5-triphenyltetrazolium chloride (TTC) staining showed the significant damage of the optic tectum in the photothrombotic treated zebrafish. Data suggest that Rose Bengal with light exposure induces reproducible photothrombotic brain damage in zebrafish, and that zebrafish can be used as a model of focal cerebral ischemia.

Disclosures: X. Yu: None. **Y.V. Li:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Program#/Poster#: 802.10/V14

Topic: C.08. Ischemia

Support: NIH R01 GM066018

Title: Mitochondrial and cytosolic hydrogen peroxide and glutathione in pyramidal cells and astrocytes in hippocampal area CA1 respond to oxygen-glucose deprivation

Authors: B. YIN[1], N. POVYSHEVA[2], G. BARRIONUEVO[2], *S. WEBER[3];
[1]Chem., [2]Neurosci., [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: A good model for studying cerebral ischemia is oxygen glucose deprivation (OGD). Abundant evidence supports the damaging nature of excess reactive oxygen species (ROS) in cells, which can be converted into less reactive H2O2; then H2O2 can be scavenged by glutathione (GSH), which is oxidized to glutathione disulfide (GSSG). Monitoring H2O2 level and glutathione redox potential (GSHRP) during OGD-reperfusion is a promising way to investigate the course of ischemic injury. We are unaware of single-cell investigations in tissue though there is a rich literature on this problem. Thus we have developed an approach using the organotypic hippocampal slice culture (OHSC), and employ green fluorescent protein (GFP)-based sensors, which are selectively sensitive to H2O2 level or GSHRP. Real-time O2 level inside OHSC (10 - 40 μm depth) is measured with a Clark-type electrode. Our study focuses on the H2O2 level and GSHRP in mitochondrial and cytosol from pyramidal cells and astrocytes in OHSC. We treated OHSCs with OGD-reperfusion (pre-oxygenation, 10 min/ OGD, 20 min/ reperfusion, 30 min), followed by fully oxidizing and reducing them for calibration. We performed ratio-metric imaging of the H2O2 and GSHRP sensors (Ex 405/488 nm; Em 500-530

nm). Fluorescence signal ratio (FSR) 405/488 is normalized to 1 at the sensor's fully oxidized state. FSR is 0.17 or 0.24 for fully reduced GSH or H2O2 sensors, respectively. For pyramidal cell mitochondria, FSRGSH is 0.46 ± 0.01, 0.37 ± 0.01, 0.55 ± 0.01, for pre-, during, post- OGD, respectively (n=6); while FSRH2O2 is 0.53 ± 0.01, 0.43 ± 0.02, 0.57 ± 0.01, for pre-, during, post-OGD, respectively (n=6). These values are statistically significant different suggesting that ROS generation is suppressed during OGD. The situation is reversed during reperfusion. Notably, the GSRRP is stable over the observation time, showing no evidence of a "burst". The peroxide sensor does not respond quickly enough to see a "burst". We also find similar changes in astrocyte mitochondria. However, cytosolic sensors, in either pyramidal cells or astrocytes do not show significant changes during OGD and reperfusion (ANOVA). Supported by NIH Grant R01 GM066018.

Disclosures: B. Yin: None. **N. Povysheva:** None. **G. Barrionuevo:** None. **S. Weber:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Program#/Poster#: 802.11/V15

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAPIIT IN204213

Title: Oxidative stress and neuronal death in non-coma hypoglycemia

Authors: *N. G. LANGUREN, T. MONTIEL, A. JULIO-AMILPAS, L. MASSIEU;
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Abstract: Type 1 diabetic patients are exposed to insulin treatment in order to regulate blood glucose levels. However, intensive insulin tretment increases their risk to present episodes of moderate and even severe hypoglycemia. Severe hypoglycemia leading to blood glucose levels below 20 mg/dl can lead to the coma state both in animals and humans, which is associated with neuronal death in vulnerable brain regions such as the cortex, the hippocampus and the striatum. However, the presence of neuronal death has also been observed in rats exposed to non-coma hypoglycemia, particularly in the cerebral cortex. The consequences of non-coma hypoglycemia in brain have not been completely elucidated and the mechanisms leading to the death of cortical neurons are unknown. The aim of the present study was to investigate whether severe non-coma hypoglycemia induces oxidative stress in discrete regions of the brain and wheter it correlates to

their vulnerability to neuronal death. Male Wistar rats were subjected to severe hypoglycemia (< 20 mg/dl) by insulin administration and were rescued with glucose before the coma, as monitored by EEG recording. Four and 24 h after insulin injection, the levels of lipoperoxidation (TBARS method), reduced glutathione (GSH, fluorometric method), and superoxide production (oxidation of dihydroethydium (DHE) were determined, while neuronal death was evaluated by Fluoro Jade B 24 h after insulin injection. Results indicate that lipoperoxidation was significantly increased in the parietal and frontal cortices, the hippocampus and the striatum of severe hypoglycemic animals at the end of the hypoglycemic period (4 h after insulin), while GSH levels were significantly diminished at this time in all brain regions. At 24 h lipoperoxidation levels remained elevated in frontal cortex but returned to control levels in the parietal cortex, the hippocampus and the striatum. Consistently, the levels of GSH remained low in frontal cortex while it recovered in the parietal cortex, the hippocampus and the striatum. The production of superoxide was increased in both cortices and the hippocampus at 24 h and the frontal cortex showed the largest increase. Consistent with these observations, degenerating neurons were observed only in the frontal cortex. These results suggest that a differential regulation of the antioxidant defense and a differential production of ROS contributes to selective brain damage induced by non-coma hypoglycemia.

Disclosures: **N.G. Languren:** None. **T. Montiel:** None. **A. Julio-Amilpas:** None. **L. Massieu:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Topic: C.08. Ischemia

Support: Veteran Affair Merit Award I01BX001696-01

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Title: The beta-pix - rac1 - cofilin pathway after brain ischemia

Authors: T. LUO, C. LIU, Y. PARK, I. SABIRZHANOVA, *B.-R. HU;
Univ. of Maryland Sch. of Med., Univ. of Maryland, Baltimore, MD

Abstract: The beta-PIX - Rac1 - cofilin pathway plays a key role in synaptic networking after brain injury. Ultimately, functional recovery, which requires synaptic networking, is a key issue for stroke patients. For that reason, understanding of the beta-PIX - Rac1 - cofilin pathway is essential for developing therapeutics to prevent brain injury and to facilitate functional recovery after brain ischemia. However, how this pathway is regulated following brain ischemia remains largely unknown. This study utilized a rat transient cerebral ischemia model to investigate this pathway. The results showed that the Rac1 GTPase was significantly upregulated and translocated to the synaptic and nuclear fractions after brain ischemia. Cool1/beta-PIX (beta-PIX hereafter), a Rac1 guanine nucleotide exchange factor, was dramatically activated by translocation to the synaptic membrane and nuclear structure initially, and then depleted during the recovery phase after brain ischemia. Correspondingly, an episode of brain ischemia led to transient activation of cofilin, a Rac1 downstream effector by dephosphorylation. This cascade of events suggests that the beta-PIX - Rac1 - cofilin pathway is transiently upregulated, which may be responsible for the excitotoxicity in the initial phase after brain ischemia. Furthermore, the depletion of beta-PIX of this pathway during the recovery phase may play a negative role in functional recovery during the late postischemic phase. Therefore, transient inhibition of the beta-PIX - Rac1 - cofilin pathway before or immediately after the onset of brain ischemia may prevent excitotoxicity, but activation of this pathway in the late postischemic phase may facilitate functional recovery.

Disclosures: T. Luo: None. **B. Hu:** None. **C. Liu:** None. **Y. Park:** None. **I. Sabirzhanova:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Topic: C.08. Ischemia

Support: NIH Grant NS073779

Title: Effect of cerebral ischemia on behavioral outcomes in insulin-treated diabetic rats exposed to recurrent hypoglycemia

Authors: V. SHUKLA, P. FUCHS, A. LIU, K. SERDAHELY, P. REDDY, *K. DAVE; Neurol., Univ. Miami Sch. Med., MIAMI, FL

Abstract: The International Diabetes Federation estimates that 382 million people suffer from diabetes worldwide. The major side effect of intensive therapy to control blood glucose levels in both type 1 and type 2 diabetics is recurrent hypoglycemic (RH) episodes. Recurrent hypoglycemia in a rat model of insulin-dependent diabetes exacerbates cerebral ischemic damage, as indexed by the number of surviving neurons in the CA1 region of the hippocampus. The goal of the present study is to determine the effect of cerebral ischemia on behavioral outcome in RH-exposed diabetic rats. Streptozotocin-induced diabetic rats were used as an animal model. Four experimental groups were examined: 1) naïve (non-diabetics), 2) insulin-treated diabetics (ITD) (diabetics on insulin therapy), 3) ITD + RH (diabetics on insulin therapy experiencing RH), and 4) ITD + RH + Glucose (control for additional insulin injected to induce hypoglycemia). Moderate RH was induced once a day for five consecutive days. Global cerebral ischemia (8 min, 2VO + hypotension) was induced the day after the last hypoglycemia treatment. Sham operated rats for each group were used as controls. Behavior was assessed using the open field test (day 13 post-ischemia), contextual fear conditioning test (day 13-14 post-ischemia), zero maze test (days 4 and 12 post-ischemia), and Barnes maze test (days 4-11). Brains were harvested on day 14 post-ischemia for histological assessments. Open field test showed no significant difference in distance traveled among four ischemia groups when compared to respective sham operated groups. Currently we are evaluating effects of ischemia in above mentioned experimental groups on contextual fear conditioning, zero maze and Barnes maze test. We are also evaluating histological outcomes in these animals. Characterization of RH on cerebral ischemia-induced behavioral impairments may help understand the nature of cerebral ischemic injury in diabetic patients.

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Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Program#/Poster#: 802.14/V18

Topic: C.08. Ischemia

Support: Fondazione Pisa

Title: Behavioral, physiological and neuroanatomical changes following a focal ischemic damage in the mouse motor cortex

Authors: *C. ALIA[1,2], C. SPALLETTI[3], S. LAI[4], A. PANARESE[4], A. GHIONZOLI[4], S. MICERA[4,5], M. CALEO[1];
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Abstract: Stroke is one of the leading causes of long-term motor disabilities and re-acquisition of motor skills is a priority for stroke survivors. To develop new effective rehabilitation protocols, it is critical to achieve a more complete understanding of the spontaneous cortical reorganization that occurs following injury. In this study, we have assessed behavioral, physiological and neuroanatomical changes in a mouse model of focal photothrombotic injury in the forelimb motor region (caudal forelimb area, CFA). At the behavioral level, spontaneous motor tasks such as the Gridwalk Test, the Schallert Cylinder Test and the Swim Test indicated robust deficits that were selective for the contralesional forelimb and persisted for at least 30 days after stroke. Reconstruction of paw trajectories during a skilled reaching task indicated that several kinematic parameters were altered by focal ischemia, and these changes persisted up to 30 days. However, the percentage of successful grasps significantly recovered starting from the third week after stroke, pointing to the development of compensatory strategies. To identify physiological and morphological underpinnings of compensation, we used intracortical microstimulation to map motor representations and immunostaining to measure levels of plasticity markers in the perilesional areas at 30 days. Intracortical microstimulation revealed significant rewiring of cortical regions posterior to the lesion, i.e. the hindlimb motor cortex. In addition, the percentage of sites producing combined, forelimb/hindlimb/tail movements was enhanced in the peri-infarct zone. Neuroanatomical analyses in the peri-infarct cortex showed a decrease in the density of perineuronal nets, as well as of somatostatin- and parvalbumin-positive interneurons. Perineuronal nets are well known plasticity brakes and a reduction in intracortical inhibition has been previously shown to promote adult cortical plasticity. Thus, reductions in these markers may underlie spontaneous reorganization of the perilesional areas. These data provide baseline information on the behavioral, physiological and neuroanatomical changes triggered by a focal cerebral ischemia in mice. Ongoing experiments in our laboratories are applying these quantitative measures to the study of post-stroke rehabilitation. In particular, we are measuring functional recovery and cortical rewiring following rehabilitation with a robotic device that we have recently described (Spalletti et al., Neurorehabil Neural Repair, 2014).

Disclosures: C. Alia: None. **C. Spalletti:** None. **S. Lai:** None. **A. Panarese:** None. **A. Ghionzoli:** None. **S. Micera:** None. **M. Caleo:** None.

Poster

802. Ischemia: Cellular Mechanisms and Neuroprotection VII

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Title: A non-ionotropic activity of GluN2A-containing NMDA receptor confers neuroprotection

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Abstract: Function of N-methyl-D-aspartate receptor (NMDAR) is believed to depend on its channel activity. GluN2A- and GluN2B-containing NMDARs GluN2ARs and GluN2BRs) are the major subtypes of NMDARs, whose activations require agonist glutamate and co-agonist glycine. Here we show that glycine alone acts through an ifenprodil-sensitive binding site in the GluN1 subunit of GluN2ARs to enhance Akt phosphorylation independent of the channel activity of GluN2ARs, indicating that the ionotropic GluN2AR has a non-ionotropic property. The non-ionotropic GluN2AR mediates a neuroprotective effect of glycine against glutamate neurotoxicity-induced neuronal death *in vitro*. In a rat cerebral ischemia model, glycine prevents neuronal death and improves functional recovery in a NMDAR channel activity-independent manner. These results suggest that as a co-agonist of ionotropic NMDAR, glycine serves as an agonist to trigger a non-ionotropic activity of GluN2ARs and confers neuroprotection. This study uncovers a previously unknown mechanism whereby GluN2AR, through its non-ionotropic activity, exerts a different effect than GluN2BR in neuronal survival.

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Poster

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Topic: C.08. Ischemia

Support: Korean Ministry of Health and Welfare Grant HI11C2138 (B110072)

Title: The multi-herbal mixture HT047 improves functional recovery after focal cerebral ischemia in rats

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Abstract: HT047 is a multi-herbal mixture consisting of Pueraria lobata and Scutellaria baicalensis which have been used together to treat stroke in traditional Korean medicine. In the present study, the effects of HT047 on functional recovery after focal cerebral ischemia were investigated. Sprague-Dawley rats were subjected to 90 min of middle cerebral artery occlusion (MCAO) followed by reperfusion. HT047 were orally administered to rats for 14 days at a dose of 30 mg/kg or 100 mg/kg, starting 3 days after MCAO. Functional recovery was assessed at weekly intervals using the rotarod test and modified Neurological Severity Score (mNSS). In rotarod test, rats were trained for 7 days prior to induction of MCAO. The latency to fall on the accelerating rotarod was recorded for 5 min at 7, 14, 21, and 28 days after MCAO. The mNSS which includes both motor and sensory tests was scored from 0 (normal) to 7 (maximal deficit) at 7, 14, and 21 days after MCAO. Rats were weighed daily the day before MCAO and over 4 weeks after MCAO. At 14 days after MCAO, treatment with HT047 100 mg/kg significantly prolonged the time that rats remained on the rotarod by 62.6% compared with the control group (210.2 ± 23.5 vs. 129.3 ± 20.8 sec, $p<0.05$). HT047 100mg/kg-treated rats showed significantly lower mNSS scores than saline-treated rats at 21 days after MCAO (3.1 ± 0.4 vs. 4.5 ± 0.5, $p<0.05$). The body weights of rats treated with HT047 100 mg/kg continued to increase and were significantly different from those of control group over time from 3 days to 28 days after MCAO. In conclusion, HT047 treatment improves functional recovery and prevents weight loss after experimental stroke.

Disclosures: J. Song: None. **D. Lee:** None. **H. Kim:** None. **H. Lee:** None. **H. Kim:** None.

Poster

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Topic: C.08. Ischemia

Support: AHA 12PRE12060489

Title: Third ventricle neural stem cell niche dynamics following focal ischemic lesion

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[1]Neurobio. and Behavior, [2]Stony Brook Univ. Med. Ctr., STONY BROOK, NY

Abstract: In addition to the well established neural stem cell reserves in the adult mammalian brain located in the dentate gyrus (DG) of the hippocampal formation and the sub-ventricular zone (SVZ) lying along the lateral ventricle wall, the 3rd ventricle lying proximal to the hypothalamus has also been shown to house limited-potential progenitors. Local α or β tanycytes (a radial glia-like cell population), which slowly differentiate in response to leptin and insulin-growth factor (IGF) signaling, add neuronal and glial cells (NG2+) to the arcuate and paraventricular nuclei. The proliferative zone of the 3rd ventricle also extends into thalamic territory, though whether adult neurogenesis occurs in these dorsal reaches of the 3rd ventricle remains an open question. We studied the response of 3rd ventricle neural stem cells (NSCs) to focal thalamic lacunar ischemia in wild-type (WT) C57Bl6/J mice and in mice lacking the high mobility group B2 (HMGB2) gene - loss of which leads to heightened neurogenesis in the adult SVZ NSC niche. We have previously reported a role for this chromatin protein (HMGB2) in the modulation of embryonic neurogenesis and in the adult SVZ. We examine the response of HMGB2-associated transcriptional and epigenetic changes following ischemia, which could lead to insights into changes occurring in conditions such as Dejerine-Roussy syndrome (thalamic pain syndrome) or heat shock/exhaustion (hypothalamic in origin) - as both are caused by injury to those particular brain regions. Supported by: AHA Predoctoral Fellowship 12PRE12060489 to RB

Disclosures: R. Bronstein: None. **S.E. Tsirka:** None.

Poster

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Topic: C.08. Ischemia

Support: BAF grant

Title: Differential CSF-derived microparticle protein expression in humans with cerebral vasospasm after aneurysmal subarachnoid hemorrhage

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Abstract: Cerebral vasospasm (CVS) and related ischemic injury is a major contributor to death and disability after aneurysmal subarachnoid hemorrhage (aSAH). Our goal is to understand the molecular mechanisms underlying CVS in order to facilitate the rational development of safe, effective and reliable diagnostic and treatment paradigms. Our hypothesis is that there are specific early changes in protein expression related to endothelial injury and inflammation that induce CVS. Cerebro-spinao fluid (CSF) was collected daily from patients with aSAH. Microparticles (MPs), micromolecules released by vesicles, red and white blood cells, platelets and endothelial cells, were isolated using serial ultracentrifugation. Differential protein expression in CSF-MPs was analyzed by a mass spec (MS) based system using isotopically tagged peptides to profile the proteins and determine their relative concentrations in individual patient samples. These proteins were correlated with patient's clinical data and used to identify candidates for biomarkers predictive of CVS. More than 150 proteins were isolated from CSF-MPs. Proteomic and molecular pathways analysis revealed marked differential expression of proteins in patients with CVS. We identified specific candidate proteins that could potentially serve as early biomarkers for CVS. ApoE, ApoD, prostaglandin D5, synaptic nuclear envelope protein 1, clusterin, α-1-acid glycoprotein, plasma protease C1 inhibitor, and prostaglandin H2 D isomerase were downregulated in patients who developed CVS. Haptoglobin, fibrinogen α and γ chain, synaptic nuclear envelope protein 2, and hemoglobin subunits α and β were upregulated. Some of these proteins are associated with immune and metabolic processes and some have been specifically associated with cerebrovascular disease states. This is the first demonstration that there is differential protein expression in CSF-MPs from VS patients.. Alone or in combination, these and other proteins may be useful as biomarkers to guide in stratifying patients into categories of risk to develop CVS. They may also deepen our understanding of the mechanisms and facilitate the development of safer and more effective therapies for CVS.

Disclosures: M.M. Szymanska: None. **W.W. Ashley:** None.

Poster

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Topic: C.08. Ischemia

Support: F31 NS081834

Title: Glial responses and neuroregeneration in a pediatric model of ischemic stroke

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Abstract: Pediatric arterial ischemic stroke affects more than 1,000 children in the United States each year, with a vast majority of surviving children suffering long-term neurologic deficit with varying degrees of disability. Initial studies suggest that recovery from stroke is greater among juveniles compared neonates or adults. We have therefore developed a middle cerebral artery occlusion (MCAO) model of pediatric stroke in order to understand repair mechanisms and cellular responses that are unique to this juvenile developmental time period. One strikingly understudied aspect of stroke, especially in juvenile subjects, is its impact on glial cells, in particular myelinating glia. Therefore, the glial responses following experimentally-induced stroke in juvenile mice (20-25 days old) were investigated. GFAP immunoreactivity steadily increased in the lesioned striatum from 24 hours to 30 days following MCAO. Similarly, Iba1-positive microglia and NG2-positive cells proliferated in the lesioned area 24 hours after stroke, and remained increased after 30 days. Surprisingly, mature oligodendrocytes and gross myelin production were unaffected during the acute (24 hours and 3 days post MCAO) and subacute (7 days) recovery phases. However, during the chronic (30 days) phase, some myelin debris and signs of axon pathology were detected in the lateral striatum, where gliosis was most severe. Taken together, these results suggest that myelinating oligodendrocytes in the pediatric brain are resistant to the initial ischemic insult but that ongoing astrogliosis and microgliosis could be harmful. Extensive neuronal cell death occurred during acute phases of recovery throughout the lesioned striatum. During subacute and chronic phases, neuronal cell death continued in the lateral striatum. However remarkable recovery of the neuronal population was observed in the medial striatum, starting 7 days after stroke and continuing at 30 days. Consistent with this, we observed increased proliferation of doublecortin-positive cells in the ipsilateral subventricular zone and increasing numbers of doublecortin-positive neuroblasts were found in medial striatum. These data suggest that partial recovery in the juvenile brain occurs after experimentally induced stroke, and that recovery may be correlated with the degree of ongoing gliosis and the proximity to germinal centers, such as the subventricular zone. Understanding the unique cellular responses in the juvenile brain could yield valuable insight into enhancing cellular resistance to ischemia as well as promoting recovery after ischemia.

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Poster

803. Ischemia: Inflammation

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Support: Italian Ministry for Education, University and Research (MIUR)

Fellowship from University College Collegio Ghislieri, Pavia, Italy.

Title: Lymphocytes bioenergetics after stroke: A functional proteomic study

Authors: *F. FERRARI[1], D. GEROLDI[2], A. GORINI[1], G. PERO[3], R. F. VILLA[1];
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Abstract: BACKGROUND - Modulation of molecular and cellular inflammatory responses is a potential target of neuroprotection but an in-depth understanding of their therapeutic implications in the brain ischemia is still lacking [1]. Moreover, stroke leads to important metabolic alterations and energy availability is required for molecular and cellular brain functions [2]. This in progress, systematic research studies the catalytic properties of cytoplasmic and mitochondrial enzymes of stroke patients' lymphocytes, indicative of cellular-molecular mechanisms of immune system engagement and predictive markers of neuronal energy transduction abnormalities. METHODS - Controls and stroke patients were divided by sex and age and the catalytic properties of these regulatory enzymes were assayed: hexokinase (HK), lactate dehydrogenase (LDH) for glycolysis; citrate synthase (CS), malate dehydrogenase (MDH) for Krebs' cycle (TCA); NADH-cytochrome c reductase (Complex I-III), succinate dehydrogenase (SDH, Complex II), cytochrome oxidase (COX, Complex IV) for Electron Transfer Chain (ETC); glutamate dehydrogenase (GlDH), glutamate-oxaloacetate- (GOT) and glutamate-pyruvate- transaminase (GPT) for amino acids/glutamate metabolism. RESULTS - Because of the little number of patients enrolled so far, results are referred only to the gender effect; even so, stroke changed lymphocytic enzyme activities of patients, particularly in females, reflecting the metabolic dysfunction of ischemic brain, like for other pathologies [3, 4]. Overall, the glycolytic and the aerobic flux in TCA and at the ETC entry level points were increased, although the COX activity was decreased because of the lack of O2 as electron terminal acceptor. Moreover, GlDH was decreased explaining on functional basis the glutamate accumulation in the early ischemic phase [5]. CONCLUSIONS - The biochemical machinery of lymphocytic bioenergetics suggests

different responses to ischemia in males and females and this functional proteomic approach may be a suitable model to study drug actions. In the research progress, we will increase the number of enrolled patients, taking into account also the age factor and considering the recovery phases, when inflammation become more relevant for patients' outcome, REFERENCES - [1] Iadecola C, Anrather J. 2011. Nat Med, 17:796-808; [2] Villa RF et al. 2013. Neurochem Int, 63:765-81; [3] Molina JA et al. 1997. Neurology, 48:636-8; [4] Leuner K et al. 2012. Mol. Neurobiol. 46:194-204; [5] Cooper AJ. 2012. Neurochem Res, 37:2439-55. AKNOWLEDGEMENT - The research was supported by MIUR and a Fellowship from University College Collegio Ghislieri, Pavia, Italy.

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Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT 80811, 226454

232656

Title: Pharmacological evaluation of the antinociceptive and anti-inflammatory activities of limonene and nerol in the oxazolone-induced colitis model

Authors: ***A. E. GONZÁLEZ-RAMÍREZ**[1], F. LOPEZ MUÑOZ[1], M. GONZALEZ TRUJANO[2], M. GONZALEZ TRUJANO[2], M. GONZALEZ TRUJANO[2], M. GONZALEZ TRUJANO[2], M. GONZALEZ TRUJANO[2], M. GONZALEZ TRUJANO[2];
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Abstract: Monoterpenes are natural products and main constituents in essential oils, plant resins and citrus. Antinociceptive, anti-inflammatory and antioxidative activities have been attributed to this kind of compounds. In a chronic inflammatory condition like ulcerative colitis, a painful disease with clinic manifestations as diarrhea, pain and abdominal cramps, ulcers, weight and

appetite loss and fatigue; it has been involved the nociceptive and inflammatory reactions linked to a Th2 immune response that leads to release of cytokines such as: IL-4, IL-5, IL-13 and IFN-γ. Because of this, the aim of this investigation was to analyze the antinociceptive and anti-inflammatory effects of the monoterpenes limonene and nerol by using oxazolone-induced colitis model in BALB/c mice. The evaluation of pathological characteristics considered Disease Activity Index (DAI) and Colonic Damage Index (CDI) as pathological markers that were used to evaluate the effect of monoterpenes and a positive control treatments; as well as secondary hyperalgesia which was determined by using Von Frey filaments as a mechanical stimulus and histological estimation in colon sections performed with hematoxylin-eosin staining. Results demonstrated that individual administration of limonene and nerol reduced the oxazolone-induced colonic damage markers with a significant decrease in DAI at a 100 mg/kg dosage and in CDI at 177.8 and 300 mg/kg. In addition, these monoterpenes reduced hyperalgesia from a dosage of 30 mg/kg. Finally, the histological analysis gave evidence that monoterpenes treatment prevented the oxazolone-induced colonic damage in lamina propia, a highly vascular layer of connective tissue under the basement membrane lining a layer of epithelium, previously observed in control subjects. In conclusion, this study gives preclinical pharmacological evidences of therapeutic properties of nerol and limonene in a painful disease like ulcerative colitis.

Disclosures: A.E. González-Ramírez: None. **F. Lopez Muñoz:** None. **M. Gonzalez Trujano:** None. **M. Gonzalez Trujano:** None. **M. Gonzalez Trujano:** None. **M. Gonzalez Trujano:** None. **M. Gonzalez Trujano:** None. **M. Gonzalez Trujano:** None.

Poster

803. Ischemia: Inflammation

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Support: CONACyT 80811,226454

Post-degree fellowship 232903

Title: Diosmin reverses hyperalgesia in a model of neuropathic pain in rats involving the opioidergic system

Authors: *A. CARBALLO[1], M. GONZÁLEZ-TRUJANO[2], F. J. LÓPEZ-MUÑOZ[1];
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Abstract: Despite of the discovery of new drugs and their possible involved mechanism of action, the treatment for neuropathic pain remain unclear; there is not single treatment that works in all conditions of neuropathic pain and its underlying mechanisms. Conventional therapy such as non-steroidal anti-inflammatory drugs, opioids, tricyclic anti-depressants, anti-convulsants and topical medicine have been associated with various adverse effects, withdrawal syndromes and multiple pathological mechanisms that are not suitable for all types of neuropathy. In psychiatric medicine, it is developing phytomedicines, including analgesics with the purpose of using complementary and alternative therapies. Diosmin (Diosmetin-7-O-rutinoside) is a flavonoid that possesses anti-diabetic, antioxidant, anxiolytic and anti-inflammatory activities. These properties suggest that diosmin is a possible candidate for the relief of neuropathic pain, which antinociceptive effect could be mediated through of an opioid pathway. In this investigation, painful neuropathy was induced in male rats using the Constriction Chronic Injury (CCI) model in order to investigate the involvement of opioid pathway in diosmin-induced effects. We tested the non-selective opioid receptors naloxone given 10 min before to diosmin or its vehicle administration in acute treatment. The evaluation of an hyperalgesic response was measured with mechanical and thermal withdrawal thresholds. Diosmin administrated at dose of 316.2 mg/kg, produced a significant inhibition of the withdrawal thresholds in CCI model in both responses. Animals receiving pre-treatment of naloxone (1 mg/kg, i.p.) reduced the antinociceptive response of diosmin in both tests. Our data showed that systemic administration of diosmin decreased the CCI-induced hyperalgesia mediated by an opioid mechanism suggesting that diosmin is a potential useful flavonoid for the therapy of neuropathic pain.

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Poster

803. Ischemia: Inflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Dyad of CD40/CD40 ligand fosters neuroinflammation and cognitive impairment: Implications for sepsis-associated encephalopathy

Authors: *F. PETRONILHO[1,2], M. MICHELS[2], A. VIEIRA[2], D. FLORENTINO[2], D. ZAPELINI[2], M. MODOLON[2], F. MINA[3], G. Z. REUS[1], D. D. LEFFA[1], T. BARICHELLO[1], F. DAL-PIZZOL[3], J. QUEVEDO[1];
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Abstract: Introduction: Sepsis is a clinical condition associated with high morbidity and mortality in patients in intensive care units. The most frequent complication of sepsis is sepsis-associated encephalopathy (SAE), characterized by decline of mental processes, impaired attention, delirium and coma. Several factors contribute to this condition such as generation of proinflammatory cytokines, blood brain barrier (BBB) permeability, oxidative stress and molecules could amplifying this responses. CD40 molecule expressed in microglia active and your ligand CD40L can exert this function in different diseases that involve neuroinflammatory process. Therefore, for all the points raised so far, the objective to propose an investigation if the dyad CD40/CD40L fosters neuroinflammation and cognitive impairment in sepsis animal model. Materials and Methodos: Male Wistar rats were subjected to cecal ligation and puncture (CLP) to induce sepsis. The animals were killed 12, 24 and 48hours after CLP surgery and removed the hippocampus to evaluate the CD40 and CD40L levels by western blotting. In another experiment, Wistar rats received antibody anti-CD40 intracerebroventricularly and were divided in sham (control), CLP, CLP + anti-CD40 100mg/kg and killed 24 hours after surgery to cytokine determination (IL-1β, TNF-α and IL-6) and BBB permeability. Ten days after sepsis, in another experiment, the animals were submitted to the habituation to the open field and aversive memory to the step-down inhibitory avoidance task. Data were evaluated by ANOVA and Tukey post hoc test. Man Whitney test was utilized for behavioral analysis with all significant at $p<0.05$. Results: We observed in the hippocampus an increase of CD40 and CD40L in 12 and 24 hours after CLP. The use of anti-CD40 reverted the increase of BBB permeability and levels of IL-1β, IL-6 and TNF-α in the hippocampus 24 hours after CLP. The anti-CD40 additionally reverted the cognitive impairment. Conclusion: The results observed in this study reinforce the role of CD40-CD40L in the neuroinflammatory response and cognitive impairment verified in SAE

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Poster

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Title: Protective role of high mobility group box 1-toll-like receptor 2 axis on ischemia-induced oligodendrocyte death

Authors: *J. CHOI[1], B. G. KIM[2,4,3];
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Abstract: Ischemic white matter injury is commonly encountered in clinical practices resulting in various neurologic disabilities ranging from sensorimotor dysfunctions to severe cognitive impairments. Demyelination with oligodendrocyte (OL) loss is a prominent histopathological feature of ischemic white matter injury. Therefore, protection of OLs from ischemic insults would be an important therapeutic strategy to overcome neurologic disabilities caused by ischemic white matter injury. Previously, we found that Toll-like receptor 2 (TLR2) expressed in OL lineage cells provides cell-autonomous protective effects. It has been shown that certain endogenous ligands can activate TLR2 in sterile pathological conditions. Among them, high mobility group box 1 (HMGB1), well known danger associated molecular pattern protein, is released from dying cells and elicit inflammation and/or promote tissue repair. In the present study, we identified HMGB1 as an endogenous TLR2 ligand to promote protective effect in ischemia-induced OL death. After oxygen-glucose deprivation (OGD), OLs released HMGB1 into bathing media as early as 5 minutes after OGD completion. Conditioned media collected from cultured OLs exposed to OGD exhibited protective activity against OGD-induced OL death in a TLR2-dependent manner. Immunodepletion of HMGB1 from the conditioned media abolished the protective activity. In addition, application of glycyrrhizin, a specific HMGB1 inhibitor, resulted in the aggravation of OGD-induced OL death, suggesting that HMGB1 released from OLs may exert endogenous protective effects on OLs through binding to TLR2.

Furthermore, exogenous recombinant HMGB1 application after OGD in OL culture also reduced OGD-induced OLs death. In primary microglia culture, HMGB1 application increased the expression of insulin-like growth factor 1 (IGF-1) a well-known OL survival factor, in a TLR2-dependent manner, suggesting a dual mode of OL protection by HMGB1. We confirmed the protective effects of HMGB1 in a endothelin-1-induced focal white matter stroke model. Animals with glycyrrhizin co-injection showed worsening neurobehavioral parameters measured by corner test and pole test compared to those with vehicle co-injection. Preliminary data also revealed the expansion of demyelinating lesion by glycyrrhizin. These results suggest that HMGB1 may act as an endogenous TLR2 ligand mediating protective effects on OLs after ischemic insult. Further studies on HMGB1-TLR2 signaling axis may reveal a novel therapeutic target to treat ischemic white matter injury.

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Poster

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Title: Spatiotemporal profile of blood monocyte subsets accumulation in the post-ischemic brain

Authors: *L. GARCIA-BONILLA, G. FARACO, J. MOORE, D. BREA, G. RACCHUMI, C. IADECOLA, J. ANRATHER;
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Abstract: Inflammation is a key component of ischemic stroke pathophysiology, participating both in tissue injury and repair (Nat Med 17:796, 2011). A key feature of post-ischemic inflammation is the infiltration of blood monocytes. There are two main monocytes subsets in the mouse blood: $CCR2^{+}/CX3CR1^{-}/Ly6C^{hi}$ "inflammatory" monocytes involved in acute inflammation, and $CCR2^{-}/CX3CR1^{+}/Ly6C^{lo}$ "patrolling" monocytes, which may play a role in repair processes (Immunity 19:71, 2003; J Exp Med 204:3037, 2007). CCR2 mediates the trafficking of $Ly6C^{hi}$ cells, while CX3CR1 of $Ly6C^{lo}$ cells. We hypothesize that inflammatory

monocytes are present in the early phase after ischemia, whereas patrolling monocytes infiltrate the brain at later times. To this end, lethally irradiated C57BL/6 mice (7 weeks old) were transplanted with $CX3CR1^{GFP/+}CCR2^{RFP/+}$ bone marrow. The restricted expression of GFP in hematogenous CX3CR1 cells allows differentiating infiltrating $CX3CR1^{GFP/+}$ monocytes from CNS-resident ($CX3CR1^{+}$) microglia. Five weeks after transplant, the chimeric mice underwent transient middle cerebral artery occlusion (MCAO) and were sacrificed 1, 2, 3, 5, 7, 14 or 28 days later (n=3-4/group). $CX3CR1^{GFP/+}$ and $CCR2^{RFP/+}$ fluorescent cells were mapped in serial sections using anatomical reconstruction and semi-automated cell counting**.** The number of $CCR2^{+}$ inflammatory monocytes in the ischemic brain peaked 3 days after MCAO ($3.8\pm0.9x10^5$ vs. $0.2\pm0.1x10^5$ cells in sham mice; $p<0.05$; mean±SE), returning toward baseline at day 7 ($0.8\pm0.2x10^5$) and 28 ($0.9\pm0.2x10^5$). $CCR2^{+}$ cells were observed mainly in the infarct core. On the other hand, $CX3CR1^{+}$ cells increased slightly at day 1 ($1\pm0.1x10^5$), 3 ($1.4\pm0.3x10^5$), 5 ($3.2\pm0.5x10^5$; $p<0.05$), and 7 ($1.8\pm0.3x10^5$, $p<0.05$) from sham ($0.6\pm0.1x10^5$), and peaked 14 days after MCAO ($7.1\pm0.8x10^5$, $p<0.05$). At day 28, $CX3CR1^{+}$ cells were still increased ($6.4\pm1.2x10^5$, $p<0.05$). $CX3CR1^{GFP/+}$ cells exhibited 3 distinct phenotypes: macrophage-like cells (amoeboid with retracted processes), microglial-like cells (with ramified processes) and perivascular cells (elongated shape). GLUT-1, PDGFRα, NeuN, and GFAP co-staining 28 days after MCAO revealed that $CX3CR1^{+}$ cells are phenotypically distinct from endothelial cells, pericytes, neurons or astrocytes. The data suggest that $CCR2^{+}$ monocytes are rapidly recruited to the injured brain, whereas $CX3CR1^{+}$ monocytes populate the infarct at later times. Although assessing the role of these monocytes subsets will require additional studies, it is conceivable that $CCR2^{+}$ cells are involved in the acute phase of the damage, while $CXCR1^{+}$ cells play a role in post-ischemic repair processes.

Disclosures: L. Garcia-Bonilla: None. **G. Faraco:** None. **J. Moore:** None. **D. Brea:** None. **G. Racchumi:** None. **C. Iadecola:** None. **J. Anrather:** None.

Poster

803. Ischemia: Inflammation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 803.09/V30

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: PhD Posgraduate Program of Pharmacology, CUCS

Title: Acute and chronic exposure to ozone produce lipid peroxidation in rat hippocampus

Authors: *M. L. MENDOZA-MAGANA, J. J. RAMIREZ-VAZQUEZ, M. A. RAMIREZ-HERRERA, J. C. FRIAS-MARQUEZ, A. A. RAMIREZ-MENDOZA, G. CAMARGO-HERNANDEZ;
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Abstract: Air pollution is a worldwide problem especially in large cities, where ozone (O3) and other toxic compounds constitute the smog. The concentration of such compounds increases due to the combustion of fossil fuels and industrial activity. Exposure to O3 is a risk factor to develop neurodegenerative diseases because of its high oxidizing power. The olfactory mucosa is the direct entryway to the CNS and the respiratory system constitutes the indirect route to the nervous system through blood stream. We evaluated the effect of an acute and chronic exposure O3 in cellular lipids of the hippocampus in young Wistar rats. The experimental group was placed in an acrylic exposure chamber with an artificial atmosphere containing O3 (0.7 ppm) and the control group was exposed to environmental air using the same chamber. The exposure acute (15 days) and chronic (60 days) was performed daily during 4 hours. Malondialdehyde (MDA) and 4 hydroxyalkenal (HAE) concentration of in hippocampus homogenates were analyzed. Lipid peroxidation was determined by a colorimetric assay as indicated by the manufacturer (Oxford Biomedical Research) and spectrophotometrically determined at 586 nm. Data were analyzed by ANOVA test and Bonferroni as post-hoc test. MDA/HAE concentration in the control group was 5.60 ± 1.85 and 5.95 ± 2.33 μM of protein in acute and chronic exposure respectively. MDA/HAE concentration in the O3 exposed group was 14.89 ± 1.52 and 21.15 ± 9.55 μM of protein in acute and chronic exposure respectively. Lipid peroxidation, as well as other assays to evaluate oxidative damage, are required to establish the oxidative status in experimental exposure to O3 as air contaminant. Neurodegenerative diseases usually bear the oxidative damage as part of their physiopathological hallmarks, but oxidative damage could play a more crucial role as initiator that triggers a cascade of events that may lead to the development of diverse diseases. It contributes to the maintenance of chronic inflammation in degenerative diseases.

Disclosures: M.L. Mendoza-Magana: None. **J.J. Ramirez-Vazquez:** None. **M.A. Ramirez-Herrera:** None. **A.A. Ramirez-Mendoza:** None. **J.C. Frias-Marquez:** None. **G. Camargo-Hernandez:** None.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.01/V31

Topic: C.08. Ischemia

Title: Distribution of ß-hydroxybutyrate (bhb) in cd-1 mice after experimental stroke

Authors: *K. A. KOCH, J. KONIETZKA, D. BERRESSEM, G. ECKERT, J. KLEIN; Pharmakologisches Inst. für Naturwissenschaftler, Goethe Univ. Frankfurt Am Main, Frankfurt Am Main, Germany

Abstract: Stroke is a major medical emergency and the second most frequent cause of death in the world. While drug treatment of stroke remains unsatisfactory, dietary approaches to stroke prevention and regeneration, such as ketogenic diet, have recently attracted much interest. (R)-3-Hydroxybutyrate (BHB) is the most important ketone body and is a precursor of acetyl-CoA which can be used as an energy source for the citric acid cycle in the brain. In the present study, we have used a common experimental stroke model, middle cerebral artery occlusion (MCAO), in mice to investigate the levels of BHB before and after brain ischemia. In female CD-1 mice, ischemia was induced in the left hemisphere with the MCAO method. After 90 min of ischemia, mice were sacrificed, blood was withdrawn, and liver and brain tissue were harvested and extracted for BHB measurements. After derivatisation with BSTFA/TMCS, the extracts were measured by GC-MS. We found that ischemia induced a 13-fold increase of BHB levels in the left (stroked) hemisphere when compared with sham-operated animals (control group: 105 ± 13,6 µM calculated for brain water; stroke group: 1385 ± 280 µM; mean ± S.E., N=6). In the right (healthy) hemisphere, an 11-fold increase was observed (control group: 99,3 ± 9,1 µM; stroked group: 1121 ± 237 µM). Liver homogenates showed the following results: control group, 258 ± 19 µM; stroked group, 3.688 ± 481 µM (calculated for intracellular water). Plasma levels of BHB showed the same tendency: control group, 61,4 ± 17 µM; stroked group: 924 ± 231 µM. Our data show massive increases of BHB levels in three compartments induced by ischemia in female CD-1 mice. The data demonstrate a strong production of ketone bodies by the liver which are accompanied by massive increases of BHB in plasma and in both hemispheres of the brain. Our current work focuses on the elucidation of the underlying interaction between brain and regulation of liver metabolism.

Disclosures: K.A. Koch: None. **J. Konietzka:** None. **D. Berressem:** None. **G. Eckert:** None. **J. Klein:** None.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.02/V32

Topic: C.08. Ischemia

Support: NIH/NINDS grants NS079345

VA Merit Review grants RX000199

VA Merit Review grants BX002346

Title: Recombinant NAMPT exerts neuroprotective effects against cerebral ischemia

Authors: *X. R. CHEN[1,2], Z. JING[1,2], A. STETLER[1], Y. LUO[3], X. JI[3], S. H. GRAHAM[1,2], G. CAO[1,2,3];
[1]Dept. of Neurol., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Geriatric Research, Educ. and Clin. Ctr., Veterans Affairs Pittsburgh Healthcare Syst., Pittsburgh, PA; [3]Xuanwu Hospital, Capital Med., Beijing, China

Abstract: The protective effects of Nicotinamide phosphoribosyltransferase (NAMPT) have been observed across multiple systems, including brain injury, using both transgenic overexpression and viral delivery of the NAMPT transgene. To assess the more clinically relevant delivery of NAMPT as a recombinant protein, we investigated the impact of recombinant NAMPT on brain ischemia/reperfusion injury. METHODS: Recombinant NAMPT preparations were generated from either bacterial E. coli (PT-E.coli) or mammalian 293T cells (PT-293T). Transient focal cerebral ischemia was induced in male C57BL/6 mice by 60 min occlusion of the middle cerebral artery (MCAO) followed by 72 h reperfusion. Three different administration paradigms were used: 1) a single dose administered i.p. delivered at 1 h, 3h and 5h after stroke, 2) a single dose administered i.c.v. at 1 h after stroke, and 3) three doses of i.c.v administration (12 h prior to, right before and 12 h after MCAO). Body weight was monitored over 3 d post-surgery, and neurological dysfunction and brain infarct volume were assessed at 72 h post-stroke. Corner test and Rotarod test were also included. RESULTS: Intraperitoneal administration of either PT-E.coli or PT-293T reduced brain infarct size in a dose-dependent manner. PT-293T markedly reduced brain damage when administered within 3 h after transient ischemia and exhibited a larger therapeutic window compared to PT-E.coli. Reduction of ischemic brain injury was correlated with reduced functional impairment in mice. A single dose of PT-293T i.c.v injection exerted better neuroprotective effect compared to i.c.v. administration of PT-E.coli in the mouse model of ischemic stroke. In the paradigm which delivered recombinant proteins three times (i.c.v.) within the same animal, PT-293T also exerted significant neuroprotective effects. CONCLUSIONS: Recombinant delivery of NAMPT provides significant protection against ischemic brain injury. We have found that PT-293T appears to exert better ischemic neuroprotection compared to PT-E.coli. Taken together, these

results provide validation of recombinant NAMPT delivery as potential neuroprotective strategy for stroke.

Disclosures: **X.R. Chen:** None. **G. Cao:** None. **A. Stetler:** None. **Y. Luo:** None. **X. Ji:** None. **S.H. Graham:** None. **Z. Jing:** None.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.03/W1

Topic: C.08. Ischemia

Support: Heracleitus II Grant. KA3396

Title: 2-Arachidonoylglycerol protects the retina from AMPA excitotoxicity *in vivo* via the activation of CB1 receptor. Involvement of the PI3K/Akt signaling pathway

Authors: *D. KOKONA[1], A. ZIMMER[2], K. THERMOS[1];
[1]Univ. of Crete, Heraklion, Greece; [2]Inst. of Mol. Psychiatry, Univ. of Bonn, Bonn, Germany

Abstract: Retinal ischemia leads to excitotoxicity and neurodegeneration that result in loss of visual acuity and blindness. It is prominent in retinal diseases such as diabetic retinopathy and glaucoma. Even today there are no efficacious treatments available for the treatment of neurodegenerative eye disease. New neuroprotective therapeutic targets are essential to protect retinal neurons and prevent blindness. Cannabinoids have been shown to protect the CNS, including the retina, from ischemic insults. An endocannabinoid system, which includes the endocannabinoids [2-arachidonoylglycerol (2-AG) and anandamide] and the cannabinoid receptors (CB1 and CB2), is present in the retina. In the present study we examined the ability of the endocannabinoid 2-AG to afford neuroprotection in the retina in an *in vivo* model of AMPA excitotoxicity, and the mechanisms involved in the neuroprotection. Sprague Dawley rats were intravitreally injected with PBS or AMPA (42nmol/eye). In previous work in our laboratory we detected the loss of retinal amacrine (cholinergic and nitric oxide synthetase containing) and horizontal cells, twenty four hours after the AMPA administration, but no loss of photoreceptors, rod bipolar or ganglion cells. Using bNOS immunoreactivity we examined the effect of 2-AG (10-8, 10-7, 10-6 M) in reversing retinal cell loss. We also used CB1 (AM251, 10-6 M) and CB2 (AM630, 10-6 M) antagonists, and JWH015 (CB2 agonist, 10-8, 10-7, 10-6 M) to elucidate the involvement of each receptor in the neuroprotection. In addition, wild type, CB1-/- and CB2-/-

C57BL/6 mice were also intravitreally administered with AMPA(21nmol/eye) or AMPA + 2-AG (10-7 M) or vehicle to validate the role of CB1 and CB2 receptors in the neuroprotection. In addition, Akt2-/- C57BL/6 mice were employed to investigate the involvement of the PI3K/Akt2 downstream signaling pathway, in the neuroprotection afforded by 2-AG. The results suggest that the endocannabinoid 2-AG protects the retina from AMPA excitotoxicity *in vivo* via a mechanism involving the CB1 receptor and the activation of the PI3K/Akt2 signalling pathway. The AMPA excitotoxicity model is useful in determining the early effects of ischemia. The pharmacologic profile of 2-AG renders it a promising therapeutic agent in retinal disease whose pathophysiology involves ischemic and excitotoxic insults. Ongoing studies are investigating other a) receptors involved in the neuroprotective effects of 2-AG, such as the TRPV receptor, and b) cannabinoids, as well as the inhibitors of the metabolic enzymes of the endocannabinoids, as possible targets of retinal therapeutics.

Disclosures: D. Kokona: None. **A. Zimmer:** None. **K. Thermos:** None.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.04/W2

Topic: C.08. Ischemia

Support: Department of Anesthesiology Research Funds, NYU Langone Medical Center

Title: A single, intravenous injection of minocycline prior to aortic occlusion is sufficient to reduce severe hind-limb motor impairment, attenuate spinal astrogliosis, and preserve neuronal cytoarchitecture in rats

Authors: E. JAFFREY[1,2], *A. SIDERIS[2], B. PISKOUN[2], T. J. J. BLANCK[3], B. DRENGER[5], E. RECIO-PINTO[4];
[1]NYU CAS, New York, NY; [2]Anesthesiol., [3]Anesthesiology, Neurosci. & Physiol., [4]Anesthesiology, Biochem. & Mol. Pharmacol., NYU Langone Med. Ctr., New York, NY; [5]Anesthesiol. and Critical Care Med., Hadassah–Hebrew Univ. Med. Ctr., Jerusalem, Israel

Abstract: The incidence of paraplegia following surgery or endovascular stenting of the thoracic aorta remains unacceptably high, despite advances in anesthetic and surgical management. Reducing the current paralysis rate is achieved by interventions which are mostly physical in nature. There is a need to find or develop pharmacological compounds administered

prophylactically and aimed at further lowering the patient risk of spinal cord ischemia. Minocycline is a tetracycline derivative, and has been used for many years in humans at low doses. There is mounting pre-clinical evidence for a new use of this old drug. Minocycline appears to be neuroprotective in several animal models of cerebral ischemia, stroke and inflammation; however, these effects are uncertain. Here, we used a model of spinal cord ischemia in which the thoracic aorta was occluded with a 2F Fogarty Catheter for 18 minutes under isoflurane anesthesia. A high intravenous dose, 10mg/kg, of minocycline hydrochloride was administered 30 minutes prior to aortic occlusion in a group of rats. Hindlimb motor function was evaluated using a modified Tarlov scale at 3 hours, 24 hours and 48 hours after reperfusion in naïve, sham, and drug-treated sham and injured animals. The occlusion time, under isoflurane anesthesia, produced severe hindlimb paralysis (Tarlov grade< 1) in 80% of rats acutely after reperfusion, and paralysis was maintained for 24 hrs and 48 hrs in 75% and 60% of animals, respectively. Interestingly, the single minocycline injection effectively mitigated severe hindlimb motor impairment (Tarlov grade ≥ 2) in 80% of animals at 3hrs after injury, and in 100% of the animals at 24 and 48 hours after reperfusion. Spinal cord tissue was collected 48 hours after reperfusion, and processed for histological staining (H&E and GFAP+). Quantification of GFAP+ areas indicated that relative to controls, ischemia induced significant astrogliosis in white matter ($p<0.0001$) and in gray matter ($p<0.0001$) of thoracolumbar spinal cord sections-areas caudal to the occlusion. Minocylcine significantly reduced astrogliosis in these areas (white matter, $p<0.05$; gray matter $p<0.0001$). Our data indicate that in addition to its reported inhibitory effects on microglia, minocycline may modulate the glial response to injury via inhibition of reactive astrocytosis. Minocycline also protected against neuronal damage as evidenced by an increased percentage of intact spinal cord tissue and decreased vacuolation in gray matter. Together, our data indicate that a single, high dose of minocycline administered prophylactically may be helpful in alleviating severe behavioral deficits in a model of spinal cord ischemia in rats.

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Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.05/W3

Topic: C.08. Ischemia

Support: Hornick Foundation at UAMS

Title: Dodecafluoropentane emulsion extends the time for effective tPA stroke therapy

Authors: *R. D. SKINNER[1], C. ARTHUR[2], A. T. BROWN[2], J. LOWERY[3], W. C. CULP[2]; [1]Dept. Neurobiol & Dev Sci. and Ctr. Translational Neurosci., [2]Dept. Radiology, [3]Dept. Lab. Animal Med., Univ. Arkansas Med. Sci., LITTLE ROCK, AR

Abstract: Nanometer-size bubbles of the perfluorocarbon Dodecafluoropentane in emulsion (DDFPe) carry large quantities of oxygen and can protect the penumbra in animal stroke models up to 24 hours. Currently the treatment of choice for ischemic stroke is the clot-lysing drug tissue Plasminogen Activator (tPA). However, due to contraindications, most commonly delays of >3 hrs (FDA) or >4.5 hrs (common use) post stroke, less than 5% of stroke patients receive IV tPA therapy. We tested the time constraint for stroke treatment using DDFPe to extend the time window for application of tPA. One hour IV delivery of DDFPe is clinically practical since, in the MagFast stroke trial, 73% of patients received IV drugs in <1 hr of onset and 97% in <2 hrs. The rabbit model was chosen because tPA transitioned into clinical care from rabbit testing. Many more patients will meet tPA criteria if DDFPe can be given early in the field. Thus, as in the phrase "Time Is Brain", effective extension of time to successful tPA treatment or other revascularization saves brain. Aim: To test the penumbra protecting ability of DDFPe to safely extend the time of tPA thrombolysis to 9 hrs after stroke, double or triple the current standard. Methods. IACUC approval was obtained. Under isoflorane anesthesia New Zealand rabbits (4-5 kg, N=23) received selective angiography followed by injection of a 4 mm blood clot in the internal carotid artery for flow directed middle cerebral artery occlusion. Angiography was repeated. Rabbits were recovered and randomly assigned to tPA (n=8), and DDFPe+tPA (n=7) groups. Standard 1 hr treatment of IV tPA (0.9 mg/kg) was given starting 9 hrs post stroke. In one group DDFPe (0.3 ml/kg of 2% emulsion) dosing began at 1 hr and continued at 90 min intervals for 6 doses; others received saline injections. Historic controls without therapy were compared (n=8). At 24 hrs Neurological Assessment Scores (NAS, 0-10) were determined. Then the brain was removed, sectioned, and stained with triphenyltetrazolium chloride. The area of stroke was measured in each section and a percent Stroke Volume (%SV) of the brain was computed. Results at 24 hrs: For NAS values, DDFPe+tPA was improved vs. controls, p=0.013, and also vs. the tPA alone group, p=0.032. The tPA only and controls were not different, p=.89. For %SV the combination treatment DDFPe+tPA was more effective, mean 0.80, vs. tPA only, mean 2.24, (p=0.0045). Conclusion. With reperfusion the combination of DDFPe+tPA therapy was more effective than tPA alone in preserving functioning brain tissue after stroke as determined by NAS and also %SV. Thus, DDFPe therapy significantly extends the time at which administration of tPA is effective by 2-3 times.

Disclosures: **R.D. Skinner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent pending. **C. Arthur:** None. **A.T. Brown:** None. **J. Lowery:** None. **W.C. Culp:** E. Ownership Interest (stock,

stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent pending.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.06/W4

Topic: C.08. Ischemia

Support: NIH NINDS

VA Merit

AHA Postdoctoral Fellowship

Title: Hsp70 inhibits dynamin expression: A new mechanism of protection in cerebral ischemia?

Authors: *N. KIM[1], J. KIM[1], J. LEE[2], M. A. YENARI[1];
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Abstract: The 70 kD heat shock protein (Hsp70) has been shown to protect brain cells from apoptosis in animal and cell-culture models of stroke. The anti-apoptotic mechanism of Hsp70 is still not completely understood, particularly in the central nervous system. In a previous study, our lab found that Hsp70 overexpressing transgenic (Tg) mice were protected from experimental stroke compared to wild type mice. One protein particularly suppressed in the Hsp70 Tg brains was dynamin. Dynamin is best known for its role in endocytic mechanisms but has also been shown to translocate Fas protein to the cell surface where it can be bound by FasL. Once activated, the Fas receptor triggers one of several extrinsic apoptosis pathways leading to caspase-dependent cell death. Our study investigates the relationship between dynamin-Fas signaling and Hsp70's anti-apoptotic role in stroke. We subjected groups of wildtype, Hsp70 transgenic (Tg), and Hsp70 knockout (Ko) mice to cerebral ischemia using distal MCAO (dMCAO). Brains were assessed for dynamin, membrane Fas, and caspase-8 expression 3d following dMCAO as well as for infarct size and neurological behavior 14d post dMCAO. We found that dynamin is upregulated by ischemia and colocalizes primarily to neurons. In addition, Hsp70 overexpression protects the brain following dMCAO as evidenced by decreased infarct volume (n=6/group, $P<0.05$), improved neurobehavioral outcomes ($P<0.05$), and decreased brain

dynamin, membrane Fas, and caspase-8 expression relative to wildtype and KO groups. Neuron cultures treated with a dynamin antagonist showed reduced cell death following oxygen glucose deprivation (OGD, $P<0.01$). We locally administered the same dynamin antagonist in a mouse model of stroke (n=7/group) and saw improved neurobehavioral outcomes. Our results indicate dynamin as a potential target for treating cerebral ischemia. Furthermore, these findings identify a novel mechanism underlying Hsp70's neuroprotective, anti-apoptotic effects and may offer significant implications toward new therapeutic strategies for stroke.

Disclosures: **N. Kim:** None. **J. Kim:** None. **J. Lee:** None. **M.A. Yenari:** None.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.07/W5

Topic: C.08. Ischemia

Support: Harvey Peters Foundation

Title: Cerium oxide nanoparticles improve survival and motor function in a *Drosophila* model of stroke

Authors: J. A. BATES, B. LOCKLER, *K. S. HOCKEY, C. A. SHOLAR, J. COOK, A. S. FREY, M. J. BILLINGS, B. A. RZIGALINSKI;
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Abstract: After stroke, return of circulation and oxygenation is known to produce abundant free radicals that impart damage to brain tissue and associated functional deficits. Based on the antioxidant activity of cerium oxide nanoparticles (CeONP) and their neuroprotective effects in other models of neurodegenerative disease, we reasoned that CeONP may be useful in treatment of neuronal deficits associated with stroke. In our prior tissue culture studies of mixed brain cells exposed to anoxia, CeONP protected neurons from cell death and maintained near-normal basal and agonist-stimulated intracellular free calcium levels. Therefore we hypothesized that CeONP would improve survival and functional outcome in a *Drosophila* model of stroke. Male and female Oregon R *Drosophila* were separated and collected upon eclosure, and fed standard fly mix or mix containing 1-200 micromolar CeONP (average particle size 10 nm) for 22 days, until approximately early mid-life in the fly. On day 23, flies were placed in empty vials and secured in a gas tent. All oxygen in the tent was evacuated and replaced with nitrogen, simulating anoxia

and stroke. Flies were kept under anoxic conditions for 2 ½ hrs, followed by return to their respective food groups in the normoxic environment. Survival was counted daily until all flies perished. Motor function was periodically assessed by measurement of negative geotaxis, which is the ability of a fly to climb to 3 different heights in response to a stimulus. Flies exposed to anoxia exhibited a significantly increased death rate as compared to controls, which was improved in groups treated with CeONP. Greater protective effects were observed for longevity in females, as compared to males. Motor function was also decreased in flies exposed to anoxia, and was significantly depressed at 6, 14, and 36 days after anoxia. However in flies fed CeONP, depression of motor function was significantly less, and had returned to levels consistent with unstroked controls by 14 days post-anoxia. Similar results were observed in flies treated with CeONP after exposure to anoxia (no pretreatment). These results demonstrate that CeONP improve survival and motor function after anoxia in *Drosophila*, suggesting that this nanopharmaceutical has potential for treatment or prevention of the neurological deficits associated with stroke.

Disclosures: **J.A. Bates:** None. **K.S. Hockey:** None. **J. Cook:** None. **C.A. Sholar:** None. **B. Lockler:** None. **A.S. Frey:** None. **M.J. Billings:** None. **B.A. Rzigalinski:** None.

Poster

804. Ischemia: Neuroprotection II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.08/W6

Topic: C.08. Ischemia

Support: NIH Grant NS050730

Title: PELP1 - a major scaffold protein for efficient non-genomic estrogen signaling in the brain

Authors: ***R. D. THAKKAR**[1], Q.-G. ZHANG[1], Y. DONG[1], H. TANG[1], R. VADLAMUDI[2], D. BRANN[1];
[1]Dept. of Neurosci. and Regenerative Med., Georgia Regents Univ., Augusta, GA; [2]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: 17β-Estradiol (E2) has been suggested to be neuroprotective against a variety of neurological disorders, although the mechanisms underlying its action are not completely understood. In order to enhance understanding in this area, our lab previously cloned a novel estrogen receptor (ER) co-regulator protein named PELP1 (proline-, glutamic acid-, and leucine-

rich protein-1) that may help mediate E2 non-genomic and genomic signaling. The purpose of the present study was to examine whether PELP1 functions as a key scaffold protein to connect ER-α with the PI3K kinase (p85) subunit, and thereby facilitate activation of the downstream pro-survival factor, Akt. To test this possibility, we employed a co-immunopreciptation approach (Duolink) that allows detection of two interacting proteins *in situ*. Using this approach, PELP1 was shown to interact with p85 and ER-α in the hippocampal CA1 region of sham, placebo- and E2-treated ovariectomized female mice 30 minutes following global cerebral ischemia. Intriguingly, E2 caused a robust increase in PELP1 interaction with p85 and ER-α, an effect that correlated with E2 induction of Akt activation and neuroprotection. As a whole, the findings suggest that PELP1 may function as an important scaffold protein to mediate E2-induced Akt pro-survival signaling in the hippocampus.

Disclosures: R.D. Thakkar: None. **Q. Zhang:** None. **Y. Dong:** None. **H. Tang:** None. **R. Vadlamudi:** None. **D. Brann:** None.

Poster

804. Ischemia: Neuroprotection II

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Program#/Poster#: 804.09/W7

Topic: C.08. Ischemia

Support: Grant-in-aids from the Ministry of Education, Science and Culture of Japan (No.23390342)

Grant-in-aids from the Ministry of Education, Science and Culture of Japan (No.24390336)

Title: Neuroprotective effects of cilostazol through the multi-mechanisms in mice permanent focal ischemia

Authors: *H. SHICHINOHE[1], T. YAMAUCHI[1], H. SAITO[1], T. ABUMIYA[1], K. HOUKIN[1], S. KURODA[2];
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Abstract: Background and Purpose: It has been reported that Cilostazol, which is phosphodiesterase (PDE) 3 inhibitor clinically used as an antiplatelet drug, could ameliorate ischemic brain injury, but the mechanisms were not clarified. We aimed to investigate how

Cilostazol affected mice permanent focal ischemia model. Methods: Male Balb/c mice were subjected to permanent middle cerebral artery (MCA) occlusion. They were treated with Cilostazol (10- mg/kg or 20- mg/kg) or vehicle 30 min and 24 h post-ischemia. Infarct volume was assessed by 2,3,5-triphenyltetrazolium chloride (TTC) method after 48 h (n=8 in each group). In histological analysis, the animals were sacrificed 6 h or 24 h post-ischemia (n=5 in each group), and then the immunohistochemistry was performed to the brain sections. Results: We showed that the treatment with Cilostazol salvaged the tissue damage in the infarct rim in a dose-dependent manner, and 20- mg/kg Cilostazol significantly reduced infarct volume to 70.1±24.4% of the control ($P<0.05$). Immunoreaction for 8-Hydroxydeoxyguanosine (OHdG), an oxidative stress marker, showed the neuronal damage by oxidative stress in peri-infarct, and the treatment with Cilostazol reduced the ratio of the damaged neurons 6 h post-ischemia (65.8±33.5% in vehicle-treated animals, 21.3±9.9% in Cilostazol-treated ones, $P<0.05$). Double staining for 8-OHdG and von Willebrand factor also showed that Cilostazol attenuated oxidative stress on the endothelial cells in peri-infarct 24 h post-ischemia, significantly ($P<0.01$). On the other hand, the immunostaining for phosphorylated cAMP response element binding protein (pCREB) showed that Cilostazol increased the ratio of the pCREB-positive neurons in peri-infarct 24 h post-ischemia significantly (74.5±8.0% in vehicle-treated animals, 93.6±2.9% in Cilostazol-treated ones, $P<0.01$). Conclusions: These findings suggested that Cilostazol could have multi-mechanisms to ameliorate tissue damage due to permanent focal cerebral ischemia. Thus, one is to attenuate oxidative stress on neurnons and endothelial cells in the infarct rim, and another is to protect neural damage with anti-apoptotic effect through the pathway of Akt/pCREB.

Disclosures: **H. Shichinohe:** None. **T. Yamauchi:** None. **H. Saito:** None. **T. Abumiya:** None. **K. Houkin:** None. **S. Kuroda:** None.

Poster

804. Ischemia: Neuroprotection II

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Topic: C.08. Ischemia

Support: Grant from Educational Commission of Anhui Province, China (KJ2012A155)

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Title: Vitexin protects brain against ischemia/reperfusion injury via modulating mitogen-activated protein kinase and apoptosis signaling in mice

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Abstract: The injury from ischemic stroke and subsequent reperfusion is a major cause of permanent disability and death in the adult worldwide. Past decades have witnessed a tremendous advance in understanding of the pathophysiology of cerebral ischemia/reperfusion (I/R) injury, however therapeutic options for acute cerebral I/R injury are still limited. Vitexin (apigenin-8-C-β-D-glucopyranoside) is a flavonoid compound present in dried leaves of hawthorn (crataegus pinnatifida). Previously we found that vitexin protects the heart against I/R injury via modulating mitogen-activated protein kinase (MAPK, including ERK, JNK and p38) and apoptosis signaling pathways. Recent study revealed that vitexin has a neuroprotective effect in primary cortical neuron culture. Whether this protective effect applies to cerebral I/R injury remains unclear. In the present study, we examined the potential neuroprotective effect of vitexin against cerebral I/R injury and the underlying mechanisms. Focal cerebral I/R model in male Kunming mice was induced by occluding the middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion for 22 h. Vitexin (3.25, 7.50, and 15.00 mg/kg) was administered via tail vein at the beginning of the reperfusion. Twenty-four hours after I/R, vitexin at 7.50 mg/kg or 15.00 mg/kg significantly reduced neurological deficits, cerebral infarct volume and histopathological damages to cerebral I/R injury. Western Blot analyses revealed that vitexin markedly upregulated p-ERK1/2 and downregulated p-JNK and p-p38. Meanwhile, vitexin increased cortical Bcl-2 expression and suppressed the overexpression of Bax in I/R injured mice. Our data indicate that vitexin protects the brain against cerebral I/R injury, and this effect may likely occur via modulating MAPK signaling and preventing cellular apoptosis. To our knowledge, we report for the first time the therapeutic application of vitexin against cerebral I/R injury which may pave a new way for developing a novel drug candidate against cerebral I/R injury in stroke patients.

Disclosures: G. Zhang: None. **L. Dong:** None. **Y. Wang:** None. **Q. Jiang:** None. **Y. Zhen:** None.

Poster

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Topic: C.08. Ischemia

Support: Grant-in-Aid (S1311011)

Title: L-carnitine enhances axonal plasticity and improves white matter lesions after chronic hypoperfusion in rat brain

Authors: *Y. UENO[1], M. KOIKE[2], Y. SHIMADA[3], H. SHIMURA[4], K. HIRA[3], Y. UCHIYAMA[2], N. HATTORI[3], T. URABE[4];
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Abstract: Chronic cerebral hypoperfusion causes white matter lesions with oxidative stress and cognitive impairment, and in turn induces limited regeneration of oligodendrocytes. However, the biological mechanisms that regulate axonal plasticity under chronic cerebral hypoperfusion have not been fully investigated. Here, we investigated whether L-carnitine, an anti-oxidant agent, enhances axonal plasticity and oligodendrocyte expression, and explored the signalling pathways that mediate axonal plasticity in a rat chronic hypoperfusion model. Adult male Wistar rats subjected to ligation of the bilateral common carotid arteries were treated with or without L-carnitine. Immunohistochemical and western blot analyses, and behavioural tests were performed after ligation of the bilateral common carotid arteries. L-carnitine-treated rats exhibited significantly reduced escape latency at 28 days after operation in the Morris water maze task. L-carnitine increased levels of phosphorylated high-molecular weight neurofilament (pNFH), concurrent with reduction of phosphorylated phosphatase tensin homolog deleted on chromosome 10 (PTEN), and increases of phosphorylated Akt and mammalian target of rapamycin (mTOR), as well as reduction of semaphorin 3A at 28 days after chronic hypoperfusion. Axonal phosphorylated Akt and mTOR protein were present in pNFH+ axons at 28 days in the L-carnitine-treated rats. In contrast, axons at 28 days in the vehicle-treated rats exhibited many fewer axonal phosphorylated Akt and mTOR proteins. L-carnitine reduced lipid peroxidation and oxidative DNA damage, and enhanced oligodendrocyte marker expression and myelin sheath thickness after chronic hypoperfusion. Additionally, L-carnitine facilitated myelination of phosphorylated NFH+ axons by myelin basic protein+ oligodendrocytes at 28 days after LBCCA. Collectively, these findings demonstrate that L-carnitine regulates the PTEN/Akt/mTOR signalling pathway and semaphorin 3A, and enhances axonal plasticity while concurrently ameliorating oxidative stress and increasing oligodendrocyte myelination of axons, thereby improving white matter lesions and cognitive impairment in a rat chronic hypoperfusion model.

Disclosures: **Y. Ueno:** None. **M. Koike:** None. **Y. Shimada:** None. **H. Shimura:** None. **K. Hira:** None. **Y. Uchiyama:** None. **N. Hattori:** None. **T. Urabe:** None.

Poster

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Title: Neuronal expression of GPR3 in rodent brain is associated with cell survival

Authors: *S. TANAKA, T. MIYAGI, I. HIDE, T. SHIRAFUJI, N. SAKAI;
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Abstract: G-protein coupled receptor (GPR) 3 belongs to a member of constitutively active Gs-coupled receptors that activate 3'-5'-cyclic adenosine monophosphate (cAMP) and is highly expressed in the brain. Previously, we have reported that the expression of GPR3 in cerebellar granular neurons (CGNs) enhances neurite outgrowth (Tanaka et al., JBC 2007) and modulates proliferation of cerebellar granule cell precursors (Tanaka et al., 2009 PLoS One); however, the physiological functions of GPR3 remain to be fully elucidated. In the present study, we investigated the survival functions of GPR3 in rodent brain. CGNs from GPR3-knockout mice demonstrated lower survival than did CGNs from wild-type or GPR3-heterozygous mice under normal culture conditions. To further investigate GPR3 function in developing cerebellar granule neurons, cerebellar sections from wild-type and GPR3 -/- pups, aged postnatal day 7, day 14, and day 21, were stained with a cleaved caspase-3 antibody. The number of caspase-3-positive cells was significantly increased in the internal granular cell layer of the cerebellum in P7, P14 and P21 GPR3 -/- mice compared with that in P7 wild-type mice. Next, we introduced a transient middle cerebral artery occlusion (tMCAO) model in wild-type and GPR3-knockout mice to determine whether GPR3 expression modulates neuronal survival after brain ischemia. After tMCAO, GPR3-knockout mice exhibited a significantly larger infarct area than did wild-type mice. Furthermore, GPR3 -/- mice indicated larger neuronal loss in the cortex and the striatum in

the ischemic hemisphere by Nissl staining, twenty-four hours after ischemia .These results indicate that intrinsic GPR3 expression is involved in neuronal survival both *in vitro* and *in vivo*.

Disclosures: **S. Tanaka:** None. **T. Miyagi:** None. **I. Hide:** None. **T. Shirafuji:** None. **N. Sakai:** None.

Poster

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Program#/Poster#: 804.13/W11

Topic: C.08. Ischemia

Support: "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022014)" Rural Development Administration, Republic of Korea

Title: Black rice extract ameliorates bilateral common carotid artery occlusion-induced memory impairment in mice

Authors: S.-N. HWANG[1,2], J.-C. KIM[1,2], *S. YOON[3,4], M. I. H. BHUIYAN[1,2], J. KIM[1,2], S. KIM[1,2];
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Abstract: Black rice is likely to be beneficial because of its nutritional facts. It has been reported that black rice contains various bioactive compounds including anthocyanin to exhibit antioxidant activities. In addition, it has been demonstrated that black rice extract significantly reduces atherogenic progression. Until now, the effect of black rice extract on memory performance has not been elucidated. Thus, the present study was carried out to demonstrate effects of chronic treatment with the extract of black rice on bilateral common carotid artery occlusion (BCCAO)-induced memory impairment in C57BL/6 mice. Black rice extract (300 mg/kg) was orally administered once a day for 21 days. BCCAO with 23-minute occlusion time was adopted at 8th day following initial administration of the extract. From 7 days after BCCAO, Morris water maze test was performed to evaluate working and reference memory performance in mice. BCCAO profoundly resulted in impairments of both working and reference memory. The administration of black rice extract significantly ameliorated BCCAO-induced memory impairments. Additionally, black rice extract seems to have significant protective effects against BCCAO-induced hippocampal neuronal death in the CA1 area. Taken together, our results

suggest that black rice contains some ingredients which may ameliorate brain ischemia-induced memory impairments.

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Poster

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Support: This research was supported by the Brain Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2012- 0005827).

Title: The effects of anti-oxidants on experimental cerebral ischemia in the rat

Authors: J. PARK[1,2], J. KIM[1], S.-T. LEE[3], K.-M. LEE[3], K. PARK[1], W. LEE[1], *J. LEE[1,2]; [1]Anat., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]BK21 Plus Project for Med. Science, and Brain Res. Inst., Seoul, Korea, Republic of; [3]Neurol., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Dehydroascorbic acid (DHA), oxidized form of ascorbic acid, is able to enter the blood-brain barrier(BBB) system. Glutathione(GSH), a natural tripeptide of glutamate, cystein, and glysine, also plays an important role in reducing oxidative stress by detoxifying reactive oxygen species(ROS) and can serve as a substrate for various peroxidases. Melatonin (N-acetyl-5-methoxytryptamine, MEL) is an endogenously produced indoleamine secreted by the pineal gland and the secretion is suppressed by light. Melatonin is a highly effective antioxidant and plays a pivotal role in reducing the free radical, and has anti-inflammatory effect. The relation of DHA and GSH is known as boosting their generation through redox cycle. Previous studies focused on the effect of antioxidants treated before ischemic injury. In this study, it is hypothesized that single or combinational treatment of antioxidants after cerebral ischemia reduce the brain infarct size. Each antioxidant was prepared as following; DHA(100mg/kg/mL), GSH(500mg/kg/mL), MEL(10mg/kg/mL) in a sodium bicarbonate/acetate buffer, pH 5.5. All rats of 6 groups(NC, EC, DHA 100mg/kg/mL, GSH 500mg/kg/mL, MEL 10mg/kg/mL, DHA 100mg/kg/mL+GSH 500mg/kg/mL+MEL 10mg/kg/mL) were subjected to ischemic condition

for 90 min. Treatments of each prepared antioxidants were administered at the start of reperfusion. All Rats were sacrificed to confirm the volume of brain infarct 1 day after ischemic injury. Unlike our expectation, it was shown that the combinational treatment of antioxidants did not have protective effects on focal cerebral ischemia, but the infarct sizes were significantly reduced in DHA or GSH treatment groups. These results show the potential of antioxidant as therapeutic drug candidate in cerebral ischemia.

Disclosures: J. Park: None. **J. Kim:** None. **S. Lee:** None. **K. Lee:** None. **K. Park:** None. **W. Lee:** None. **J. Lee:** None.

Poster

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Program#/Poster#: 804.15/W13

Topic: C.08. Ischemia

Title: The glial neuropeptide ODN worsens excitotoxic and ischemic cell death *in vivo*

Authors: *R. LAMTAHRI[1,2], B. LEFRANC[1], D. VAUDRY[1], J. LEPRINCE[1], J. CHUQUET[2]; [1]INSERM U982, Mont Saint Aignan, France; [2]Univ. of Rouen, INSERM U982, Rouen, France

Abstract: ODN is a biologically active fragment corresponding to the amino-acid residues 33-50 of diazepam binding inhibitor (DBI). This neuropeptide, is synthesized by astrocytes and is known to be involved in various neurobiological processes such as neurogenesis, central glucose sensing and calcium dynamics in astrocytes. We have recently shown that ODN protects cultured astrocytes and neurons from a lethal oxidative stress. The objective of the present work was to test, *in vivo*, the neuroprotective potential of ODN in stroke and in NMDA-induced brain damage. Focal cerebral ischemia was induced in mice by the transient occlusion of the middle cerebral artery (MCA) for 60 min. On average, both groups underwent a similar reduction of blood flow in the MCA territory as measured by laser-doppler flowmetry (control 40.30.6% *vs* treated 45.70.5%, respectively). ODN (1 µg/3 l) or its vehicle solution was injected in the ipsilateral ventricle 20 min before the onset of ischemia. For NMDA induced brain damage, mice received a mix of NMDA (20 nmol) and ODN (1 µg in 0.5 l) or its vehicle solution into the right striatum. In both model, the volume of the lesion was quantified 48 h later. In cerebral ischemia, administration of ODN resulted in an increase of the infarction volume (51%; $P<0.05$) accompanied by an increase in the brain edema volume (42%; $P<0.05$) although ODN is not toxic *per se*. For the excitotoxic lesion model, the co-administration of ODN and

NMDA also resulted in an increase in the lesion volume (87%; $P<0.05$). Our measurement of physiological parameters shows that this deleterious effect of ODN cannot be explained by a hyperthermic, a cerebrovascular or a cardiovascular effect induced by central ODN administration. Therefore, the neuroprotective potential of ODN seen *in vitro* seems overtaken *in vivo* by an exacerbation of excitotoxic processes likely to be caused by it's inverse agonist effect on the GABA-A receptor.

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Poster

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Topic: C.08. Ischemia

Support: ITMAT

Title: Intranasal salvinorin A reduces cerebral ischemia/reperfusion injury in mouse middle cerebral artery occlusion model

Authors: C. CHEN[1], C. XI[1], J. MA[1], T. ABEL[2], *R. LIU[1];
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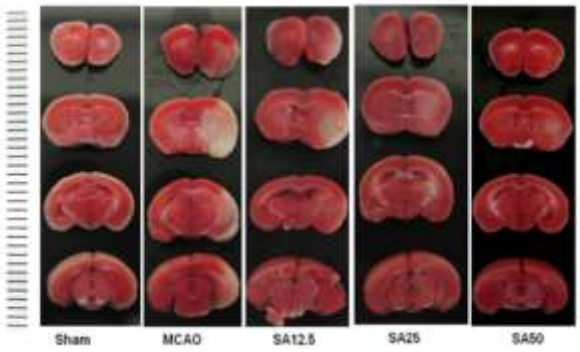
Abstract: Aims: In this study, we investigated the effect of intranasal administration of SA in focal cerebral ischemic injury and the related mechanisms. **Methods:** The middle cerebral artery occlusion (MCAO) model was established using C57/BL6 male adult mice. The 120 minutes occlusion of MCA was induced by silicon-coated suture followed by 24 hr reperfusion. SA of different doses was administered intranasally after 120 min ischemia and when reperfusion was established. Norbinaltorphimine (2.5mg/kg, i.p.), a KOR antagonist, was given prior to administration of SA for an additional group given 50 ug/kg of SA. After 24 hr reperfusion, neurobehavioral outcome was investigated, infarct volume and Evans blue extravasations were determined. Nissl staining was used for the evaluation of neuronal morphological changes. Immunohistochemistry and western blot were performed to detect the activated caspase-3, eNOS, iNOS, IL-10 and TNF-alpha to investigate the apoptotic and inflammatory changes. **Results:** SA reduced infarct volume (as indicated in figure 1 using tetrazolium chloride staining) and improved neurological deficits dose dependently. Meanwhile,SA at the dose of 50ug/kg

protected the vascular integrity shown through the Evans blue extravasations. Immunohistochemistry and western blot analysis demonstrated that SA decreased the expression of cleaved casepase-3, iNOS, IL-10 and TNF-alpha in the penumbra areas, but increased the expression of eNOS. All these effects were blocked by Norbinaltorphimine. **Conclusions:** Salvinorin A alleviated ischemic brain injury by activation of KOR and eNOS, inhibition of iNOS, caspase-3 and inflammation factors such as IL-10 and TNF-alpha, suggesting that SA is neuroprotective. **Figure 1:** Tetrazolium chloride staining shows that intranasal salvinorin A (SA) reduced infarct volume (white area) dose dependently. Sham, control group without ischemia, MCAO, middle cerebral artery occlusion group; SA12.5; SA25; or SA50 represent middle cerebral artery occlusion with 12.5 ug/kg, 25 ug/kg, or 50 ug/kg (SA).



Disclosures: **C. Chen:** None. **R. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); intellectual property rights. **C. Xi:** None. **J. Ma:** None. **T. Abel:** None.

Poster

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Topic: C.08. Ischemia

Support: Conacyt Grant 57204

Title: Effect of mannitol on neurogenesis in a model of ischemic stroke

Authors: ***Y. CRUZ**[1], J. KIM[1], V. GÁLVEZ[1], E. GARCÍA-VENCES[1,2], A. IBARRA[1]; [1]facultad de ciencias de la salud, Univ. Anáhuac, México, Mexico; [2]INMEGEN, México, Mexico

Abstract: Stroke is a neurological disorder caused by the occlusion of cerebral arteries. The occlusion results in diminished cerebral blood flow triggering a series of events such as excitotoxicity, apoptosis and inflammation that eventually leads to neuronal death. Mannitol is used in the treatment of many neurological conditions. This molecule functions as an osmotic agent and therefore diminishes edema; it also is capable of neutralizing free radicals and reducing tissue damage. Studies have shown that mannitol facilitates the entry of neurotrophic factors into the brain and increases the efficacy of migration and mitotic activity of many cell types. Thus, we investigated if mannitol is capable of promoting neurogenesis in a rodent model of middle cerebral artery occlusion (MCAO). Two experiments were carried out. The first evaluated neurogenesis in the acute phase (7 days post-MCAo) and the second during the chronic phase of stroke (2 months post-MCAo). The neurological deficit was evaluated weekly. Neurogenesis was evidenced using immunofluorescence techniques utilizing the following markers: BrdU (bromodeoxyuridine) which incorporates into the cell during the proliferative stage and DCX (doublecortin) which stains microtubules expressed by young neurons during their migration progress. Neurogenesis was analyzed at the subventricular zone, hippocampal dentate gyrus, and cerebral cortex. The results showed that mannitol promotes a speedier neurological recovery and a marked tendency to increase neurogenesis in the analyzed regions ipsilateral to the lesion, in both the acute and chronic post-MCAo phases. Keywords: cerebral ischemia, stroke, neurogenesis, and mannitol.

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Poster

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VA Merit Award

Uehara Foundation

NIH Grant HL090606

Title: The sphingosine 1-phosphate receptor agonist FTY720 (Fingolimod) provides neuroprotection against experimental stroke in aged rodents

Authors: *M. A. YENARI[1], M. KAWABORI[2], Z. ZHENG[2], J. S. KARLINER[3];
[1]Neurol, Univ. California, San Francisco and San Francisco Veterans Affairs Med. Ctr., San Francisco, CA; [2]Neurol., [3]Cardiol., SF VAMC and UCSF, San Francisco, CA

Abstract: The sphingosine-1-phosphate (S1P) receptor agonist FTY720 (fingolimod) has anti-inflammatory properties, and is indicated for use in advanced multiple sclerosis. Recent studies now show that it decreases reperfusion injury in heart, liver, and kidney. There is accumulating evidence that FTY720 is also effective against experimental ischemic brain injury through multiple immunomodulatory actions, such as limiting lymphocyte production, trafficking, and apoptosis. S1P acts through several sphingosine kinases (SK), and prior work has suggested that it may act largely through SK2 in the brain, and SK2 and SK1 in the heart. However, it is still unknown whether FTY720 can alleviate ischemic injury in aged animals and under conditions of permanent middle cerebral artery occlusion (MCAO), or whether it acts through SK1. In this study, male sphingosine kinase 1 knockout (SK1-KO) and wildtype (WT) mice (C57/BL6 background) between 52-61 weeks of age were subjected to permanent distal MCAO. FTY720 (1 mg/kg IP) was given at the time of MCAO. Lesion size, behavioral indices and markers for inflammation were studied. Lesion size and behavior were no different between WT and SK1-KO mice; however, both WT and SK1-KO mice given FTY720 showed significant improvement in motor function and reduction of infarct size (WT; 28% reduction, KO; 48% reduction vs vehicle treated controls) at day 1. Apoptotic cells were significantly decreased in both treated groups compare to non-treated groups, but there was no difference in microglial activation in any of the groups. These data suggest that activation of sphingosine 1-phosphate receptor by FTY720 is neuroprotective, and is not dependent on SK1. The mechanism is also independent of any microglial activation, but does protect against permanent MCAO (brain ischemia without reperfusion) in older mice.

Disclosures: M.A. Yenari: None. **M. Kawabori:** None. **Z. Zheng:** None. **J.S. Karliner:** None.

Poster

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Support: CONACYT grant 381861/253971

Title: Effect of low dose of recombinant human erythropoietin (rhEpo) administered intranasally on MAPK-mediated signaling regulating the transition of G1 / S on brain ischemia/reperfusion injury in adult rats

Authors: *R. CASTAÑEDA ARELLANO, A. FERIA-VELASCO, M. UREÑA GUERRERO, M. RIVERA-CERVANTES;
Univ. de Guadalajara, Zapopan, Mexico

Abstract: The erythropoietin in the nervous system is a neuroprotective potential factor for cerebral ischemic damage due to binding specific erythropoietin-receptor and that this interaction is associated at survival-mechanisms through signaling pathways that lead to the pro-apoptotic-neuronal regulation. Especially when high doses are administered of recombinant human erythropoietin (rhEpo) increases both endogenous Epo and its receptor expression that stimulates the survival and cellular proliferation. The purpose of this work was to assess whether a rhEpo-low dose (500UI/Kg) intranasal administered induces cell survival through MAPK-pathway that regulates G1/S phase transition and could exert a neuroprotective effect. Results shown using a low dose of rhEpo led to decrease the neuronal loss and neurodegenerative pattern caused by ischemia both in CA1 and dentate gyrus of hippocampus. This protective mechanism was mainly by increased MEK1-ERK1/2-Elk-1-c-Jun pathway and decreased p38-pathway expression leading to regulation of cell cycle effectors such as p16 and retinoblastoma. It is proposed as a major survival pathway that determines the neuronal fate of re-entry to cell cycle and consequently apoptosis.

Disclosures: R. Castañeda Arellano: None. **A. Feria-Velasco:** None. **M. Ureña Guerrero:** None. **M. Rivera-Cervantes:** None.

Poster

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Support: Colciencias Contrato No.244-2010

Title: Glucosamine neuroprotection in a rat model of cerebral ischemia: Molecular mechanisms at play

Authors: *J. RENGIFO[1], A. VERA[1,3], C. F. CARDOZO[1], M. J. CRUZ[1], L. I. MOSQUERA[1], E. VIVEROS[1], C. A. ARANGO[2];
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Abstract: A growing number of studies demonstrate that supplementation of glucosamine (which increases protein O-GlcNacylation levels) has cytoprotective effects in multiple organ systems. More than 3,000 nuclear and cytoplasmic proteins are now known to be transiently modified by the O-linked attachment of the monosaccharide b-N-acetylglucosamine (O-GlcNAc) to their serine and/or threonine residues and, like phosphorylation, O-GlcNAcylation has proven to be a highly dynamic and ubiquitous protein modification. Indeed, for many proteins it has been demonstrated that phosphorylation and O-GlcNAcylation are reciprocal modifications that regulate protein function. In multiple forms of cellular stress, including ischemic stress, it has been demonstrated that an acute increase of O-GlcNAc levels is an endogenous response to stress and that strategies that augment O-GlcNAc levels are pro-survival, whereas those that reduce O-GlcNAc levels are deleterious. Results from our group, and others, show that glucosamine administration reduces the size of the infarct in the rat Middle Cerebral Artery Occlusion (MCAO) model. Here we evaluate the participation of several proteins in the neuroprotection generated by glucosamine; MAP-2 and Tau, cytoskeleton associated proteins, and Akt, GSK-3b and Bcl-2, proteins implicated in the regulation of apoptosis. Using western blotting and fluorescent inmunohistochemistry we studied the protein levels of expression, phosphorylation and O-GlcNAcylation in the cerebral cortex, hippocampus and substantia nigra of the rat brain. There was no significant difference in the expression levels of these proteins in the ischemic rat brain with or without glucosamine treatment. But for some of these proteins, our results indicate that glucosamine supplementation in ischemia reduces protein phosphorylation at some residues, indicating that an O-GlcNAcylation/phosphorylation interplay is likely to be an important regulator of the glucosamine generated neuroprotection.

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Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.21/W19

Topic: C.08. Ischemia

Title: Mechanisms of neuroinflammation in rat model of subarachnoid hemorrhage

Authors: *C. TOSUN[1], C. HONG[2], D. KURLAND[2], V. GERZANICH[2], M. SIMARD[2]; [1]Univ. of Maryland In Baltimore, BALTIMORE, MD; [2]Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Subarachnoid hemorrhage (SAH) is a life-threatening condition and affects 27,000 people annually in the United States. The mortality rate approaches 30% acutely in hospitalized patients with SAH and survivors exhibit long-term neurological deficits. Recently, our laboratory has provided evidence that the sulfonylurea receptor type 1 (Sur1) and transient receptor potential channel member 4 (Trpm4) is transiently upregulated and co-associate to form the Sur1-Trpm4 channel in humans and rats following SAH. Previous work has implicated that opening of this channel is responsible for tissue swelling, cell death and formation of hemorrhagic transformation in animal models of CNS injury. Activation of TLR4 signaling pathways also upregulate the Sur1/Trpm4 channel and can play a major role in fine tuning the activities of inflammatory cells. Here, we tested whether pharmacologically blocking the Sur1/Trpm4 channel with glibenclamide would reduce SAH-induced neuroinflammation. 50ul of fresh non-heparinized autologous tail blood was injected bilaterally into the subarachnoid space of the entorhinal cortex. 48 hour after the injection of blood sections were labelled for markers of neuroinflammation. Vehicle treated rats had significantly higher numbers of microglia labelled with Iba-1 in the entorhinal cortex when compared to shams. Glibenclamide treatment following SAH significantly reduced the number of microglia, and were not different from shams. Vehicle treated rats had prominent astrogliosis in the entorhinal cortex marked by GFAP, and treatment with glibenclamide was sufficient to reduce the activation of astrocytes. Similarly, the number of neutrophils in the same area was increased in vehicle treatment, whereas glibenclamide treated rats had significantly fewer neutrophils. In contrast, the number of Iba-1 positive microglia was higher in glibenclamide treated rats when compared to vehicles in the hippocampus. The dynamic balance between M1/M2 microglial phenotypes has been shown to be critical following CNS injury and can be either responsible for a cytotoxic response or critical in reconstitution/repair and immunosuppression. Labeling with specific microglial markers revealed that the prominent phenotype of microglia in the hippocampus is M2 in glibenclamide treated rats, while the majority of microglia is M1 in vehicle treated rats 48 hours after SAH. Taken together, specifically blocking the SUR1-Trpm4 channel following SAH is sufficient to reduce neuroinflammation in the entorhinal cortex. Glibenclamide can switch the microglial phenotype in the hippocampus and can be critical in repair mechanisms.

Disclosures: C. Tosun: None. **C. Hong:** None. **D. Kurland:** None. **V. Gerzanich:** None. **M. Simard:** None.

Poster

804. Ischemia: Neuroprotection II

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Topic: C.08. Ischemia

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Title: Omega-3 polyunsaturated fatty acids enhance cerebral angiogenesis and provide long-term protection after stroke

Authors: *L. HAN[1,2,3,4], Y. SHI[2], Y. SHI[4], L. ZHANG[2], L. ZHANG[4], F. ZHANG[2], F. ZHANG[4], X. HU[2], X. HU[4], W. ZHANG[2], R. K. LEAK[2], R. K. LEAK[4], Y. GAO[2], Y. GAO[4], J. CHEN[2], J. CHEN[4];
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Abstract: Stroke is a devastating neurological disorder and one of the leading causes of death and serious disability. After cerebral ischemia, revascularization in the ischemic boundary zone provides nutritive blood flow as well as various growth factors to promote the survival and activity of neurons and neural progenitor cells. Enhancement of angiogenesis and the resulting improvement of cerebral microcirculationare key restorative mechanisms and represent an important therapeutic strategy for ischemic stroke. In the present study, we tested the hypothesis that post-stroke angiogenesis would be enhanced by omega-3 polyunsaturated fatty acids (n-3 PUFAs), a major component of dietary fish oil. To this end, we found that transgenic *fat-1* mice that overproducen-3 PUFAs exhibited long-term behavioral and histological protection against transient focal cerebral ischemia (tFCI). Importantly, *fat-1* transgenic mice also exhibited robust improvements in revascularization and angiogenesis compared to wild type littermates, suggesting a potential role for n-3 fatty acids in post-stroke cerebrovascular remodeling. Mechanistically, n-3 PUFAs induced upregulation of angiopoietin 2 (Ang 2) in astrocytes after tFCI and stimulated extracellular Ang 2 release from cultured astrocytes after oxygen and glucose deprivation. Ang 2 facilitated endothelial proliferation and barrier formation *in vitro* by potentiating the effects of VEGF on phospholipase Cγ1 and Src signaling. Consistent with these

findings, blockade of Src activity in post-stroke *fat-1* mice impaired n-3 PUFA-induced angiogenesis and exacerbated long-term neurological outcomes. Taken together, ourfindings strongly suggest that n-3 PUFA supplementation is a potential angiogenic treatment capable of augmenting brain repair and improving long-term functional recovery after cerebral ischemia.
Key words: angiogenesis, angiopoietin 2, astrocyte, neuroprotection, omega-3 polyunsaturated fatty acids, stroke, VEGF

Disclosures: **L. Han:** None. **Y. Shi:** None. **Y. Shi:** None. **L. Zhang:** None. **L. Zhang:** None. **F. Zhang:** None. **F. Zhang:** None. **X. Hu:** None. **X. Hu:** None. **W. Zhang:** None. **R.K. Leak:** None. **R.K. Leak:** None. **Y. Gao:** None. **Y. Gao:** None. **J. Chen:** None. **J. Chen:** None.

Poster

804. Ischemia: Neuroprotection II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 804.23/W21

Topic: C.08. Ischemia

Support: CONACYT-CB-182266

Title: Resveratrol increases phosphorylated AMPK in cerebral ischemia

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Abstract: Cerebral Ischemia results from occlusion of a major cerebral artery and involves a sequence of molecular events that can induce either cell death or cell survival through diverse mechanisms. Decrease of cerebral blood flow restricts the delivery of substrates predominantly oxygen and glucose with consequent decline in the metabolism of energy. Interestingly, neuronal cell death can be prevented by modulating the energetic state of the cell. Antioxidants such as resveratrol (RSV) exert a neuroprotective effect in rats subjected to ischemia by increasing mitochondrial function through mechanisms closely associated with glucose metabolism and activation of ATP generating pathways in which AMP-activated kinase (AMPK) is involved. Objective: We investigated whether treatment with RSV regulates AMPK phosphorylation

following an ischemic cerebral event. Methods: Male Wistar rats (250 to 350g) were subjected to transient middle cerebral artery occlusion (MCAO) for 2 h followed by reperfusion (R). A dose of RSV (1mg/kg; i.v.) diluted in 50% ethanol, was given at the beginning of R. The rats were sacrificed at different times of R (0, 15, 30, 60 and 120 min). The AMPK and the phosphorylated AMPK (pAMPK) levels were evaluated by Western Blotting. Rat primary neuronal culture (from E18 embryos) was used to assed the mitochondrial membrane potential ($\Delta\Psi m$) using the fluorescent probe JC- 1 (3 μM). Results: MCAO does not change total AMPK but increased pAMPK levels. R induced an increase on pAMPK level after 15 min (93%) that peaks after 120 min (145%) of R. RSV increased pAMPK in control group (74.6 %). RSV also induced an increased after MCAO and R. The peak of pAMPK induced by RSV was observed after 15 minutes of R (203.3%). Significant changes were observed in the $\Delta\Psi m$ after treatment with RSV. Discussion: pAMPK levels were increased after MCAO and remained elevated up to 120 min of R. RSV induced an additional increase on pAMPK levels in MCAO groups. This transient effect of RSV was observed after short times of R. It is possible that the RSV mechanism of action might be related to AMPK activation since this enzyme modulates the energetic state of cell which is relevant to survival after ischemia

Disclosures: **N. Pineda-Ramirez:** None. **A. Ortiz-Plata:** None. **J. Pedraza-Chaverri:** None. **P. Aguilera:** None. **M. Espinoza-Rojo:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 805.01/W22

Topic: C.08. Ischemia

Title: Cortical microcirculation in a mouse model of subarachnoid hemorrhage

Authors: ***K. SCHUELLER**, N. PLESNILA;
ISD, Munich, Germany

Abstract: Subarachnoid hemorrhage (SAH) is a subtype of stroke, which leads to a mortality of 21% in patients within the first day after the bleeding (Pobereskin, 2001). Recently the term of early brain injury has been established to describe the cerebral pathophysiology following SAH. It may be caused by elevated intracranial pressure, inflammatory processes and cerebral ischemia. After SAH pearl- string- like constrictions of pial arterioles, called microvasospasms, were detected in humans (Uhl et al., 2003) as well as in mice (Friedrich et al., 2011). The effect

of these microvasospasms on blood flow dynamics in parenchymal vessels has not been investigated. The aim of the current study was to test whether microvasospasms lead to microcirculatory dysfunction and thereby induce cerebral ischemia after SAH. The deep cerebral microcirculation was measured 3 h after the hemorrhage by 2 photon microscopy. SAH surgery was performed by endovascular Circle of Willis perforation as described previously (Feiler et al., 2010). Cerebral vessels were visualized by fluorescent plasma dyes and scanned for different parameters. Physiologic parameters were monitored during the procedure and did not differ between the SAH and SHAM surgery group. Regional cerebral blood flow was reduced significantly after SAH, indicating that cerebral ischemia develops early after the bleeding. The overall arteriolar vessel diameter as well as the blood flow velocity were significantly reduced compared to the baseline condition. Additionally the volume of perfused microvessels in deep cerebral tissue diminished significantly after SAH which indicates parenchymal perfusion deficits. In line with this finding the number of sticking leukocytes increases in deep microvessels after SAH. With this *in vivo* 2 photon microscopy study we show that microvasospasms lead to ischemia in the cerebral cortex of mice. Spastic vessels reduce cerebral blood flow and cause perfusion deficits as well as leukocyte plugging. Taken together our findings demonstrating severe dysfunction of cerebral microvessels could serve as an explanation for the ischemic brain damage observed after SAH. Thus restoration of microcirculatory flow could be a novel therapeutic target after SAH.

Disclosures: K. Schueller: None. **N. Plesnila:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Topic: C.08. Ischemia

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Title: Adenosine a2b agonist inhibits hemorrhagic transformation induced by tpa after cerebral ischemia

Authors: *Q. LI[1,2], C.-F. CHANG[2], X. HAN[2], R. KOEHLER[2], Y. ZHAI[1], J. WANG[2];
[1]Dept. of Neurology,Shanghai Ninth People's Hosp., Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China; [2]Dept. of Anesthesiol. and Critical Care Medicine,, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Thrombolysis with tissue plasminogen activator (t-PA) is currently the only pharmacologic treatment approved for patients with ischemic stroke. However, some researchers believe that tPA is associated with hemorrhagic transformation and aggravates brain damage after reperfusion. Therefore, it would be beneficial to identify a drug that could be administered with tPA to reduce the risk of hemorrhage. The aim of this study was to explore whether adenosine A2b agonists reduce the risk of hemorrhage after tPA use for ischemic stroke. Male Sprague-Dawley rats (anesthetized with 2% isoflurane in 30% oxygen and 70% air) were subjected to transient middle cerebral artery occlusion by endovascular occlusion of the left middle cerebral artery. At 1.5 hours after ischemia onset, we administered Bay 60-6583, a selective A2b agonist (1 mg/kg via intravenous [i.v.] injection) combined with an intravenous perfusion of tPA (10 mg/kg, i.v.). The suture was withdrawn after drug treatment at 2 hours after ischemia onset. At 24 hours after reperfusion, we used Western blotting to assess expression of the tight junction proteins claudin-5, zonula occludens-1 (ZO-1), and matrix metalloproteinase-9 (MMP-9). Blood-brain barrier (BBB) permeability, as demonstrated by albumin extravasation, was evaluated by Western blotting and immunohistochemistry. We evaluated hemorrhagic transformation in the rats with the intraparenchymal brain hemoglobin assay and used a battery of tests to evaluate neurologic deficits. Administration of tPA after ischemia led to an increase in hemorrhagic transformation and BBB permeability. This effect was associated with an increase in MMP-2/9 activity, which led to aggravation of the post-ischemic degradation of ZO-1 and claudin-5. tPA also aggravated ischemia-induced neurologic deficits. Combining Bay 60-6583 administration with tPA preserved the expression of ZO-1 and claudin-5, reduced hemorrhagic transformation and BBB permeability, and improved neurologic function. Our results show that A2b adenosine receptor activation is an important pathway by which tPA-induced damage to the BBB can be inhibited after cerebral ischemia. Thus, using A2b agonists as adjuvants to tPA could be a promising strategy for decreasing the risk of hemorrhagic transformation during treatment of ischemic stroke.

Disclosures: Q. Li: None. **C. Chang:** None. **X. Han:** None. **R. Koehler:** None. **J. Wang:** None. **Y. Zhai:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Program#/Poster#: 805.03/W24

Topic: C.08. Ischemia

Support: INSERM

Lower Normandy

Title: tPA interacts with the ER stress response to protect neurones during cerebral ischaemia

Authors: M. LOUESSARD, D. VIVIEN, C. ALI, *B. D. ROUSSEL;
Cyceron, INSERM U919, Caen, France

Abstract: Stroke is the third biggest killer and the leading cause of acquired disability in adults. The only drug approved to treat ischaemic stroke is tissue plasminogen activator (tPA). However, tPA exerts multiple and antagonistic effects within the brain parenchyma. In experimental models of ischaemia it has been shown that the endoplasmic reticulum stress (ER stress) pathway is activated and it has been suggested that it may modulate positively or negatively neuronal fate, depending on its duration. To date, the potential influence of tPA on ER stress has never been addressed in appropriate models. The aim of the study was to decipher the potential link between tPA and ER stress during stroke, and its implications in ischaemia-induced cell death. We used primary cultures of neurones under oxygen and glucose deprivation (OGD) in the presence or not of tPA to characterize the ER stress response. We measured by western blots the phosphorylation level of the eukaryotic initiation factor 2 alpha (eIF2 alpha) and by qPCR the expression levels of CHOP, GADD34 and Bip, which are ER stress response markers. Neuronal death was quantified by measuring lactate dehydrogenase release . As expected, OGD induced an ER stress response, as evidenced by the phosphorylation of eIF2 alpha, and the increased expression of CHOP, GADD34 and Bip. The ER stress response decreased in the presence of tPA, suggesting that tPA acts as an ER stress inhibitor during OGD. Moreover, OGD-induced neuronal death also decreased in the presence of tPA. Guanabenz an inhibitor of ER stress also protected neurones against OGD and no additive effect was observed with it. This suggests a direct role of tPA on OGD-induced neuronal death via an inhibition of the ER stress response. We report here for the first time a direct modulation of the ER stress response by tPA, showing beneficial effects on OGD-induced cell death. We need to understand the mechanisms of action of tPA on the ER stress response, to propose a strategy of neuroprotection in an *in vivo* model of stroke. Indeed we suggest a new neuro-trophic pathway for the tPA.

Disclosures: M. Louessard: None. **B.D. Roussel:** None. **D. Vivien:** None. **C. Ali:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Title: Neuroprotection by inhibition of p53 after asphyxic cardiac arrest involves autophagic and apoptotic mechanisms in rat hippocampus

Authors: *D. CUI[1,2], H. SHANG[4,3], W. NI[1], Y. XU[1], X. ZHANG[1], W. JIANG[1], U. BHALALA[2];
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Abstract: Objective: This study focuses on the contributions of the p53 signaling pathway and the molecular mechanism of autophagy activation and cell death in hippocampus following cerebral ischemia-reperfusion (I/R) triggered by asphyxic cardiac arrest(CA) . Materials and Methods: Male Sprague-Dawley rats weighing between 300 and 350 g were implanted with a cannula aimed at the left cerebroventricle for subsequent i.c.v. administration of PFT, BFA or 3-MA. Rats were intubated orotracheally with a 14-gauge cannula. After a 10-min equilibration period following surgery, asphyxia was induced by stopping mechanical ventilation and clamping the tracheal tubes at the end of expiration. After 8-min asphyxia, CPR was initiated by administering an i.v. bolus injection of epinephrine (0.02 mg/kg) and 5% (w/v) sodium bicarbonate (1mmol/kg), followed by mechanical ventilation and thoracic compressions. Main Results : Morphological and biochemical results showed activation of autophagy in hippocampal cells as evident by the increased formation of the autophagosome, the expression of active lysosomal cathepsin B and D and the microtubule-associated protein light chain 3 (LC3), and the conversion of LC3-I to LC3-II. CA upregulated the expression of tumor suppressor protein 53 (p53) and its target genes, including Bax, p53-upregulated modulator of apoptosis (Puma), and

damage-regulated autophagy modulator (DRAM). CA-induced elevations in pro-apoptotic proteins Bax and Puma and autophagic proteins LC3-II and DRAM were significantly reduced by the p53 specific inhibitor pifithrin-α (PFT). PFT also reduced hippocampal neuronal damage caused by CA. Similarly, the autophagy inhibitor 3-methyladenine (3-MA) and the lysosomal inhibitor bafilomycin A1 (BFA) significantly inhibited hippocampal neuronal damage. These results indicate that p53 exerts effects on both autophagy and apoptosis signaling pathways and that autophagy plays a significant role in the process of hippocampal neuronal cell death induced by cerebral I/R following asphyxic CA. Conclusions: Our data indicate that in addition to necrosis, cell death induced by CA contains both autophagy and apoptosis. Activation of autophagy preceded the apoptotic process and is mediated, at least partially, by p53. Autophagy contributes to CA-induced hippocampal neuronal death. Further clarification of beneficial and detrimental roles of autophagy in CA will shed new light on elucidating pathogenesis of cerebral ischemic diseases involving protein misfolding.

Disclosures: D. Cui: None. **W. Ni:** None. **Y. Xu:** None. **X. Zhang:** None. **W. Jiang:** None. **H. Shang:** None. **U. Bhalala:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.08. Ischemia

Support: AHA Grant 0555266B

Title: Do allografts of rat umbilical cord blood cells decrease infarct size to the same extent as human umbilical cord blood cell xenografts after middle cerebral artery occlusion in the rat?

Authors: *A. E. WILLING, J. D. NEWCOMB, S. GARBUZOVA-DAVIS, P. R. SANBERG; Ctr. Excellence Aging & Brain Repair, Neurosurg., Univ. South Florida, TAMPA, FL

Abstract: A single systemic injection of human umbilical cord blood (HUCB) cells delivered at the 48 hours post-stroke decreases infarct volume 80% and improves behavioral recovery after middle cerebral artery occlusion (MCAO). These improvements are associated with a reduction in both local inflammation in the brain and inhibition of the systemic immune response to the stroke. Our main hypothesis is that the mononuclear cell (MNC) fraction of the umbilical cord blood (UCB) decrease infarct size and improve stroke outcome by decreasing the

immune/inflammatory responses after stroke. Further, these cells will produce these effects regardless of whether they transplanted within species (rat (R) UCB cells into rat; allograft) or across species (HUCB into rat; xenograft). In order to address this hypothesis, we examined 1) the cellular composition of the MNC fraction of RUCB and HUCB cells compared to adult R MNCs and H MNCs; and 2) whether RUCB cells will decrease infarct size even more than xenografted HUCB cells. In the first study, samples of RUCB, HUCB, adult rat and human MNC underwent flow cytometric analysis to determine the distribution of T cells, B cells, monocytes and stem cells within the MNC fraction. HUCB cells had significantly more naïve T cells ($p < 0.001$) and significantly fewer cytotoxic T cells ($p < 0.05$) and stem cells ($p < 0.001$) than RUCB. When the RUCB and HUCB MNCs were injected intravenously 48 hours after MCAO and infarct size measured two weeks later, the smallest infarcts were present after HUCB administration, while infarcts in the RUCB group were similar to MCAO only group. These differences in the percentage of naïve T cells, cytotoxic and stem cells between RUCB and HUCB may have contributed to the differential effects of the allografted RUCB and the xenografted HUCB cells on infarct size. Alternatively, differences in immune functional capacity of the allografted and xenografted cells may have contributed to the observed results.

Disclosures: **A.E. Willing:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Saneron CCEL Therapeutics, Inc. **J.D. Newcomb:** None. **S. Garbuzova-Davis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Saneron CCEL Therapeutics, Inc. **P.R. Sanberg:** A. Employment/Salary (full or part-time):; Saneron CCEL Therapeutics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Saneron CCEL Therapeutics, Inc.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 805.06/W27

Topic: C.08. Ischemia

Title: The origin of self-renewing stem cells in the cortex after stroke

Authors: *M. FAIZ[1], N. SACHEWSKY[2], R. DESAI[1], C. MORSHEAD[2], A. NAGY[1]; [1]Lunenfeld-Tanenbaum Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada; [2]Univ. of Toronto, The Donnelly Ctr., Toronto, ON, Canada

Abstract: Under physiological conditions, adult neural stem cells (NSCs) in the SVZ give rise to neuroblasts that migrate to the olfactory bulb and give rise to new neurons. Brain injury elicits a response from NSCs and causes a deviation in the migration of progenitor cells to sites of damage. In response to damage, resident astrocytes also respond and contribute to the process of ongoing gliosis. Recently, it has been suggested that reactive astrocytes have a potential for self-renewal and multipotency and show neurosphere formation *in vitro*. However, both endogenous stem cells from the SVZ or de-differentiated reactive astrocytes have so far been limited in their potential to regenerate the brain. In order to increase the availability of cells present at the site of injury, recent effort has been focused on using the power of cellular reprogramming to convert astrocyte to NSC or neurons. Here, we investigate the origin of cortical-derived stem cells following a mild cortical stroke. Lineage tracing using inducible Nestin-CreER^{T2};tdtomato^{fl} mice showed that cortical-neurospheres are derived from SVZ precursor cells that migrate to the lesion site early after an insult. A small population of stem cells persists at later time points post stroke, while the majority of cells rapidly differentiate and give rise to a subpopulation of reactive astrocytes (derived from SVZ cells) that contributes to the ongoing gliosis. Lastly, we show that forced expression of transcription factor, Ascl1 can efficiently reprogram SVZ-derived reactive astrocytes to neurons, a novel way to boost endogenous neurogenesis.

Disclosures: M. Faiz: None. **N. Sachewsky:** None. **R. Desai:** None. **C. Morshead:** None. **A. Nagy:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Topic: C.08. Ischemia

Support: NIH NS085272

Title: Glutamate metabolism by inducible glutamate oxaloacetate transaminase protects stroke-affected brain

Authors: *S. KHANNA, S. GNYAWALI, J. WEIST, S. ROY, C. K. SEN, C. L. RINK; Surgery, Ohio State Univ., COLUMBUS, OH

Abstract: Glutamate serves multi-faceted (patho)physiological functions in the CNS as the most abundant excitatory neurotransmitter and under pathological conditions (i.e. ischemic stroke) as potent neurotoxin. The current work stems from our key observation demonstrating that induction of glutamate oxaloacetate transaminase (iGOT), a glutamate metabolizing enzyme in the brain, can attenuate stroke-induced injury. We have recently reported that by correcting the hypoxia component of stroke injury by supplementing oxygen (OH, oxygenated hypoxia) during stroke GOT level were significantly increased and glutamate levels were markedly reduced in stroke-affected brain. We hypothesize that under OH extracellular glutamate is taken up directly by neural cells and metabolized through a truncated TCA cycle. NMR spectroscopy was employed for *in vitro* and *in vivo* experiments to assess glutamate metabolism. For *in vitro* studies, primary cortical neuronal cells were incubated with [U13C]Glu for 4h under control and OH conditions. Under control conditions, greater proportion of [U13C] glutamate was metabolized and re-synthesized to [1,2,3-13C]Glu. In OH, less [U13C] was re-synthesized and [U13C]Aspartate was emerged as new metabolite supporting metabolism of glutamate via truncated TCA cycle. Next, 9.4T NMR spectroscopy was performed to assess spatial resolution of Glu and ATP distribution during stroke in mouse brain penumbra. Glutamate concentration was lower in penumbra stroke hemisphere of mouse receiving 100% inhaled oxygen. On the other hand, stroke-related loss of high energy phosphates were rescued by 100% inhaled oxygen. In this work we have identified that increased GOT in brain attenuates stroke injury by metabolizing extracellular glutamate via a truncated TCA cycle.

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Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Topic: C.08. Ischemia

Support: Soochow University Research starting funding Q 421500113

Doctoral Fund of Ministry of Education of China K521507713

Title: The roles of HIF-1alpha and VEGF in blood brain barrier damage during acute cerebral ischemia

Authors: *X. JIN[1], Y. SUN[2], Y. LIU[2], H. SHU[2];
[1]Inst. of Neurosci., Soochow Univ., Jiangsu, China; [2]Inst. of Neurosci., Soochow Univ., Suzhou, China

Abstract: Disruption of the blood brain barrier (BBB) is an antecedent event to intracerebral hemorrhage (ICH) in ischemic stroke. Interestingly, our previous studies showed that BBB disruption occurred within the thrombolytic time window of acute cerebral ischemia and tight junction protein occludin was damaged. However, the mechanism of this acute BBB disruption remains less well known. In the present study, we sought to determine whether hypoxia-inducible factor 1α (HIF-1α) and vascular endothelial growth factor (VEGF) play a role in ischemia induced BBB damage within the thrombolytic time window. Rats were subjected to 2 hrs filament occlusion of the middle cerebral artery (MCAO), followed by 10 min reperfusion. Successful MCAO was confirmed by 2,3,5-triphenyltetrazolium chloride (TTC) staining. Evans blue was injected to observe BBB damage, and HIF-1α and VEGF was detected by western blot and Immunohistochemistry. Extravasation of Evans blue was seen in brain sections after 2 hrs MCAO, with the tracer leakage limited to subcortical regions and the piriform cortex, indicating BBB damage, the expression of VEGF and HIF1α markedly increased at where the BBB was damaged, the tight junction protein occludin was degraded. Pretreatment with YC-1 (2 mg/kg, intra-vein, 30 min and 24 h before MCAO) significantly decreased VEGF expression, prevented the degradation of occludin and alleviated BBB damage. Taken together, early acute cerebral ischemic lead to HIF1α activate VEGF, which eventually leads to disruption of tight junction proteins BBB disruption. These findings may illustrate the effect of HIF1α and VEGF in early ischemic BBB injury, and may provide new ideas and strategies to extend the time window of thrombolysis, reduce cerebral hemorrhage caused by thrombolytic.

Disclosures: **X. Jin:** None. **Y. Sun:** None. **Y. Liu:** None. **H. Shu:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

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Topic: C.08. Ischemia

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Chinese NSF grant 81371306

Title: Nicotinamide phosphoribosyltransferase protects white matter injury in cerebral ischemia

Authors: *Z. JING[1,2], J. XING[1,2], X. CHEN[1,2], R. A. STETLER[1], Z. WENG[1], Y. GAN[1], F. ZHANG[1], Y. GAO[3], R. K. LEAK[4], J. CHEN[1,2], G. CAO[1,2];
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Abstract: Nicotinamide phosphoribosyltransferase (NAMPT) has been implicated in neuroprotection against ischemic brain injury, but the mechanism underlying its protective effect remains largely unknown. To further examine the protective effect of NAMPT against ischemic stroke and its potential mechanism of action, we generated a novel NAMPT transgenic mouse line. Transgenic mice and wild-type littermates were subjected to transient occlusion of the middle cerebral artery (MCAO) for 60 min. NAMPT overexpression significantly reduced infarct volume and improved long-term neurological outcomes compared to littermates. Interestingly, NAMPT transgenic overexpression significantly increased the area of myelin expression following ischemia compared to wild-type ischemic littermates, indicating that NAMPT protects against white matter injury. These data suggest a novel role for NAMPT in the protection of white matter following ischemic injury.

Disclosures: Z. Jing: None. **J. Xing:** None. **G. Cao:** None. **R.A. Stetler:** None. **F. Zhang:** None. **Z. Weng:** None. **Y. Gan:** None. **J. Chen:** None. **Y. Gao:** None. **R.K. Leak:** None. **X. Chen:** None.

Poster

805. Ischemia: Cellular Mechanisms and Neuroprotection VIII

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 805.10/W31

Topic: C.08. Ischemia

Support: NIH Grant R01 NS060017

Title: Proinflammatory role of Receptor Interacting Protein-3 (RIP3) in Ischemia induced neuronal cell death

Authors: *D. C. GILLIS, S. KIM, J. LI;
Vet. Integrative Biosci., Texas A&M Univ., College Station, TX

Abstract: Pro-inflammatory role of Receptor Interacting Protein-3 (RIP3) in Ischemia induced neuronal cell death D. Christopher Gillis, Sunja Kim, Jianrong Li Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843 Ischemic tissue damage to the developing brain leads to death of the CNS resident cells and adverse long-term neurological deficits. Recent revelation that certain necrotic cell death is regulated by the Receptor Interacting Protein-3 (RIP3) suggests that programmed necrotic cell death mechanism may contribute to ischemic brain injury. As RIP3 is also implicated in promoting pro-inflammatory responses, we investigated the role of RIP3 in ischemia-induced neuronal damage as well as inflammation-induced potentiation of the neuronal damage. When we stimulated organotypical hippocampal slices with lLipopolysaccharide (LPS), a TLR4 ligand, we found that $RIP3^{-/-}$ slices exhibited decreased production of proinflammatory cytokines and chemokines such as IL-1β, IL-6, CCL2, CCL3, and CCL5 as compared to wild type $RIP3^{+/+}$ slices. LPS pretreatment markedly exacerbated Oxygen Glucose Deprivation (OGD)-induced CA1 neuronal death in wild type hippocampal slices, but to a much less extent in $RIP3^{-/-}$ slice cultures. These results demonstrate that pro-inflammatory microglial activation perpetuates neuronal damage after OGD by activating the RIP3 pathway. To determine the role RIP3 plays *in vivo*, we used a mouse neonatal hypoxia-ischemia model (HI), which involves permanent ligation of the right common carotid artery followed by exposure to a low oxygen environment. This experimental model revealed a potential role for microglia in ischemic brain injury. Further analysis of respective brain samples from $RIP3^{-/-}$ versus $RIP3^{+/+}$ mice is ongoing, and preliminary results show differences in neuronal populations. Finally, we seek to examine the developing brain of from $RIP3^{-/-}$ versus $RIP3^{+/+}$ mice after systemic pro-inflammatory challenge using LPS.
Supported in part by the National Institutes of Health Grant R01 NS060017.

Disclosures: D.C. Gillis: None. **S. Kim:** None. **J. Li:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.01/W32

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: A intramural grant from the University of Mississippi Medical Center

Title: Hyperoxia activates microglia but reduces apoptosis in the neonatal rat brain

Authors: *Y. PANG[1], L.-T. TIEN[2], X. DAI[3], J. SHEN[1], Z. ZHU[1], A. BHATT[1], L.-W. FAN[1]; [1]Univ. Mississippi Med. Ctr., Jackson, MS; [2]Med., Fu Jen Catholic Univ., New Taipei City, Taiwan; [3]Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Oxygen supplement is routinely used to care for premature infants due to their lung immaturity. High oxygen levels (hyperoxia) are well known to cause damage to retina in premature infants by generating excessive reactive oxidative species. Recent clinical data suggest that hyperoxia is associated with long-term adverse neurological outcomes in premature infants; however, the underlying mechanisms remain elusive. The aims of this study were to test: 1. whether hyperoxia exposure affects programmed neuronal death, which is an important developmental program in controlling neuronal numbers; and 2. whether hyperoxia exposure affects glial development. Rat pups at postnatal P4 were exposed to 85% oxygen for 48 h. On P6, brain sections were prepared for TUNEL and immunohistochemical studies. Our results showed there were extensive TUNEL+ cells in the control P6 rat brain, which were mostly abundant in the deep subcortical nuclei such as the basal ganglia, thalamus, and nucleus accumbens. Hyperoxia exposure significantly reduced the number of TUNEL+ as well as caspase-3+ cells in all these brain regions. In contrast, hyperoxia exposure markedly activated microglia in the periventricular region, indicated by increased amoeboid microglia labeled with Iba1 antibody. Hyperoxia also enhanced the immunoreactivity of Rip, a marker for immature oligodendrocytes. Our data suggest neonatal exposure to hyperoxia may lead to aberrant early neurodevelopmental programs, resulting in adverse neurological outcomes.

Disclosures: Y. Pang: None. **L. Tien:** None. **X. Dai:** None. **J. Shen:** None. **Z. Zhu:** None. **A. Bhatt:** None. **L. Fan:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.02/W33

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: HL111621

GM083108

Title: Computational modeling of cytokine signaling in microglia

Authors: *W. D. ANDERSON, H. MAKADIA, J. SCHWABER, R. VADIGEPALLI; Thomas Jefferson Univ., PHILADELPHIA, PA

Abstract: Neuroinflammation due to glial activation has been linked to many CNS diseases, including Alzheimer's disease, epilepsy, neuropathic pain, and neurogenic hypertension. While etiologies vary, correlation between neuroinflammation and disease suggests that CNS inflammation may underlie the genesis and/or perpetuation of many pathological states. Because precision in the mechanistic understanding of how intercellular cytokine interactions coordinate the inflammatory milieu is limited in statistical analyses of expression data, we employed a dynamic modeling approach that permits the assessment of system control mechanisms using robustness analyses and targeted perturbations. We developed a microglial cytokine interaction network model to assess potential novel mechanisms of glial activation by employing Delay Differential Equations. The model has six nodes, 12 activating connections (e.g., TNFα promotes TGFß production), and 12 inhibitory connections (e.g., TGFß inhibits TNFα production), all of which were validated based on available literature. A model fit to cytokine data from LPS-stimulated cultured microglia demonstrated that the model captures the dynamics of experimentally observed cytokine production. Variance-based parametric sensitivity analysis suggested that that TNFα is highly sensitive to negative feedback from TGFß and IL-10. Consistent with experimental data, the model showed tolerance in the TNFα response to repeated doses of LPS. While both TGFß and IL-10 inhibit TNFα via feedback, IL-10 knockout (KO) enhanced TNFα tolerance whereas TGFß KO hyper-sensitized the TNFα response to repeated LPS stimuli. The contrasting effects of TGFß versus IL-10 occurred because the IL-10 KO enhanced the initial TNFα peak response, which resulted in subsequent TGFß activation and robust negative feedback, thereby rendering TNFα unresponsive to a second dose of LPS. Thus, IL-10 decreased tolerance by restraining the influence TNFα on TGFß. The mechanism of TNFα tolerance to LPS involved TGFß up-regulation following the initial LPS dose, consistent with data. Simulations examining adaptation of the TNFα response to LPS suggested that TGFß controls adaptation by modulating the steady-state response to LPS, with negligible effect on the peak TNFα response. In contrast, IL-10 primarily controlled the TNFα peak amplitude following LPS, without an effect on adaptation. In summary, our model simulations and analysis results predict that TGFß and IL-10 have prominent and contrasting roles in regulating microglia activation, suggesting potential for targeted therapeutic interventions against neuroinflammation.

Disclosures: W.D. Anderson: None. **H. Makadia:** None. **J. Schwaber:** None. **R. Vadigepalli:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.03/W34

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH P01 HD016596

NIH R01 NS085165

Title: Idebenone stimulates mitochondrial respiration and attenuates nitric oxide production by activated microglial cells

Authors: *E. A. BORDT[1,2], P. STUDLACK[1], M. HANSCOM[1], S. X. GE[1,2], B. M. POLSTER[1,2];
[1]Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Univ. of Maryland Shock, Trauma and Anesthesiol. Res. (STAR) Organized Res. Ctr., Baltimore, MD

Abstract: Microglia, the innate immune cells of the brain, survey their environment and maintain a healthy milieu through clearance of debris. Microglia enter an activated state following brain injury and release various proinflammatory factors such as nitric oxide and reactive oxygen species, which in excess are toxic. Using the Seahorse XF24 Extracellular Flux Analyzer to measure cellular oxygen consumption, we found that respiration was impaired following activation of rat HAPI microglial cells with lipopolysaccharide (LPS) and interferon-γ (IFN-γ). We tested the hypothesis that the cell permeable short chain Coenzyme Q analogue idebenone will bypass respiratory inhibition following microglial activation and reduce the release of proinflammatory mediators. Co-treatment of HAPI microglial cells with idebenone (20-40 μM) resulted in a marked decrease in the release of nitric oxide in response to LPS plus IFN-γ. In addition, idebenone partially rescued the respiration of activated cells when added 8-18 hours post LPS/IFN-γ treatment. Because idebenone is thought to shuttle reducing equivalents from cytoplasmic NAD(P)H to complex III of the electron transport chain, we tested the effect of electron transport chain inhibitors on the ability of idebenone to impair activation-induced nitric oxide production. The complex III inhibitor antimycin A but not the complex I inhibitor rotenone attenuated the ability of idebenone to diminish the LPS plus IFN-γ-induced release of nitric oxide without themselves altering nitric oxide release, consistent with the possibility that idebenone influences microglial activation by restoring mitochondrial electron transport. Overall, our results indicate that idebenone attenuates proinflammatory microglial nitric oxide

production, possibly through a mitochondrial mechanism. The safety of idebenone in humans is already well established. Our findings suggest that idebenone may be clinically useful for a number of additional neurodegenerative disorders with a neuroinflammatory component.

Disclosures: E.A. Bordt: None. **P. Studlack:** None. **M. Hanscom:** None. **S.X. Ge:** None. **B.M. Polster:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.04/W35

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CTSA grant KL2TR000057

Title: Liver X receptor-dependent inhibition of microglial inducible nitiric oxide synthase

Authors: *U. OH, J. MCVOY;
Neurol., Virginia Commonwealth Univ. Sch. of Med., Richmond, VA

Abstract: The nuclear receptor liver X receptor (LXR) exerts transcriptional control over reverse cholesterol transport and inflammatory response in cells of the myeloid lineage, suggesting that LXR may be a potential target in a number of chronic neuroinflammatory and neurodegenerative diseases where persistent microglial activation has been implicated in the pathogenesis. Questions regarding endogenous LXR activation in the central nervous system (CNS), mechanism of transcriptional repression and the response to LXR agonist in the setting of established CNS inflammation remain. The effect of LXR activation on microglia and CNS inflammation was studied using a synthetic LXR agonist in cultured microglia, microglial cell line and experimental allergic encephalomyelitis (EAE), an animal model of CNS inflammation. LXR activation inhibited iNOS expression and the production of nitric oxide in lipopolysaccharide (LPS)-stimulated microglia. Inhibition of microglial response to interferon-γ was less reliable. LXR-dependent iNOS repression was associated with inhibition of histone 4 acetylation and of NF-kappaB p50 binding at the iNOS promoter. The addition of histone deacetylase inhibitor or HDAC3 siRNA partially reversed the LXR-dependent inhibition of nitric oxide production, suggesting that the histone deacetylase activity is mechanistically important for LXR-dependent transcriptional repression. Analysis of CNS gene expression in EAE showed that LXR and LXR-dependent genes expressions were downregulated in the setting of CNS

inflammation. Nevertheless, administration of LXR agonist GW3965 during the effector phase of EAE improved clinical disease and reversed the diminished LXR-dependent gene expression with respect to the genes involved in reverse cholesterol transport. However, the gene expression of iNOS and other inflammatory genes were not significantly inhibited by LXR activation in the CNS. In conclusion, LXR activation exerts transcriptional repression of iNOS in LPS-stimulated microglia, in part through associated HDAC activity, but endogenous CNS LXR activity was downregulated in the setting of EAE. The administration of LXR agonist reversed the downregulation of reverse cholesterol transport, but suppression of inflammatory genes in the CNS was less reliable.

Disclosures: U. Oh: None. **J. McVoy:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.05/W36

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR grant 6218

Title: The role of macrophage scavenger receptor A (SR-A) in macrophage polarisation

Authors: *A. D. GREENHALGH[1], A. KRONER[2], S. DAVID[2];
[1]McGill Univ., Montreal, QC, Canada; [2]Res. Inst. of the McGill Univ. Hlth. Ctr., Montreal, QC, Canada

Abstract: Uncontrolled inflammation after central nervous system (CNS) injury and disease can result in excessive tissue damage. Timely resolution of inflammation relies on the phagocytic removal of dead and damaged cells and their components. Macrophages of both microglial and monocytic origin perform this role in the CNS by clearing material through a myriad of cell surface receptors. The class A macrophage scavenger receptor (MSR-A) is the prototypic member of a family of membrane receptors collectively termed scavenger receptors. Receptors of this group recognise modified lipoproteins and apoptotic cells and are expressed on both peripheral macrophages and microglial cells. Here we investigate the influence of the SR-A in macrophage polarization. Preliminary results using mice lacking SR-A show that two receptors involved in the pro-inflammatory response; the co-stimulatory signalling receptor CD86 and the Fc receptor CD16, are more highly expressed is bone marrow-derived macrophage (BMDM)

cultures treated with LPS, compared to BMDMs from wild type (WT) controls. Conversely, enzymes involved in suppressing inflammation; Arginase-1 and sphingosine kinase, are down regulated in SR-A null BMDMs which phagocytose myelin, compared to WT controls. These results suggest that signalling at the SR-A has an anti-inflammatory effect and further studies are under way to assess its role in the context of CNS injury.

Disclosures: A.D. Greenhalgh: None. **A. Kroner:** None. **S. David:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.06/X1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Research Council grant no. 2010-4389

VINNOVA, grant No. 2009-00236

Swedish Brain Foundation, the Human Frontier Science Program

Title: The role of Galectin-3 in α-synuclein-induced microglial activation

Authors: *A. BOZA-SERRANO[1], J. REYES[2], N. REY[3], H. LEFFLER[2], L. BOUSSET[4], U. NILSSON[5], P. BRUNDIN[3], J. VENERO RECIO[6], M. BURGUILLOS[7], T. DEIERBORG[2]; [1]Exptl. Neuroinflam. Lab., [2]Lund Univ., Lund, Sweden; [3]Van Andel Inst., Grand Rapids, MI; [4]Lab. d'Enzymologie et Biochimie Structurales, Gif-sur-Yvette, France; [5]Ctr. for Analysis and Synthesis, Lund, Sweden; [6]Univ. de Sevilla, Sevilla, Spain; [7]Karolinka Institutet, Stockholm, Sweden

Abstract: Abstract Background: Parkinson's disease (PD) is the most prevalent neurodegenerative motor disorder. The neuropathology is characterized by intraneuronal protein aggregates of α-synuclein and progressive degeneration of substantia nigra dopaminergic neurons. It is believed that neuroinflammation contributes to the neurodegenerative process. Previous studies have shown that extracellular α-synuclein aggregates activate microglial cells and induce inflammation. The signaling pathways involved in α-synuclein-mediated microglia activation are poorly understood. Galectin-3 is a member of a carbohydrate-binding protein family. It is involved in inflammation in different tissues and is expressed in the brain. Therefore, we investigated whether galectin-3 is involved in the microglia activation triggered by α-

synuclein. **Results:** We cultured microglial (BV2) cells and induced cell activation by addition of exogenous α-synuclein monomers and aggregates to the culture medium. This caused a significant increase in the levels of proinflammatory mediators including the inducible Nitric Oxide Synthetase (iNOS), interleukin 1 Beta (IL-1β) and Interleukin-12 (IL-12). We then reduced the levels of galectin-3 expression using siRNA or pharmacologically inhibited galectin-3 activity using bis-(3-deoxy-3-(3-fluorophenyl-1*H*-1,2,3-triazol-1-yl)-β-D-galactopyranosyl)-sulfane. Both these approaches led to a significant reduction in the observed inflammatory response induced by α-synuclein. We confirmed these findings using primary microglial cells obtained from galectin-3 null mutant mice. Finally, we performed injections of α-synuclein in the olfactory bulb of wild type mice and observed that some of the α-synuclein was taken up by activated microglia that were immunopositive for galectin-3. **Conclusions:** We show that α-synuclein aggregates induce microglial activation and demonstrate for the first time that galectin-3 plays a significant role in microglia-induced activation by α-synuclein. These results suggest that genetic down-regulation or pharmacological inhibition of galectin-3 might constitute a novel therapeutic target in α-synucleinopathies that includes PD. **Keywords:** Microglia, Galectin-3, Neuroinflammation, α-synuclein, Parkinson's Disease

Disclosures: A. Boza-Serrano: None. **J. Reyes:** None. **N. Rey:** None. **H. Leffler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock Options. **L. Bousset:** None. **U. Nilsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock Options. **P. Brundin:** None. **J. Venero Recio:** None. **M. Burguillos:** None. **T. Deierborg:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.07/X2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sema3A-Plexin-A1 interaction is crucial for Toll-like receptor-mediated microglial activation in the development of lipopolysaccharide-induced encephalopathy

Authors: *T. ITO[1], K. YOSHIDA[2], T. NEGISHI[2], K. YUKAWA[2];
[1]Physiol., Meijo Univ., Nagoya / Aichi, Japan; [2]Meijo, Nagoya, Japan

Abstract: Recent studies have suggested that semaphorins, a family of repulsive axon guidance molecules, may exert a crucial role in maintaining brain homeostasis by controlling microglial activity. Sema3A secreted in higher amounts from injured neurons is suggested to attenuate excessive inflammatory responses by inducing microglial apoptosis through its interaction with Plexin-A1 receptors on activated microglia. To disclose the *in vivo* role of Plexin-A1-mediated signaling in lipopolysaccharide (LPS)-induced injury in mouse brain, we examined the neuroinflammatory changes initiated by LPS administration to the cerebral ventricles of both wild-type (WT) and Plexin-A1-deficient (-/-) mice. WT mice administered LPS displayed significantly higher expression of COX-2, IL-1β, TNF-α, and iNOS in the hippocampus, and significantly greater ventricular enlargement and intracerebral infiltration of leukocytes, as compared with the saline-treated mice. In contrast, Plexin-A1-/- mice administered LPS did not show significantly increased COX-2, IL-1β, TNF-α, and iNOS in the hippocampus as compared with the saline-treated mice. Plexin-A1-/- mice administered LPS did not exhibit significant increases in ventricle size or infiltration of leukocytes into the brain, as compared with the saline-treated mice. In WT, but not in Plexin-A1-/- primary microglia treated with LPS, Sema3A induced significantly more NO generation than in the immunoglobulin G control. Further studies with BV-2 microglial cells demonstrated the crucial role of Sema3A-Plexin-A1 signaling through ERK activation for the Toll-like receptor-induced NO production in activated microglia. These results reveal the crucial role of the Sema3A-Plexin-A1 interaction in the Toll-like receptor 4-mediated signaling of the LPS-induced activation of microglia. Thus, our results reveal the essential role of Plexin-A1-mediated signaling in the development of LPS-induced neuroinflammation in mice, suggesting the possible application of microglial control of the semaphorin-plexin signaling system to the treatment of LPS-induced encephalopathy and other psychiatric diseases associated with neuroinflammation.

Disclosures: T. Ito: None. **K. Yoshida:** None. **T. Negishi:** None. **K. Yukawa:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.08/X3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: IFNAR1 signaling regulates the microglial phenotype in response to peripheral LPS challenge

Authors: *J. M. TAYLOR, K. M. BRODY, P. J. CRACK;
Dept of Pharmacol., Univ. of Melbourne, Melbourne, Australia

Abstract: Neuroinflammation has been proposed to contribute to the neuronal cell damage following injury, infection or disease. We hypothesise that type-1 interferon (IFN) signalling is a key mediator of the neuroinflammatory response. Mice lacking the type-1 IFN receptor (IFNAR1) (n=6) were given a single intraperitoneal (i.p) injection of lipopolysaccharide (LPS) (2mg/kg). A temporal analysis of the early neuroinflammatory changes was undertaken by QPCR, western analysis and immunohistochemistry. QPCR confirmed increased expression of IFN-[[Unsupported Character - Symbol Font ]] and the downstream mediator, IRF7 in wildtype mice at 6 and 24 hours post-LPS injection (compared to sham levels), a response that was decreased in the IFNAR1-/- brains. Increased Iba-1 immunoreactive microglial cells were identified in the substantia nigra of both wildtype and IFNAR-1-/- mice at 6 and 24 hours post-LPS injection. Significantly, at both time-points the microglial number and activation was reduced in the IFNAR1-/- brains compared to wildtype mice. Upon activation, microglia can polarise to form two differing phenotypes; M1 (damaging) or M2 (neuroprotective). QPCR identified reduced expression of both M1 (iNOS and CD32) and M2 (Arg-1, YM1, CD206 and TGF-[[Unsupported Character - Symbol Font ]]) microglial markers in the IFNAR1-/- brains at 6 hours post-LPS suggesting an altered neuroinflammatory phenotype. This study confirms type-1 IFN signaling plays a role in regulating the cellular environment involved in the neuroinflammatory response. This modulation has implications in both acute and chronic neuropathologies where neuroinflammation may be a critical event in perpetuating the neuronal cell death.

Disclosures: J.M. Taylor: None. **K.M. Brody:** None. **P.J. Crack:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.09/X4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Doxycycline reduces the inflammatory response of LPS-treated microglial cells

Authors: S. B. SOCIAS[1,2], I. OUIDJA[3], F. SANTA CECÍLIA[4], J. E. SEPÚLVEDA DÍAZ[3], T. M. CUNHA[4], E. DEL-BEL[5], *S. HUNOT[3], P. P. MICHEL[3], R. RAISMAN-VOZARI[3];

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Abstract: We previously demonstrated that the antibacterial drug doxycycline confers neuroprotection in a Parkinson disease mouse model by restraining glial cell activation (Lazzarini et al, Glia, 2013). In the present study, we wished to explore further the potential of doxycycline against neuroinflammation, using microglial cell-enriched cultures prepared from post-natal (P1) mouse brain. More specifically, we evaluated the impact that a 4 hour pretreatment with doxycycline exerts on microglial cells exposed for the next 24 hours to the bacterial inflammogen LPS (1 ng/ml). Our results show that the calcium-binding protein Iba-1 used as a marker of microglial cell activation, was detectable in 80% Mac-1+ cells after LPS treatment and that this number dropped to 20% with an optimal concentration of doxycycline, i.e., 200μM. Coherent with this result, doxycycline diminished the release of two pro-inflammatory cytokines, TNF-α and IL-1β and that of nitric oxide, a gaseous mediator of neuroinflammatory responses. Finally, doxycycline was also found highly effective in a situation where microglial cells were exposed to 10 ng/ml LPS, i.e., a concentration of the inflammogen required to adequately stimulate the production reactive oxygen species in microglial cells. Taken together our results suggest that doxycycline could operate as an efficient protective agent through a repressive effect on microglial cells.

Disclosures: S.B. Socias: None. **R. Raisman-Vozari:** None. **P.P. Michel:** None. **J.E. Sepúlveda Díaz:** None. **I. ouidja:** None. **S. Hunot:** None. **T.M. Cunha:** None. **F. Santa Cecília:** None. **E. Del-Bel:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.10/X5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Peter Deane Trust (to K.B.)

National Institutes of Health Grant AI082329 (to N.P.)

Title: APOBEC1-mediated RNA editing in microglia and its consequences for inflammation in the brain

Authors: N. PAPAVASILIOU[1], Y. CHUNG[2], K. GAGNIDZE[2], J. GAL-TOTH[2], V. RAYON[1], *K. BULLOCH[3];
[1]Lab. of Lymphocyte Biol., The Rockefeller Universituy, New York, NY; [2]Neuroimmonology and Inflammation Program, Lab. of Neuroendocrinology, [3]Neuroimmunology and Inflammation Program, Labs of Mol Imm. and Neuroendo., The Rockefeller Univ., New York, NY

Abstract: Microglia are heterogeneous population of brain immune cells important for homeostatic and inflammatory processes. We have previously shown that RNA editing by deaminase APOBEC1 in the transcriptome of bone marrow derived mouse macrophages leads to the generation of populations that are heterogeneous, and functionally diverse, enabling rapid population adaptation to different environmental settings. Here we demonstrate that APOBEC1 catalyzes editing both *in vivo* and *in vitro* in immune cells derived from the CNS. Among the edited transcripts in BV2 microglia cell line are genes particularly relevant for the development of neurodegenerative diseases, such App and adam10, as well as inflammatory genes, cd36, b2m, Lamp1 and Lamp2. Using brain immune cells of monocytic lineage isolated from the olfactory bulbs of vesicular-stomatitis virus (VSV) infected mice, and sorted based on the expression of CD45int/CD11b+/CD11c- and CD45int/CD11b+/CD11c+, we confirmed that APOBEC1 is also functional *in vivo*. Moreover, immuno-histochemical analysis of the APOBEC1 KO mice brains showed signs of demyelination and neurodegeneration, suggesting an important role for this RNA editing enzyme in the mechanisms of homeostasis in the CNS. The extent of APOBEC1 mediated editing in the CNS and the consequences for the inflammatory response (both acute and chronic) will be discussed, especially in the context of diseases of the aging brain.

Disclosures: N. Papavasiliou: None. **Y. Chung:** None. **K. Gagnidze:** None. **J. Gal-Toth:** None. **V. Rayon:** None. **K. Bulloch:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.11/X6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: IL-1 signaling exacerbates brain injury following seizurogenic exposure to the nerve agent soman (GD) in mice

Authors: *T. F. BOWENS[1,2], M. A. GUIGNET[2], J. F. IRWIN[2], K. LAITIPAYA[2], M. D. WEGNER[3], E. A. JOHNSON[2];
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Abstract: Exposure to organophosphorus compounds can initiate status epilepticus (SE), which can lead to severe progressive neuropathology and behavioral impairment for survivors of exposure to chemical warfare nerve agents (CWNAs). While treatments are available to ameliorate SE, the therapeutic window for control is relatively short, with no approved treatments available to address the neurodegenerative process. Following exposure, brain damage resulting from SE activates microglia and astrocytes to upregulate inflammatory cytokines, including the pro-inflammatory cytokine interleukin-1 (IL-1). This proinflammatory environment creates a positive feedback loop that potentiates neurodegeneration, which can potentially damage healthy neuronal tissue. Inhibition of neuroinflammation reduces acute neurodegeneration in various CNS injury models and may also be beneficial following CWNA exposure. A soman (GD) model was developed using wild-type and IL-1 signaling knockout mouse strains (i.e., IL-1R and IL-1Ra) to validate IL-1 signaling as a viable neuroprotective target. Results showed significant neuroprotection in various brain regions in strains that had impaired IL-1 signaling. In addition, the IL-1 signaling inhibitor anakinra produced post-exposure neuroprotection and replicated results seen in the knockout mice. These results show that inhibition of IL-1 signaling can afford neuroprotection after GD exposure. Therefore, research into neuroprotective strategies is important to improve brain pathology and potentially improve behavioral outcomes.

Disclosures: T.F. Bowens: None. **M.A. Guignet:** None. **J.F. Irwin:** None. **K. Laitipaya:** None. **M.D. Wegner:** None. **E.A. Johnson:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.12/X7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NASU Biotechnology Grant

DFFD Grant F46.2/001

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NASU Grant for Young Scientists

STCU Grant 5510

Title: Cell-type-specific inflammatory-induced changes in dorsal horn synaptic transmission

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Abstract: Persistent peripheral inflammation alters trafficking of AMPA receptors (AMPARs) at the synapses between primary afferents and dorsal horn neurons that contributes to the inflammatory pain maintenance. However, how this alteration changes the synaptic activity within the dorsal horn circuitry and synaptic AMPARs, particularly, in different neuronal populations still remain unknown. Here we found that Complete Freund's Adjuvant (CFA) - induced peripheral inflammation prominently augmented excitatory synaptic drive to the lamina II dorsal horn neurons characterized by intrinsic adapting firing properties and apparently decreased that to the tonic firing lamina II neurons, proving thus different roles of these types of dorsal horn interneurons in pain processing. Persistent peripheral inflammation differentially changed excitatory and inhibitory synaptic drives in these neuronal populations, shifting a balance between excitation and inhibition of lamina II neurons within the dorsal horn circuitry towards excitation in the adapting firing but towards inhibition in the tonic firing neurons. Peripheral inflammation differentially adjusted AMPAR pool at the synapses of the adaptingfiring and tonic firing lamina II neurons, assuming different mechanisms for trafficking of synaptic AMPARs in these neuronal populations during persistent inflammatory conditions. Our results thus reveal differential, neuron-type specific rearrangements in synaptic drive within the dorsal horn circuitry and in the synaptic AMPAR pool in different types of lamina II neurons that may contribute to the inflammatory pain maintenance.

Disclosures: P. Belan: None. **V. Krotov:** None. **O. Kopach:** None. **N.V. Voitenko:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R01GM057226 (to KJT)

NIH grant R01GM089807 (to VP)

Title: Brain region-specific alterations in the expression of cytokines, microglia and astrocyte markers, and cholinergic system components during lethal inflammation

Authors: H. A. SILVERMAN[1,4], M. DANCHO[1], A. REGNIER-GOLANOV[5], M. NASIM[6], M. OCHANI[1], P. S. OLOFSSON[1], M. AHMED[7], E. MILLER[2,4], S. S. CHAVAN[1], E. GOLANOV[8], C. N. METZ[3,4], K. J. TRACEY[1,4], *V. PAVLOV[1,4];
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Abstract: Exacerbated cytokine release and dysfunctional brain-peripheral immune communications are important features of several inflammatory and autoimmune disorders. Dysregulated peripheral immune responses and cytokine production activate brain immune responses and deteriorate neuronal function. Peripheral cytokine release is controlled by neural mechanisms and brain cholinergic signaling plays an important role in this regulation. Using qPCR, we aimed to provide insight into alterations in gene expression of cytokines, microglia and astrocyte markers, and cholinergic system constituents in the brain (cortex, brainstem, cerebellum, hippocampus, hypothalamus, thalamus, and striatum), during lethal murine endotoxemia. In addition, serum cytokines, gross brain cell morphology and microglia morphology were evaluated. Significant increases in serum IL-1β, IL-6 and other cytokine levels 4h following LPS (8 mg/kg, i.p.) injection highlighted the magnitude of the systemic inflammatory response, which did not however result in gross morphological alterations in the brain (H&E staining). Brain *Il1b* and *Il6* mRNA expression were differentially increased in endotoxemic mice: the highest magnitude of *Il1b* mRNA expression upregulation was determined in cortex (144-fold), and the lowest in cerebellum (29-fold); the highest *Il6* mRNA expression was observed in cerebellum (173-fold), and the lowest in striatum (29-fold). *Gfap* mRNA expression was upregulated in cortex, cerebellum, brainstem, hippocampus and thalamus. Surprisingly, *Iba1* mRNA expression was decreased in some brain regions, in parallel with microglia activation, indicated by Iba1 immunostaining. Brain cholinergic system alterations were revealed by: *Chat* mRNA expression downregulation in striatum; *Ache* mRNA expression downregulation in cortex and upregulation in hippocampus; and *Chrm1* (M1 muscarinic acetylcholine receptor) mRNA expression downregulation in cortex and brainstem of endotoxemic mice. No *Chat* or *Chrm1* mRNA expression was detected in cerebellum. These

results identify region-specific *Il1b* and *Il6* mRNA expression upregulation during lethal, endotoxin-induced inflammation. Our results also indicate that trends in alterations of microglia marker gene expression and protein staining do not necessarily correlate. We also reveal specific alterations in the brain cholinergic system in these settings. These findings contribute to better understanding the bidirectional brain-periphery communication during inflammatory conditions, which may have therapeutic implications. This study was funded in part by NIH/NIGMS.

Disclosures: **H.A. Silverman:** None. **M. Dancho:** None. **A. Regnier-Golanov:** None. **M. Nasim:** None. **M. Ochani:** None. **P.S. Olofsson:** None. **M. Ahmed:** None. **E. Miller:** None. **S.S. Chavan:** None. **E. Golanov:** None. **C.N. Metz:** None. **K.J. Tracey:** None. **V. Pavlov:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 806.14/X9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: Neuroinflammation and neuroprotection following acute soman-induced seizure in mice

Authors: ***J. F. IRWIN**[1], M. A. GUIGNET[2], K. LAITIPAYA[2], T. F. BOWENS[2], M. D. WEGNER[3], E. A. JOHNSON[2];
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Abstract: Severe neuropathology and behavioral impairment are a result of prolonged status epilepticus (SE) caused by organophosphorus compounds such as soman (GD). GD, a potent acetylcholinesterase inhibitor, causes prominent cell death in the hippocampus, thalamus and piriform cortex, leading to the activation of the neuroinflammatory cascade. Neuroinflammation can exacerbate tissue injury or promote healing, depending on the intricate interaction of multiple cells, inflammatory factors and receptors as the injury progresses, a fact that has complicated the development of effective neuroprotective therapies for CNS injury models such as SE. TNF signaling, a prominent proinflammatory pathway activated in response to injury, can worsen tissue damage. Therefore, this study investigated the temporal and regional upregulation of TNFα in the brain and the effects of the TNF signaling pathway on the acute development of tissue injury up to 24 hours after GD-induced seizure onset. A wild-type mouse model was

developed for GD exposure that maximized SE-induced brain damage. Various background-matched TNF pathway knockout mouse strains were used to identify their effects on acute neurodegeneration, mortality, seizure onset and other physiological variables following GD exposure. Results indicate that the loss of normal TNFR signaling does confer neuroprotection on various brain regions and can alter seizure onset times and mortality rates. These results along with neuroinflammation profiles from the mouse model guided the selection of pathway-specific drugs for testing. These studies describe a rational and relatively rapid therapeutic strategy for the development of effective post-exposure GD neuroprotectants and show that neuroprotection is possible following GD exposure with a longer therapeutic window than is afforded by conventional treatments.

Disclosures: **J.F. Irwin:** None. **M.A. Guignet:** None. **K. Laitipaya:** None. **T.F. Bowens:** None. **M.D. Wegner:** None. **E.A. Johnson:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

Location: Halls A-C

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Program#/Poster#: 806.15/X10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministerio de Economia y Competividad

Fundació La Marató to RLV

Title: Characterization of inflammatory response after mouse spinal cord injury

Authors: *I. FRANCOS-QUIJORNA[1], R. LOPEZ-VALES[2];
[1]Univ. Autonoma De Barcelona, Bellaterra, Spain; [2]Univ. Autonoma De Barcelona, Barcelona, Spain

Abstract: Inflammatory response plays an essential role to protect the body after injury. Inflammation, however, must be a highly regulated response, otherwise, it may lead to tissue damage and/or to chronic inflammation, as observed after spinal cord injury (SCI). Despite the contribution of inflammation to SCI, the recruitment of the different immune cell populations and the expression at the protein levels of cytokines and chemokines in the mouse contused spinal cord has not been fully characterized. Understanding the factors that impede the clearance of immune cells after SCI is likely to be critical for the development of new therapeutic

strategies. Herein we assessed the changes in the main inflammatory cell types and cytokine/chemokine expression in the mouse contused spinal cord. Our results show that neutrophils are the earliest inflammatory cells to invade the injured spinal cord. They peak at 24 hours post-injury and decline progressively up to day 28. Microglial cells peak at day 7 post-injury and slowly decline up to day 28. Microglia, however, is the most abundant immune cell in the contused spinal cord from day 3. The infiltration of monocytes peaks in the contused spinal cord at day 3, and decreases in numbers up to day 14. Few lymphocytes are present within the injured spinal cord for the first week. Strikingly, there is a great influx of lymphocytes into the contused spinal cord at day 21, and their proportion remain elevated up to day 28. The increase of lymphocytes at later stages of spinal cord injury is mainly due to the recruitment of B cells, and to minor degree, to the invasion of CD4 and CD8 T cells. Regarding cytokine/chemokine expression, our results reveal that all these pro-inflammatory mediators are up-regulated within the first 24 hours, peaking between 6 and 12 hours post-injury. IL6 and G-CSF are the most abundant cytokines at this early phase, and could play a key role in triggering the activation of glial cells and the recruitment of granulocytes. Beyond 24 hours, most of the cytokines were undetectable, except for some of them, such as M-CSF, IL-1α and IL9, that remain elevated until 28 days. Interestingly, the expression of M-CSF peaks at day 3 post-injury, suggesting its importance in monocyte and microglia expansion in the contused spinal cord. Contrary to most cytokines, the expression of chemokines remains at high levels for the first 3-7 days, and some of them, such as CXCL9, CXCL10 and eotaxin, are overexpressed up to day 28. In conclusion, our data suggest that the lasting up-regulation of some cytokines and chemokines in the injured spinal cord might be responsible, in part, in the failure of immune cell clearance.

Disclosures: I. Francos-Quijorna: None. **R. Lopez-vales:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP # 2011/13563-8

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CNPq

Title: Microglial activation in cardiovascular autonomic centers of diet-induced obese mice

Authors: *L. J. CHAAR, A. COELHO, W. T. FESTUCCIA, V. R. ANTUNES;
Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Obesity-associated sympathetic overactivity to different organs could be the linking factor between excessive fat accumulation and hypertension. Moreover, it has been shown that the hypothalamic inflammation induced by high fat diet can alter the neurotransmission of brain autonomic centers leading to hypertension. Here we screened the hypothalamus and brainstem of diet-induced obese mice for inflammatory factors that could be involved in changes in the mean arterial pressure (MAP). Ethical Committee for Animal Research of ICB-USP, #026, p.126; V.02. Male mice C57BL6 were submitted to high fat (HF; 60% kcal lipids) or control (C) diet for 8 weeks and body weight (BW), GTT, and plasma levels of leptin, insulin, triglycerides (TG) and free fatty acids (FFA) were measured. Femoral catheterization was performed to MAP recordings and spectral analysis in conscious freely moving mice. Hypothalamic and brainstem mRNA of inflammatory factors was quantified by qPCR. Immunoperoxidase for microglia followed by squeletonization were also performed. HF diet increased BW gain (31%), fasting plasma levels of glucose (C: 177±8 vs HF: 200±6 mg/dL); leptin (C: 8±3 vs HF: 17±5 ug/ml), insulin (C: 43±7 vs HF: 672±127 pmol/L), TG (C: 1.0±0.4 vs HF: 2.2±0.6 mmol/L), FFA (C: 0.2±0.1 vs HF: 0.7±0.2 nmol/L), and induced severe glucose intolerance (AUC, C: 23265 vs HF: 37633 u.a.). HF diet led to two different hemodynamic phenotypes: obese hypertensive (OH: 123±2 mmHg, n=2) and obese resistant to hypertension (OR: 106±4 mmHg, n=5) when compared to C (110±6 mmHg, n=5). OH mice had higher LF/HF (P=0.05) than C and OR. HF-diet elicited an increase of mRNA levels of CD86 in the brainstem (+30%, n=9) and CCL5 in the hypothalamus (+47%, n=10) when compared to C diet. Moreover, HF diet induced IBA+ cells accumulation at the caudal portion of the nucleus of the solitary tract (NTS) without changes at any specific hypothalamic nuclei. Taking together, our data indicate that HF induced obesity affected MAP and increased mRNA levels of CD86, a co-stimulatory molecule involved in microglia activation, and CCL5, a chemokine known to recruit leukocytes to inflammatory sites, respectively in the brainstem and hypothalamus. Further studies are required however to determine whether changes in gene expression are related to alterations in MAP.

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Poster

806. Microglia in Neuroinflammation and Neuroprotection

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Program#/Poster#: 806.17/X12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: Neuroinflammation and neuroprotection following acute soman-induced seizure in mice

Authors: ***E. A. JOHNSON**[1], M. A. GUIGNET[1], J. F. IRWIN[1], K. LAITIPAYA[1], T. F. BOWENS[1], M. D. WEGNER[2];
[1]Pharmacol., [2]Comparative Pathology, US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Exposure to organophosphorus compounds such as soman (GD) can initiate status epilepticus (SE) that can lead to progressive brain damage and behavioral impairment. GD, a potent acetylcholinesterase inhibitor and chemical warfare nerve agent (CWNA), causes prolonged SE activity and cell death in the hippocampus, thalamus and piriform cortex. Current treatments can ameliorate GD-induced SE, though treatment effectiveness diminishes rapidly following exposure. Therefore, neuroprotective strategies that can be used at later time points are needed to reduce neurodegeneration and improve cognitive outcomes. One strategy involves modulation of the neuroinflammatory response, a prominent feature in CWNA-induced brain injury. Specific inhibition of this response can reduce acute neurodegeneration in various CNS injury models. This study focused on one class of neuroinflammatory factors, the C-C chemokine family (*i.e.* CCL2 and CCL3), to investigate whether inhibition of this pathway would be a viable neuroprotective strategy. C-C chemokines were significantly upregulated in multiple brain regions following seizurogenic GD exposure in wild-type (wt) mice. Multiple background-matched C-C signaling knockout mouse strains were also exposed and showed significant changes in neuropathology, mortality, seizure onset and other relevant physiological responses compared to wt mice. These results along with data from other KO strains can help guide the selection of pathway-specific drugs for neuroprotection testing. These studies describe a rational and relatively rapid therapeutic strategy for the development of effective neuroprotectants and show that neuroprotection is possible with a longer therapeutic window than conventional treatments after GD exposure.

Disclosures: E.A. Johnson: None. **M.A. Guignet:** None. **J.F. Irwin:** None. **K. Laitipaya:** None. **T.F. Bowens:** None. **M.D. Wegner:** None.

Poster

806. Microglia in Neuroinflammation and Neuroprotection

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-NIDCR #DE021888 (OJI)

Title: Toll-like receptor 4 regulates prooxidant-mediated release of prostaglanding E2 and resovin in macrophages

Authors: *Y. ZHANG, O. J. IGWE;
Univ. of Missouri-Kansas City, Kansas City, MO

Abstract: **Background:** Oxidative stress can lead to inflammation, which is a mediating factor in many human diseases. Toll-like receptor 4 (TLR4) has been shown to be essential in mediating inflammatory responses induced by prooxidants via-a-vis oxidative stress. However, the mechanism(s) involved in are not clear**. Objective:** This study examines the mechanism by which oxidant stress can initiate, propagate and maintain the inflammatory process through TLR4 activation. **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. The TLR4 signaling pathway inhibitor, CLI-095, was used to block TLR4 signaling. RAW-Blue cells were used to determine the activation of TLR4-coupled NF-κB pathway by measuring the levels of secreted embryonic alkaline phosphatase (SEAP) release with QUANTI-Blue assay. We also used primary murine peritoneal macrophages (PM) isolated from mice expressing TLR4 (TLR4-WT) and mice with complete deletion of TLR4 (TLR4-KO). Accumulation of intracellular reactive oxygen species (ROS) in the primary culture was quantified by immunofluorescence (IF). We used ELISA to measure the contents of prostaglandin E2 (PGE2) and resolvin D1 (RsD1) released into the media following treatments with prooxidants and LPS-EK. **Results:** We found that pro-oxidants and LPS-EK evoked increased level of SEAP from Raw-Blue cells, which was abolished by CLI-095. This suggests that enhanced SEAP release was specifically due to TLR4 activation. Compared with PM derived from TLR4-WT mice, intracellular ROS accumulation was significantly lower in PM derived from TLR4-KO mice upon treatments with prooxidants or LPS-EK. In addition, prooxidants and LPS-EK enhanced PGE2 and RsD1 release in PM derived from TLR4-WT but had no effect in PM derived from TLR4-KO. Furthermore, the ratio of PGE2 to RsD1 was increased by prooxidants and LPS-EK. **Conclusion:** Taken together, our data indicate that prooxidants induced NF-κB activation through TLR4 stimulation. Therefore, oxidant stress can serve as an initiator of inflammatory process. This can lead to an imbalance between pro-inflammatory mediator PGE2 and pro-resolution mediator RsD1 maintaining diseases.

Disclosures: **Y. Zhang:** None. **O.J. Igwe:** None.

Poster

807. Neuroinflammation

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Program#/Poster#: 807.01/X14

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Fundació La Marató to RLV

Title: IL37 exerts anti-inflammatory effects after spinal cord injury

Authors: *M. COLL[1], R. LOPEZ-VALES[1], I. FRANCOS-QUIJORNA[1], E. SANTOS-NOGUEIRA[1], P. BUFLER[2], C. DINARELLO[3];
[1]Univ. Autonoma De Barcelona, Bellaterra, Spain; [2]Dr. von Hauner Children's Hosp. Ludwig-Maximilians-University, Munich, Germany; [3]Univ. of Colorado, Denver, CO

Abstract: Inflammatory response plays an essential role in protecting the body after injury or invasion by microorganisms. Regardless of the tissue in which it occurs, inflammation is a highly regulated event that undergoes resolution in a timely fashion, otherwise, it may lead to tissue damage or even to the development of an inflammatory disease. After spinal cord injury (SCI) an inefficient control of the inflammatory response occurs, which contributes to tissue damage and functional impairments. This is particularly harmful in the central nervous system (CNS) due to its limited ability to replace the damaged neurons and promote axonal regeneration, leading to irreversible deficits. Therefore, targeting inflammation could be a valuable approach to promote neuroprotection and functional recovery in SCI. Interleukin 37 (IL37) is one of the eleven members of the IL-1 family. Contrary to most IL1 cytokines, IL37 exerts potent inhibition on innate immunity. Although the mouse homolog of IL37 has not been found yet, previous works demonstrate that mice overexpressing human IL37 (hIL37tg mice) undergo significant resistance against several inflammatory insults, such as shock response, colitis, hepatitis and psoriasis. However, there is no information on the role of IL-37 in CNS pathologies yet. To evaluate whether IL-37 exerts beneficial actions after CNS trauma, we performed a contusion lesion into the spinal cord of WT or hIL37tg mice. mRNA transcripts of IL-37 were almost undetectable in the uninjured spinal cord of hIL37tg mice. However, IL37 was significantly up-regulated in the spinal cord of hIL37tg mice from 12h to 28 days post-injury, the latest time point examined. IL37 reduced the protein levels of several pro-inflammatory cytokines and chemokines in the spinal cord at 24 hours post-injury, such as IL6, KC, RANTES, G-CSF, among others, but did not alter the expression of the anti-inflammatory cytokines IL5 and IL10. Moreover, IL37

dampened the recruitment of neutrophils and monocytes in the contused spinal cord. In line with the anti-inflammatory actions of IL37, we found that IL37tg displayed reduced locomotor deficits and spinal cord tissue damage. Our data demonstrate for the first time that IL37 acts a suppressor of the inflammatory response in the CNS and leads to beneficial effects after spinal cord trauma.

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Poster

807. Neuroinflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Programme grant from the Health Research Council of New Zealand

Hugh Green Foundation

Title: The transcription factor C/EBPδ regulates human brain pericyte inflammatory responses

Authors: J. RUSTENHOVEN[1], T. PARK[1], D. JANSSON[1], R. OLDFIELD[2], P. S. BERGIN[3], E. W. MEE[3], R. L. M. FAULL[1], *M. DRAGUNOW[1];
[1]Univ. of Auckland, Auckland, New Zealand; [2]Lab. Plus, Auckland, New Zealand; [3]Auckland City Hosp., Auckland, New Zealand

Abstract: Neuroinflammation contributes to the development and progression of epilepsy, traumatic brain injuries, stroke and many neurodegenerative diseases. Transcription factors are critical regulators of brain inflammation through modulating inflammatory gene expression. Induction of the transcription factor C/EBPδ has been observed in the brain of Alzheimer's disease and amyotrophic lateral sclerosis patients; with both diseases having a significant inflammatory component. Previous studies utilising rodent microglia and astrocytes have suggested a pro-inflammatory role for C/EBPδ through induction of COX-2, IL-6 and iNOS expression. Whilst these glial cells are essential in regulating the brains immune response, recent evidence suggests that pericytes, a cell type lining the brain vasculature, can also contribute to brain inflammation. Utilising human brain tissue, our lab has previously established *in vitro* cell cultures of primary brain pericytes. We sought to investigate the function of C/EBPδ induction in

the human brain pericyte inflammatory response. Naive pericytes display low C/EBPδ expression which is significantly induced by a diverse range of immunogenic stimuli (IFNγ, IL-1β, LPS and TNFα) as determined by immunocytochemistry and RT-qPCR. Nuclear C/EBPδ induction was observed as early as four hours after inflammatory treatments and remained elevated for up to three days. In order to examine the function of C/EBPδ induction in pericytes, RNAi was employed to attenuate the IL-1β induced expression of this transcription factor. Interestingly, following siRNA mediated knock-down of C/EBPδ induction, the expression of several pro-inflammatory genes (MCP-1, ICAM-1 and IL-8) was enhanced whilst others (IL-6 and COX-2) were reduced, as determined by immunocytochemistry and RT-qPCR. C/EBPδ induction in pericytes therefore appears to confer a specific inflammatory phenotype which is neither exclusively pro/anti-inflammatory. Whilst the C/EBPδ induced expression of IL-6 and COX-2 may produce a pro-inflammatory response, these mediators have also been shown to attenuate inflammation in certain contexts. Furthermore, MCP-1, ICAM-1 and IL-8 act to recruit peripheral immune cells across the vasculature into the brain. Induction of C/EBPδ in pericytes following an immunogenic challenge may therefore act to limit peripheral immune infiltration highlighting a possible anti-inflammatory role for this transcription factor in brain pericytes.

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Poster

807. Neuroinflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1 NS45748

Title: Redox control of neuroinflammation by post-translational activation of glutamate cysteine ligase

Authors: *P. BHUYAN, L.-P. LIANG, M. PATEL;
Univ. of Colorado Denver, Aurora, CO

Abstract: Neuroinflammation and oxidative stress are hallmarks of several neurodegenerative diseases such as Parkinson's disease. However, whether and how the redox processes control neuroinflammation is relatively unknown. We determined the impact of modulating the

glutathione (GSH) redox status on markers of neuroinflammation *in vitro*. Cellular GSH levels were elevated by a novel approach i.e. post-translational activation of glutamate cysteine ligase (GCL), the rate limiting enzyme in GSH biosynthesis. A series of thiol-containing compounds were examined for their ability to increase intracellular GSH levels in a murine microglial cell line (BV2). The most potent compound to significantly increase intracellular GSH levels was identified as 2,3-dimercapto-1-propanol (DMP). DMP increased GCL activity and decreased LPS-induced production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and KC/GRO) in BV2 cells in a concentration-dependent manner. DMP's ability to elevate GSH levels and attenuate LPS induced pro-inflammatory cytokine production was inhibited by buthionine sulfoximide, an inhibitor of GSH biothynethsis suggesting a post-translational mechanism of GCL activation by DMP. Finally, we determined if DMP increased GSH levels and attenuated neuronal damage in a rat dopaminergic N27 cell line. DMP treatment in N27 cells increased GCL activity and GSH levels similar to the magnitude observed in BV2 cells. Furthermore, DMP inhibited paraquat- induced cell death in N27 cells. The data demonstrate that elevation of intracellular GSH levels by post-translational activation of GCL inhibits production of pro-inflammatory cytokines and exerts neuroprotection. This suggests the feasibility of modulating neuroinflammation via redox processes. Elevation of GSH via post-translational activation of GCL may be an attractive approach to target inflammation in age-related neurodegenerative diseases in which adaptive responses may be impaired.

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Poster

807. Neuroinflammation

Location: Halls A-C

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotection by long-chain fatty acids in trauma: the control of neuroinflammation

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Abstract: Ethanolamides of long-chain fatty acids are a class of endogenous lipid mediators generally referred to as N-acylethanolamines (NAEs). NAEs include anti-inflammatory and

analgesic palmitoylethanolamide (PEA) and the endocannabinoid anandamide. It has recently been demonstrated that PEA exerts neuroprotection in central nervous system (CNS) pathologies such after spinal cord injury (SCI) and traumatic brain injury (TBI), disorders that are associated with a high morbidity and mortality and with no specific therapeutic treatments. In recent studies, we have demonstrated that treatment with PEA significantly reduced inflammatory secondary events associated with SCI and TBI. Since oxidative stress is considered to play an important role in neuroinflammatory disorders, in the present work we studied a new composite, a formulation including PEA and the antioxidant compound luteolin (Lut), subjected to an ultramicronization process, co-ultraPEALut. We investigated the effect of co-ultraPEALut in both an *ex vivo* organotypic spinal cord culture model and an *in vivo* model of SCI as well as in *in vivo* Controlled cortical impact (CCI) model of TBI. For the organotypic cultures, spinal cords were prepared from mice at postnatal day 6 and were cut into transverse slices of 400 μm thickness. After 7 days of culturing, the slices were mechanically injured and the co-ultraPEALut was applied at different concentrations 1 hour before damage. For *in vivo* model of SCI, SCI was induced through spinal cord compression at four-level T5 to T8 and co-ultraPEALut (1 mg/kg ip) was administered at 1 and 6 hours after SCI. At 24 hours after SCI, mice were sacrificed and the spinal cords were collected for further evaluation. Moreover, we performed the CCI model in mice where a craniotomy was made and a cortical contusion was produced on the exposed cortex and the impact tip was advanced farther to produce injury of moderate severity. Pretreatment with co-ultraPEALut significantly reduced COX-2 and iNOS expression, restored nNOS expression and protected cells by cell death (MTT assay) in spinal cord organotypic cultures. Moreover, we demonstrated *in vivo* that co-ultraPEALut 1 mg/kg reduced the severity of trauma induced by compression to the spinal cord and improved the motor activity evaluated at 10 days post-injury; also in TBI coultraPEALut-treated mice had lower infarct volumes and better outcomes in neurological and behavioral tests. The present study demonstrates that coultraPEALut association possess neuroprotective effects on modulating SCI and TBI-asscoiated neuroinflammation and could be a therapeutic target in traumatic diseases.

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Poster

807. Neuroinflammation

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Program#/Poster#: 807.05/X18

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: C13-FAI-03.10.10

Title: Oxidative stress and increased inflammatory response is associated with APP over-expression in astrocytes acutely exposed *in vitro* to MMA III

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Abstract: Human exposure to inorganic arsenic (iAs), has been associated with an increased risk for Alzheimer's disease (AD) development, however the associated mechanisms have not been fully described. AD's neuropathological features include extracellular neuritic amyloid plaques (AP) formed by Aβ peptides derived from precursor amyloid protein (APP) excised by the consecutive action of β and γ secretases. Intracellular neurofibrillary tangles (NFT) which are composed of hyperphosphorylated Tau protein are also characteristic in AD brain tissue. Interestingly, APP over-expression and an increase in p-Tau have been previously demonstrated after *in vitro* or experimental exposure to arsenite. As it is known, neuroinflammation involving the induction of oxidative stress, underlines AD as well as other neurological diseases development. Importantly, iAs and its methylated metabolites (MMAIII/V, DMAIII/V) are known to induce oxidative stress and inflammatory responses in different cells and experimental models. Therefore As-induced toxicity in the brain, could involve its ability to induce neuroinflammation once it crosses the brain blood barrier (BBB) through the glucose transporter 1 (GLUT-1). Previously, we showed an important increase in ROS production soon after brain microvascular endothelial cells were exposed to 25, 50 and 100 nM of the monomethylated metabolite of arsenic (MMAIII) in an *in vitro* BBB model. In this work, we determined the effect of MMAIII on rat astrocyte from primary cultures. Toxicity assays based on lactate dehydrogenase (LDH) release showed no toxic effect in astrocytes exposed up to 1μM MMAIII; however exposure to 50 nM and higher led to astrocytes reactivation in terms of morphologic changes that are commonly correlated with a surrounding inflammatory milieu, as is observed in cells stimulated with LPS. Other set of cells were exposed to 50 to 250 nM MMAIII for 24 h and assayed for reactive oxygen species (ROS) production and for cytokines gene expression by RT-PCR. Results showed a significant increase in ROS production after 4 h in cells exposed to all MMAIII concentrations. Consistently, genes codifying for pro-inflammatory cytokines MIF, IL1-β and TNF-α were over-expressed up to 7.76, 19.1 and 9 times correspondingly, after 4h exposure to 50 and 250nM MMAIII, when compared with not exposed astrocytes. In these cells APP gene expression increased 2.3 times in cells exposed to 250nM MMAIII in correlation with inflammatory cytokines over-expression. These results suggest that arsenic-induced inflammation could importantly accounts for neurodegeneration and AD development in exposed populations.

Disclosures: **C. Escudero-Lourdes:** None. **I. Rojas-Barajas:** None. **E.E. Uresti-Rivera:** None. **M. Torres-Ramos:** None.

Poster

807. Neuroinflammation

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Additional scientific contributions from Junwei Hao, Ning Su and Yan Gan

Title: Roles for nicotinic acetylcholine receptors in modulation of inflammation and immunity

Authors: *R. J. LUKAS[1], A. SIMARD[2], B. J. MORLEY[3], Q. LIU[1], L. LUCERO[1], P. WHITEAKER[1], F.-D. SHI[1];
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Abstract: Following on earlier suggestions for roles of nicotinic acetylcholine receptors (nAChR) in the immune system, our initial studies with the late Dom Deluca characterized nAChR subunit gene expression in the mouse and human immune systems using thymic organ culture models and demonstrated effects of nicotinic ligands on T cell development and differentiation. That work suggested that nicotine induces T cell anergy and inhibits T cell differentiation, contributing to general immunosuppressive effects. Subsequent work using experimental autoimmune encephalomyelitis (EAE) in mice as a model for multiple sclerosis (MS) has led to identification of roles for different nAChR subtypes in different stages in disease progression and recovery. This is consistent with refined evaluation of nAChR subunit

expression, always validated by other studies, in different immune cell types in the periphery or infiltrating into the brain, such as T cells, B cells, dendritic cells, peripheral macrophages, and brain microglia. Overall, nicotine exposure at behaviorally-relevant concentrations protects against EAE, delaying disease onset, attenuating the magnitude of disease signs, and facilitating recovery. Studies using combinations of nAChR subunit knockout, pharmacological approaches and adoptive transfer techniques indicate or suggest hypotheses: (1) that disease exacerbating effects are mediated by peripheral nAChR containing α9 subunits (α9*-nAChR) and attenuated by α9 subunit knockout or nicotine's antagonism of α9*-nAChR, (2) that α7-nAChR, probably expressed by brain cell types, play neuroprotective roles that become particularly evident in the absence of α9*-nAChR, and (3) that β2*-nAChR are involved in recovery from EAE, perhaps by facilitating oligodendroglial precursor cell maturation leading to remyelination. Effects on indices of inflammation and immune responsiveness and evidence from studies using reduced preparations correlate well with more gross measures of disease status. Collectively, these studies suggest exciting and novel therapeutic strategies to reduce disease-exacerbating or to enhance disease-ameliorating inflammatory and/or immune processes. Such strategies have the potential to improve treatment of maladies such as MS, lupus and arthritis, and even could be deployed in treatment of stroke, brain cancer or neurodegenerative diseases.

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Poster

807. Neuroinflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSC 102-2320-B-039-026-MY3

Title: Anti-neuroinflammatory effects of paeonol in microglial cells

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Abstract: Accumulating evidence suggests that inflammatory processes in the central nervous system mediated by microglial activation play important roles in several neurodegenerative

disorders. Therefore, development of methods for microglial inhibition is considered an important strategy in the search for neuroprotective agents. Paeonol is a major phenolic component of Moutan Cortex, widely used as a nutrient supplement in the Chinese medicine. In this study, we investigated the effects of paeonol on cultured microglia cells stimulated by inflammagens. Paeonol significantly inhibited the release of nitric oxide (NO) and the expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Treatment with paeonol also reduced reactive oxygen species production and inhibited an ATP-induced increase cell migratory activity in the microglia. Furthermore, the inhibitory effects of neuroiflammation by paeonol were found to be regulated by phosphorylated adenosine monophosphate-activated protein kinase-α (AMPK-α) and GSK 3β. Administration with AMPK or GSK 3 inhibitors both reverses the inhibitory effect of neuroiflammation by paeonol in microglial cells. Furthermore, paeonol also showed significant anti-neuroinflammatory effects and improvement in the Rotarod performance in mice model as well. The present study is the first to report a novel inhibitory role of paeonol on neuroinflammation and presents a new candidate agent for the development of therapies for inflammation-related neurodegenerative diseases.

Disclosures: **C. Lin:** None. **C. Tsai:** None. **Y. Liu:** None. **D. Lu:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS085165

NIH P01 HD016596

Title: Inhibitors of the mitochondrial fission GTPase Drp1 attenuate proinflammatory microglial activation by an unknown mechanism

Authors: ***B. M. POLSTER**[1], E. A. BORDT[1], B. A. ROELOFS[2];
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Abstract: Microglia, the innate immune cells of the central nervous system, shift from a surveying to an activated, proinflammatory state following brain injury. Using the bacterial endotoxin lipopolysaccharide (LPS) and the proinflammatory cytokine interferon-gamma (IFN-gamma) to induce the activation of rat HAPI microglial cells *in vitro*, we found that mitochondria fragmented into small donut shapes after approximately four hours of treatment and failed to recover normal morphology by 18 hours. We hypothesized that mitochondrial fission promotes microglial activation in rat HAPI cells and primary rat cortical microglia. Mdivi-1 and dynasore, putative inhibitors of the mitochondrial fission GTPase Drp1, partially and dose-dependently (37.5-75 micromolar) attenuated production of nitric oxide by activated HAPI or primary microglia while completely blocking the secretion of tumor necrosis factor-alpha (TNF-alpha) at the same concentrations. However, we were unable to demonstrate inhibition of the activation-induced fragmentation of mitochondria by mdivi-1. Using the Seahorse Extracellular Flux Analyzer to measure cellular oxygen consumption, we found that mdivi-1 but not dynasore partially impaired HAPI microglial respiration. The ability of mdivi-1 to inhibit respiration was unlikely related to its ability to impede markers of activation because partial complex I inhibition by the drug rotenone failed to mimic the effect of mdivi-1. Dynasore was previously suggested to inhibit internalization of the LPS receptor toll-like receptor 4 and effects of Drp1 inhibitors on microglial activation steps independent of mitochondrial fragmentation are under investigation. Overall, results indicate that two small molecule inhibitors of the mitochondrial fission GTPase Drp1 impair microglial activation by an unknown mechanism. Interestingly, inhibition of TNF-alpha secretion was more effective than that of nitric oxide production, suggesting that nitric oxide may be upstream of TNF-alpha or that production of the two proinflammatory mediators is regulated independently.

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Poster

807. Neuroinflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI Grant Number 24590724

Title: Enhanced expression of glycoprotein non-metastatic melanoma B in macrophages and microglia in inflamed rat brain

Authors: J.-J. HUANG, *S. YOKOYAMA;
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Abstract: The glycoprotein non-metastatic melanoma B (Gpnmb), which possesses a type-I transmembrane protein-like structure, has been identified in various types of normal cells including melanocytes, osteoclasts, osteoblasts, and dendritic cells in blood, as well as in various carcinoma cells. Several studies have described that Gpnmb is abundantly expressed in highly invasive glioblastomas, implying its involvement in tumor progression and metastasis. We have demonstrated in the central nervous system (CNS) of rats that Gpnmb is produced by macrophages and microglia in normal neural tissues (Huang et al., Brain and Behavior 2, 85-96, 2012). We have also reported that Gpnmb is present in normal rat sciatic nerve and up-regulated after axotomy (Yokoyama and Yanagida, Soc. Neurosci. Abstr., 438.21, 2012; Yokoyama, Soc. Neurosci. Abstr., 813.15, 2013). In this study, we further examined whether inflammatory stimulation had any effects on Gpnmb expression in the CNS. Bacterial endotoxin lipopolysaccharide (LPS) from Escherichia coli serotype O127:B8 was dissolved in sterile phosphate-buffered saline (PBS; pH 7.4) and injected intraperitoneally at a dose of 0.1 mg/kg of body weight. After injection of LPS, we observed that Gpnmb-immunoreactive (IR) in the area postrema, which is one of the circumventricular organs, was prominent compared with that in rats injected with PBS. This change became obvious 8 hours after the LPS injection and was more widespread after 24 hours. The Gpnmb-IR cells were positive for the macrophage/microglia marker OX42, and frequently observed in the vicinity of vessels. The Gpnmb-IR in these cells was localized to cytoplasmic vesicles. Furthermore, an intraperitoneal LPS injection increased the number of Gpnmb and OX42 double-positive cells in the area postrema, indicating infiltration of blood-borne macrophages through this region. These results suggest that Gpnmb, which is expressed in microglia and macrophages in inflamed brain, plays an important role in the regulation of immune/inflammatory responses.

Disclosures: J. Huang: None. **S. Yokoyama:** None.

Poster

807. Neuroinflammation

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Title: Hmox1 and EP2 signaling regulate IL-13 - induced death of activated microglia: A potential pathway for resolution of inflammation

Authors: Y. FU[1], *M.-S. YANG[1], K.-J. MIN[2], T. GANESH[1], N. LELUTIU[1], E. JOE[2], R. DINGLEDINE[1];
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Abstract: Acute inflammation protects the body from foreign invasions but chronic inflammation can be harmful, so understanding the mechanisms by which inflammation is aborted is an important question. Microglia, the brain's resident innate immune cells that mediate neuroinflammation in response to injurious events, can be activated by an endogenous Toll-like receptor 4 (TLR4) ligand, high-mobility group box protein B1 (HMGB1). HMGB1 was recently found to be released by injured neurons, causing long-lasting microglia activation. We examined the control of microglial death *in vitro* by pathways that are engaged during inflammation. We elicited microglia activation *in vitro* by lipopolysaccharide (LPS, 10-100ng/ml) or HMGB1 (100ng/ml) at 2h, judged by induction of mRNA encoding COX2 and TNFα. We confirmed that interleukin-13 (IL-13, 20ng/ml), an anti-inflammatory cytokine, caused death of LPS - activated microglia by 6 days. We also found that IL-13 upregulated cyclooxygenase (COX2) expression and downregulated Heme Oxygenase 1 (Hmox1) expression at both mRNA and protein levels between 6h and 72h after exposure. Tricarbonyldichlororuthenium (II) dimmer used as a source of CO (CO-releasing molecule, CORM, 10μM), a product of Hmox1, reduced IL-13 induced COX2 expression at protein level. CORM also reduced IL-13 induced microglia death by 18.9%, and a Hmox1 inducer, cobalt protoporphyrin IX (CoPP, 2.5μM), reduced IL-13 induced microglia death by 26%. Moreover, a novel antagonist for the EP2 receptor of prostaglandin E2 (PGE2) reduced IL-13 induced microglia death by 75%, suggesting a role for both Hmox1 and EP2 in regulating microglia death. Taken together, these results suggest that Hmox1 and EP2 signaling regulate IL-13 induced death of activated microglia *in vitro*. We propose that timely induction of microglia death by regulating IL-13 or EP2 signaling may be a potential way to control the degree of microglia activation and reduce chronic inflammation.

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Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Defense Medical Research and Development Program (0130-10-00003-00002)

Military Center of Excellence Research Award (306514-1.00-63671)

Title: WWL70 attenuates inflammatory response by blocking prostaglandin production in microglial cells

Authors: *M. TANAKA[1], J. WEN[1], S. MORAN[2], Y. ZHANG[1];
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Abstract: Modulation of the endocannabinoid (eCB) system has been shown to play a therapeutic role in several pathological settings. Recently several studies have indicated that inhibition of eCB hydrolysis leads to enhancement of eCB signaling and reduction of inflammatory eicosanoid production. We previously reported that WWL70, an inhibitor of a 2-arachidonyl glycerol (2-AG) hydrolase α,β-hydrolase domain 6 (ABHD6), suppresses neuroinflammation and ameliorated neuronal injury in the animal models of traumatic brain injury and multiple sclerosis. In this study we investigated the anti-inflammatory mechanism of WWL70 *in vitro* using microglial cells. WWL70 was found to efficiently inhibit the accumulation of prostaglandins in LPS-activated microglia. Consistent with the recent view that 2-AG is a major substrate for arachidonic acid (AA) in the brain, addition of 2-AG dramatically increased the production of prostaglandins, which was also significantly inhibited by WWL70. However, the production of prostaglandin facilitated by AA was also significantly reduced by WWL70, suggesting alternative inhibitory effect(s) on the metabolic pathway from AA to prostaglandins, which is mediated mainly by cyclooxygenase 2 (COX2) and prostaglandin synthase (PGES). In addition, when cells were treated with a newly developed and a selective inhibitor for ABHD6, KT182, or knocked down of ABHD6 using siRNA, there was no reduction on prostaglandin production observed. Currently we are investigating whether WWL70 can directly inhibit the enzyme activities of COX2 or PGES. In terms of prostaglandin production, the effect of WWL70 was not reversed by the CB receptor antagonists, suggesting that WWL70 attenuates prostaglandin biosynthesis independently on CB receptor activation. Thus, it is likely that the anti-inflammatory and neuroprotective effects of WWL70 *in vivo* might be due to its inhibition on prostaglandin biosynthesis in microglia cell. The role of ABHD6 in the control of neuroinflammation and neurodegeneration needs to be further investigated.

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Poster

807. Neuroinflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Anti-inflammatory effects of ketamine on microglia activation

Authors: *T. KHAYRULLINA[1], M. H. SCHMIDT[1], R. G. W. STAAL[1], N. PLATH[2], T. MÖLLER[1];
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Abstract: Ketamine is a NMDA receptor antagonist that has been used as an anesthetic, antinociceptive and anti-depressive agent. Recently, much interest has focused on ketamine's non NMDA-related characteristics, for instance its anti-inflammatory properties. It has been reported that ketamine may reduce TLR4 stimulated microglial cytokine release, suggesting that ketamine may work by reducing neuroinflammation in a paradigm of bacterial endotoxin induced microglial activation. The goal of our study was to determine whether ketamine affects other microglial functions downstream of TLR4 activation. First, we set out to replicate previous reports showing that ketamine inhibits the TLR4 stimulated release of pro-inflammatory cytokines. Rat primary microglia were stimulated with control standard endotoxin (CSE), a purified form of LPS that activates TLR4 signaling. When administered prior to CSE (3EU/ml), ketamine significantly reduced the amount of the pro-inflammatory cytokines IL-1b, IL-6 and TNFa as well as nitric oxide. Ketamine was tested from 3-100uM, with minimally effective doses 10-30uM, suggesting a possibly non-NMDAR mediated mechanism. Ketamine did not show any cytotoxicity at the doses tested. Next we investigated the effects of ketamine on CD68, a marker of phagocytosis which is upregulated upon TLR4 activation in microglia. Ketamine produced a dose-dependent decrease of the CSE-induced CD68 levels. Together these data suggest that ketamine inhibits several parameters of microglial responses downstream of TLR4 activation, albeit at very high doses. To test whether ketamine regulates microglial activation pathways other than TLR4, we stimulated microglia with IFNg a known inducer of microglial MHC II expression. Treatment of microglia with IFNg significantly increased cell surface expression of MHCII over non-stimulated cells. Ketamine did not affect MHCII expression on microglia in conditions with or without IFNg. Ketamine also did not affect IFNg-induced TNFa and IL-6 levels. The data suggest that the anti-inflammatory effects of ketamine are not likely to intersect with the IFNg signaling pathway. In summary, our data suggests that ketamine at the doses tested can suppress TLR4 mediated, but not IFNg induced microglial activation. It is of

note that the ketamine concentrations used in our *in vitro* studies are in the high uM range. Additional studies are necessary to investigate whether these concentrations are reached in the CNS at clinically relevant ketamine doses and indeed affect microglia physiology.

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Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Role of P2X7 in a model of neuroinflammation associated with microglial activation: Ex-vivo and in-vivo correlates

Authors: P. ACTON[1], C. COTTO[1], W. ECKERT[2], J. Y. MA[2], B. LORD[2], P. BONAVENTURE[2], A. WICKENDEN[2], P. KING[1], B. SAVALL[2], N. CARRUTHERS[2], M. LETAVIC[2], T. LOVENBERG[2], *A. BHATTACHARYA[2];
[1]Discovery Sci. Imaging, Janssen Pharmaceut. Companies of Johnson & Johnson, SPRING HOUSE, PA; [2]Neurosci., Janssen Pharmaceut. Companies of Johnson & Johnson, SAN DIEGO, CA

Abstract: ATP-gated ion channel P2X7, expressed predominantly in the CNS glial cells, is activated during various CNS disorders that involve neuroinflammation associated with microglial activation. Microglial activation is assessed by studying pro-inflammatory cytokine burden such as IL-1b, up-regulation of markers for microglia (CD68) & astrocytes (GFAP) and the translocator protein TSPO. It is plausible ATP released during episodes of CNS disorders associated with glial activation (epilepsy, MS, AD, ALS, brain injury, mood disorders) may reach high concentrations to activate P2X7 leading to the release of IL-1b, which in turn may activate the microglial activation cascade. The aim of this study was to understand the role of P2X7 in such a process. Kainic acid was chosen as the stimulus to induce microglial activation in rats. In a dose response study, we observed robust changes in morphology of glial cells (astrocytes and microglia), neurodegeneration and TSPO up-regulation 7 days post a single administration of 8 mg/kg (ip) dose of kainic acid, indicating increased glial tone. In an effort to understand the in-vivo time course of microglial activation, rats were subject to PET scan of TSPO, using [F-18]PBR06 at various time points post kainic acid challenge. Robust PET uptake

was observed 5 days after kainic acid challenge and was maintained to 7 days (last time point studies). We then dosed rats with an orally bioavailable, high-affinity, CNS permeable P2X7 selective antagonist to test the effect on kainic acid-induced neuroinflammation on both ex-vivo (immunohistochemistry, TSPO autoradiography) and in-vivo (TSPO PET scan) endpoints. The P2X7 antagonist (10 mg/kg, oral dose; qd for 8 days), significantly attenuated the PET signal; it also produced reversal of kainic acid induced structural changes in the ventricle as assessed by MRI. To our surprise we failed to generate similar robust efficacy on the ex-vivo endpoints of neuroinflammation, primarily due to the inherent variability in the signals. The TSPO PET data is encouraging and suggestive of a critical role of P2X7 in modulating microglial activation that may have therapeutic benefits.

Disclosures: **P. Acton:** A. Employment/Salary (full or part-time):; Janssen R&D LLC. **C. Cotto:** A. Employment/Salary (full or part-time):; Janssen R&D. **W. Eckert:** A. Employment/Salary (full or part-time):; Janssen R&D. **J.Y. Ma:** A. Employment/Salary (full or part-time):; Janssen R&D. **B. Lord:** A. Employment/Salary (full or part-time):; Janssen R&D. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Janssen R&D. **A. Wickenden:** A. Employment/Salary (full or part-time):; Janssen R&D. **P. King:** A. Employment/Salary (full or part-time):; Janssen R&D. **B. Savall:** A. Employment/Salary (full or part-time):; Janssen R&D. **N. Carruthers:** A. Employment/Salary (full or part-time):; Janssen R&D. **M. Letavic:** A. Employment/Salary (full or part-time):; Janssen R&D. **T. Lovenberg:** A. Employment/Salary (full or part-time):; Janssen R&D. **A. Bhattacharya:** A. Employment/Salary (full or part-time):; Janssen Research & Development LLC.

Poster

807. Neuroinflammation

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FWO G.0675.12

KU Leuven OT08/40

KU Leuven OT12/79

Title: Chronically reactive microglia in peroxisomal multifunctional protein 2 deficiency lose surveillance markers, upregulate mTOR driven pathways but are not neurotoxic

Authors: *M. BAES[1], S. VERHEIJDEN[1], L. BECKERS[1], A. CASAZZA[2,3], O. BUTOVSKY[4], M. MAZZONE[2,3];
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Abstract: The functional diversity and molecular adaptations of reactive amoeboid microglia in the chronically inflamed central nervous system (CNS) is poorly documented. We previously showed that mice lacking multifunctional protein 2 (MFP2), a pivotal enzyme in peroxisomal β-oxidation, persistently accumulate reactive myeloid cells in the gray matter of the CNS. Here, we defined the signature of Mfp2-/- microglia by *ex vivo* FACS, microarray and immunohistochemical analysis. We found that the increased numbers of myeloid cells derive from proliferation of resident microglia and not from monocyte recruitment. Transcriptome analysis on freshly isolated microglia from Mfp2-/- brain revealed downregulation of many markers that define the unique signature of microglia in homeostatic conditions. Mfp2-/- microglia are immunologically activated but they do not show signs of neurotoxicity nor of phagocytotic activity. In agreement, neurons are conserved in Mfp2-/- brain but they release their inhibitory control on microglia as Cx3cl1 and CD200 are down regulated. Although the initiating signals remain unknown, we show that this reactive phenotype of microglia is governed by mammalian target of rapamycin (mTOR) activation leading to metabolic adaptations compatible with proliferation and growth. In conclusion, our data underscore that chronic activation of microglia not necessarily leads to overt neurotoxicity although we cannot exclude that loss of their surveillance functions contributes to CNS disease progression.

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Poster

807. Neuroinflammation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 807.16/Y5

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH DA027113

Title: Studying anti-inflammatory signaling by alpha7 nicotinic receptors in heterologous expression systems

Authors: *R. H. LORING, B. GARG, A. KULKARNI, G. THAKUR;
Pharmaceut. Sci., Northeastern Univ., Boston, MA

Abstract: Significant literature (e.g. Wang et al., *Nat. Med.* 10: 216, 2004; Saeed et al., *J. Exp. Med.* 201: 1113, 2005, Dowling et al., *Mol. Med.* 13: 576, 2007) suggests that alpha7 nicotinic receptors (a7 nAChRs) play anti-inflammatory roles in innate immune function on macrophages and leukocytes, in part by blocking the transcription factor, nuclear factor kappa-light chain-enhancer of B cells (NFkB). We tested whether a7 nAChRs directly interact with NFkB signaling in a heterologous expression system in the rat pituitary cell line GH3. Wild type GH3 cells do not bind alpha-bungarotoxin (aBT) or show evidence of rat a7 nAChRs by either PCR or Western blots with Abcam polyclonal antibody Ab23832 (Ab832). 1-40 ng/ml rat tumor necrosis factor alpha (TNF) causes up to a 30 fold increase in secreted alkaline phosphatase (SEAP) in GH3 cells transfected with a plasmid consisting of an NFkB promoter driving SEAP (NFkB-SEAP). However, GH3 cells transfected with rat a7 nAChRs show significant aBT binding, and the presence of a7 is confirmed by both PCR and Western blots with Ab832. Nicotine had no significant effect on GH3 cells transfected with both a7 and NFkB-SEAP, but selective a7 drugs such as the agonist PNU282987 (PNU) or the active enantiomer of the allosteric agonist 4BP-TQS (GAT107) blocked TNF-stimulated SEAP secretion over the concentration range of 50-300 microM. However, PNU and GAT107 both block TNF-stimulated SEAP in GH3 cells transfected with NFkB-SEAP alone, suggesting that these compounds have direct effects on NFkB signaling or SEAP secretion. These data suggest that components are lacking in GH3 cells to allow a7 nAChR interactions with NFkB signaling. To see if the a7 anti-inflammatory effects are cell type dependent, we are also investigating a7 nAChR anti-inflammatory signaling in mouse J774A1 and RAW264.7 macrophage cell lines when stimulated with lipopolysaccharide. In addition, these results suggest that the direct effects of high concentrations of PNU or GAT107 on NFkB signaling or SEAP secretion complicate the use of these drugs to study anti-inflammatory effects mediated through a7 nAChRs.

Disclosures: R.H. Loring: None. **B. Garg:** None. **A. Kulkarni:** None. **G. Thakur:** None.

Poster

807. Neuroinflammation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 807.17/Y6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: HFE H67D polymorphism is associated with reduced severity of cerebral malaria and increases survival with Plasmodium berghei ANKA

Authors: *J. R. CONNOR[1], D. F. LEITNER[1], M. LANDMESSER[2], E. NEELY[1], J. A. STOUTE[2];
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Abstract: Cerebral malaria (CM) occurs in about 5 million individuals a year with Plasmodium falciparum. CM results in myelin damage, axon injury, blood brain barrier breakdown, and long term neurological deficits in survivors. Iron deficiency commonly occurs with CM, but iron supplementation is a controversial issue in these patients. It is thought that iron loading may increase parasitemia and severity of infection. In this study, we evaluated whether an animal model with genetic iron overload (H67D HFE) had increased severity of infection which would lead to reduced survival. We tested this with the animal model for CM using Plasmodium berghei ANKA. We found that H67D mice had lower parasitemia on day 6 post infection (p.i.) and survived longer than infected WT mice. We monitored hematological parameters throughout the infection. Hematocrit (Hct), hemoglobin (Hgb), and mean corpuscular volume (MCV) were elevated in H67D mice pre-infection. However, Hct, Hgb, and red blood cells (RBCs) decreased in infected H67D mice compared to uninfected H67D until day 14 p.i. These parameters decreased to WT levels from day 7-10 p.i. MCV began to increase from day 12-14 p.i., which indicates an increase in reticulocytes that replaced lost RBCs. We have previously found that Semaphorin4A (Sema4A) induces apoptosis of oligodendrocytes. In this model of CM, Sema4A was increased in the brain of infected WT mice but there was a smaller increase in H67D infected mice. The 33% of surviving H67D mice had the lowest level of Sema4A. We also evaluated iron management proteins in whole brain and myelin. In whole brain, Hft and Tim2 (T cell immunoglobulin and mucin domain containing 2), the receptor for Sema4A, were higher in H67D mice pre-infection and both decreased after infection. However, Hft remained increased in infected H67D when compared to infected WT. Transferrin receptor (TfR) in whole brain was decreased in H67D mice pre-infection compared to WT and remained decreased after infection compared to infected WT, while TfR decreased in WT mice after infection compared to uninfected WT. TfR in the myelin fraction was decreased in H67D mice pre-infection but was no longer significantly different after infection. One treatment option that has been considered for CM is erythropoietin (Epo) which becomes elevated following anemia that is a consequence of malaria. Epo not only promotes erythropoiesis, but also has anti-apoptotic and immunomodulatory effects. We evaluated plasma Epo and found that it was elevated in H67D surviving mice. These data suggest that elevated Epo may be at least partially responsible for the improved outcome in H67D mice and suggests a mechanism that involves lower brain Sema4A.

Disclosures: **J.R. Connor:** None. **D.F. Leitner:** None. **M. Landmesser:** None. **E. Neely:** None. **J.A. Stoute:** None.

Poster

807. Neuroinflammation

Location: Halls A-C

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Program#/Poster#: 807.18/Y7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT Grant no.57204

Title: Inflammatory gene expression in rats submitted to spinal cord injury immunized with A91 and Cop-1

Authors: ***E. E. GARCIA-VENCES**[1,2], L. BLANCAS - ESPINOZA[3], M. GOLDBERG-MUROW[2], A. FLORES-ROMERO[4], R. SILVA-GARCÍA[4], A. IBARRA ARIAS[2];
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Abstract: **Introduction:** After spinal cord injury (SCI), many known auto-destructive mechanisms that are harmful for the patient take place, reducing the probability of neuronal regeneration. The expression of inflammatory genes modulates these mechanisms, hence, proinflammatory genes lead to an inflammatory response, oxidative stress and apoptosis induction, which ends with axonal demyelination and degeneration. Diverse studies are investigating a possible strategy to modulate this auto-destructive response and to promote neuroprotection. A novel approach, is the immunization with modified neural peptides such as A91 and Cop-1 derived from the 87-99 immunogenic sequence of myelin basic protein (MBP); this sequences are recognized by T-lymphocytes, causing its activation and deviation to a Th2 phenotype, which leads to expression of antiinflammatory genes; this phenomena is known as protective auto-reactivity (PA). **Objective:** Analyze the effect of A91 and Cop-1 on CASP-3, TGF-β, IL-12 and IL-6 gene expression in rats with moderate and severe SCI. **Methods:** Gene expression analysis was performed in Fischer 344 rats with SCI by contusion (moderate or severe), immunized 1 hour after the lesion with A91 and Cop-1, using as control the immunization with OVA (ovalbumin) and PBS (phosphate buffer saline) as vehicle. Seven days

post-lesion, was performed the total RNA extraction from the injured spinal cord using the method TRIzol-isopropanol (Invitrogen); RNA integrity was checked by electrophoresis and spectrophotometry. The following step was cDNA synthesis using Thermoscript Plus Reverse Transcription protocol (Invitrogen) to perform subsequently q-PCR with SYBRGreen protocol (Roche). **Results:** The results after the moderate injury showed a pattern of neuroprotection by decreasing the expression of CASP-3 and IL-6 in the immunized groups with A91 and Cop-1 compared with PBS and OVA ($P< 0.05$; Kruskal Wallis); increasing the expression of TGF-β only in Cop-1 group ($P=0.216$ Kruskal Wallis). The contrary pattern was observed in severe injury where the immunization with any antigen caused an increased expression of CASP-3, IL-12 y TGF-β compared with PBS ($P<0.05$). **Conclusions:** Immunization with A91 or Cop-1 decreases the expression of certain proinflammatory genes having their own mechanism of neuroprotection, being possible to correlate this with the reduction of lipid peroxidation and apoptosis in previous studies of our investigation group.

Disclosures: E.E. Garcia-Vences: None. **L. Blancas - Espinoza:** None. **M. Goldberg-Murow:** None. **A. Flores-Romero:** None. **R. Silva-García:** None. **A. Ibarra Arias:** None.

Poster

807. Neuroinflammation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 807.19/Y8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Programme grant from the Health Research Council of New Zealand

Hugh Green Foundation

Gravida: National Centre for Growth and Development

Title: Proinflammatory cytokines alter signalling in human brain pericytes

Authors: *D. JANSSON[1], J. RUSTENHOVEN[1], R. L. OLDFIELD[3], P. S. BERGIN[4], E. W. MEE[4], R. L. M. FAULL[2], M. DRAGUNOW[1];
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Abstract: Brain inflammation is thought to be a contributing factor in brain pathologies such as epilepsy, stroke, Parkinson's disease, motor neurone disease, Huntington's disease and

Alzheimer's disease. Previous studies of brain inflammation have focused on principal immune cells such as microglia and astrocytes. While these cell types play an important role in inflammatory pathways, new research suggests that microvascular brain pericytes, which play a vital role in blood-brain barrier (BBB) maintenance and permeability, are also key participants of immune signalling in the central nervous system. We have established an *in vitro* cell culture system of α-smooth muscle actin, platelet-derived growth factor receptor-β (PDGFRβ), and NG2 positive primary pericytes derived from adult human brain tissue. We have previously shown that these cells may play an active role in the pro-inflammatory response by inducing changes in chemokine, cytokine and cell receptor expression at the gene and protein level. Studies have revealed that PDGFRβ is necessary for pericyte survival, as PDGFRβ knock-out animal models show loss of brain pericytes. Depletion of pericytes surrounding the brain vasculature is associated with impaired BBB function and therefore increased permeability to potentially toxic molecules and cells. Indeed, BBB breakdown and pericyte loss is a common feature of several neuroinflammatory diseases. However, whether changes in PDGFRβ expression are a cause or a consequence of pericyte dysfunction in neurodegeneration remains to be determined. We investigated the effects of pro-inflammatory pathways on PDGFRβ expression and regulation in primary adult human brain pericytes. We show that when pericytes have been pre-treated with low levels of chronic pro-inflammatory cytokines there is a change in both ligand stimulated and basal PDGFRβ expression, internalization and hence activation compared to controls. Further investigation will give insight into mechanisms and potential therapeutic interventions to prevent pericyte cell loss and therefore BBB breakdown and the neurotoxicity that ensues.

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Poster

807. Neuroinflammation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 807.20/Y9

Topic: C.17. Drugs of Abuse and Addiction

Support: VIEP

Title: Chronic alcohol consumption in combination with energy drinks causes an inflammatory response and neurodegeneration in rat

Authors: *A. D. DIAZ[1], D. CORDERO[1], S. TREVINO[1], V. TOXQUI[1], M. GONZALEZ-CORONEL[1], L. AGUILAR[1], G. CARMONA[2], J. MORENO-RODRIGUEZ[2], P. AGUILAR-ALONSO[3], G. FLORES[4], J. GUEVARA[5];
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Abstract: Energy drinks (ED), possess high concentration of caffeine and taurine, which often consumed in combination with ethanol, due to the popular belief that caffeine and taurine may counteract some of the intoxicating effects of ethanol. However, scientific research suggests that chronic use of these psychoactive substances in combination with alcohol trigger an inflammatory response mediated by pro-inflammatory cytokines (IL-1β and TNF-α) and iNOS both in the central and peripheral system modifying the behaviour of individuals and causing cell death (apoptosis). Currently, the mechanism of toxicity caused by the ED-ethanol interaction in the brain, are not well known. In the present study we evaluated the effects of chronic alcohol consumption in combination with energy drinks on the inflammatory response and neurodegeneration in rat. We used male Long Evans rats (200-250g). Were formed, 4 experimental groups (n=10 per group): 1) control (water); 2) Ethanol (40%); 3) ED (Red-bull) and 4) Ethanol + ED. Animals were orally administered for 2 months, in the course of treatment was evaluated, motor activity in closed field (day 0, 30 and 60). After treatment, the animals were sacrificed; the brains were removed for immunohistochemistry and fluorescence immunoassay (ELISA) to identify markers of inflammation (GFAP, IL-1β, TNF-α, iNOS, nitric oxide) and neurodegeneration (caspase-3 and -9, synaptophysin) in the temporal cortex and hippocampus, respectively. The results showed a progressive deterioration in motor activity during the administration of ethanol + ED. Biochemical and histological studies revealed that chronic ethanol + ED caused an increase in reactive gliosis, IL-1β, TNF-α, iNOS and nitric oxide, in the temporal cortex and hippocampus, respectively, also show immunoreactivity positive for caspase-3 and 9, and a decreases synaptophysin in temporal cortex and hippocampus. The results suggest that chronic consumption of alcohol in combination, cause an inflammatory response, which induces neurodegeneration in hippocampus and cerebral cortex, which affects the motor behaviour of the rats. Therefore, it is necessary to show caution in the consumption of these drinks combined with ethanol, so it is necessary to report urgently on the risks it is exposed to the health of consumers of these substances, especially young people.

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Poster

807. Neuroinflammation

Location: Halls A-C

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Program#/Poster#: 807.21/Y10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS65167

ES10586

ES078327

Title: PKCδ mediates lipopolysaccharide-induced inflammatory response in astrocytes

Authors: N. SINGH, V. LAWANA, H. JIN, *V. ANANTHARAM, A. KANTHASAMY, A. KANTHASAMY;
Biomed Sci, Iowa Ctr. for Advanced Neurotoxicology, Iowa State Univ., Ames, IA

Abstract: Parkinson's disease(PD) is a progressive neurodegenerative disease that is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. Accumulating evidence suggest that both oxidative stress and inflammation are co-contributors in the LPS-induced progressive and delayed loss of nigrostriatal dopaminergic neurons in neuro-inflammation models of PD. We have previously showed that TNFα-induced dopaminergic neuronal injury may be mediated via activation of PKC delta(PKCδ)-dependent cell signaling events; however the exact contribution of astroglial PKCδ in the induction of an inflammatory response remains elusive. It is well known that excessive microglial activation exerts toxic effects on bystander neurons, particularly nigrostriatal dopaminergic neurons. Besides microglia, astrocytes also expresses Toll like receptors (TLRs) and several kinases, including ERK, JNK, p38 mitogen activated protein kinase (MAPK), and several isoforms of PKC including PKCδ. In this study we examined the role of PKC delta in regulating bacterial endotoxin, LPS-induced pro-inflammatory mediator generation in mouse astrocytes obtained from WT and PKCδ knock out (KO) astrocytes.Here we show that PKCδ regulates the induction of proinflammatory response in murine astrocytes.Upon treatment of WT astrocytes with LPS, a pronounced induction in the mRNA expression levels of pro inflammatory cytokine and chemokines such as TNFα, IL1β, IL-12, COX-2, MMP 9, MCP-1 and CXCL10/IP10 were evidenced; however PKCδ KO astrocytes displayed a blunted inflammatory response thereby supporting a role for PKCδ in the induction of proinflammatory gene expression in activated astroglia. More importantly, the expression levels of NADPH oxidase (NOX 1) and iNOS, were significantly down regulated in LPS- treated PKCδ KO astrocytes as compared to drug treated wild type controls. In a similar fashion, LPS-induced phosphorylation of ERK1/2 and p38 MAPK, as well as NFκB transactivation were attenuated in PKCδ KO astrocytes as compared with wild type controls. Our finding identifies PKCδ as a major regulator of pro-inflammatory response and

suggest that PKCδ contributes to the induction of proinflammatory mediators, presumably via induction of ROS generation and activation of MAPK in activated astrocytes.

Disclosures: **N. Singh:** None. **V. Lawana:** None. **H. Jin:** None. **V. Anantharam:** None. **A. Kanthasamy:** None. **A. Kanthasamy:** None.

Poster

807. Neuroinflammation

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC Grant 195814317

Title: Systemic lipopolysaccharide administration alters cortical neuromodulation by increasing monoamine-oxidase A and acetylcholinesterase activities

Authors: ***L. K. BEKAR**, M. ZHI, G. SAWICKI, M. E. LOEWEN;
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Abstract: Lipopolysaccharide (LPS)-mediated sickness behavior is known to be a result of increased inflammatory cytokines in the brain. Although effects of cytokines on various neural pathways are known, further exploration of the mechanisms underlying cytokine induced behavioral changes is needed. Several studies have demonstrated cytokine-mediated increases in brain excitation by loss of GABAA-mediated inhibition through receptor internalization or inactivation. Given that inflammatory signaling pathways, reactive oxygen species and stress are also known to increase monoamine oxidase-A (MAO-A) and acetylcholinesterase (ACh-E) activity, we assessed the impact of systemic LPS on neuromodulator-mediated shaping of a simple cortical network. Extracellular field recordings of evoked excitatory post-synaptic potentials in adult mouse somatosensory cortical slices were used to evaluate effects of a single systemic LPS challenge on neuromodulator function one week later. Neuromodulators were administered transiently as a bolus (100 µl) to the bath perfusate immediately upstream of the recording site to mimic phasic release of neuromodulators and enable assessment of response temporal dynamics. LPS-mediated neuroinflammation resulted in loss of both spontaneous and evoked inhibition as well as alterations in the temporal dynamics of neuromodulator effects on a paired-pulse paradigm. The effects on neuromodulator temporal dynamics was sensitive to the MAO-A antagonist clorgyline (for norepinephrine and serotonin) and the ACh-E inhibitor

donepezil (for acetylcholine), suggesting inflammatory-mediated increases in MAO-A and ACh-E as the potential source of altered neuromodulation in LPS-treated animals. The results show that systemic LPS treatment leads to longer-term loss of cortical inhibition and decrease in neuromodulator action as a result of increased MAO-A and ACh-E activity. Given the significant role of neuromodulators in behavioral state and cognitive processes, it is likely that an inflammatory-mediated change in neuromodulator action plays a role in LPS-induced sickness behavior and could help define the link between infection and depression.

Disclosures: **L.K. Bekar:** None. **M. Zhi:** None. **G. Sawicki:** None. **M.E. Loewen:** None.

Poster

807. Neuroinflammation

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Program#/Poster#: 807.23/Y12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R01 NS063878

NIH grant R01 NS077873

Title: Chemokine CCL3 inhibition of long-term potentiation in rat hippocampal slices

Authors: ***G. TU**[1,2], J. ZHANG[2], H. LIU[2], J. LIU[2], H. XIONG[2];
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Abstract: Chemokine (C-C motif) ligand 3 (CCL3, also known as MIP-1α) is produced by many types of cells and its cognate receptor has been detected in neurons in several brain regions including the hippocampus, suggesting CCL3 play a role in the central nervous system (CNS), in addition to its well established role in the immune system. Studies have shown that elevated CNS levels of CCL3 are associated with several neurological disorders. However, how elevated levels of CCL3 cause neurological disorders is not well understood. In this study, we investigated the effects of CCL3 on synaptic transmission and plasticity in the CA1 region of hippocampal slices prepared from 15-30d old male Sprague Dawley rats. Bath application of CCL3 produced a significant reduction in field excitatory postsynaptic potentials (fEPSP) and long-term potentiation (LTP), a well-known synaptic mechanism for learning and memory. The CCL3-mediated reduction of fEPSP and LTP was attenuated by Maraviroc, a specific CCR5 blocker. Addition of picrotoxin, a $GABA_A$ receptor antagonist, to the bath did not alter the CCL3-

mediated reduction on fEPSP and LTP, indicating that CCL3-associated reduction was not a consequence of activation of GABAergic interneurons. These results demonstrated that CCL3 alters synaptic transmission and plasticity via CCR5 which may have implications for pathogenesis of neurological disorders.

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Poster

807. Neuroinflammation

Location: Halls A-C

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Program#/Poster#: 807.24/Y13

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Tumor necrosis factor-related apoptosis inducing ligand/glucocorticoid-induced TNF receptor ligand redundancy in neurodegenerative processes

Authors: G. DI BENEDETTO[1,2], N. RONSISVALLE[1], *P. GUARNERI[3], R. BERNARDINI[1], G. CANTARELLA[1];
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Abstract: Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), a proapoptotic/proinflammatory cytokine of the TNF superfamily abundantly expressed by injuried neurons, has been proven to be a potent mediator of neurotoxicity related to various noxious stimuli, eventually setting into motion the apoptotic machinery in neuronal cells. Evidence shows that neutralization of the TRAIL death pathway in human neuronal cells is eventually associated with partial abrogation amyloid beta-related neurotoxicity. Similarly to TRAIL, the cytokine Glucocorticoid-induced TNF receptor ligand (GITRL) is able to transduce apoptotic signals. In spite of an array of reports suggesting redundant, additive effects between TRAIL and an array of cytokines, scanty data are, so far, available about a TRAIL/GITRL crosstalk. Here, we investigated possible interactions between TRAIL and the GITRL system in an *in vitro* model of neurodegeneration, using the human cortical neuronal cell line HCN-2. To accomplish this task, HCN-2 cells were treated with TRAIL in different experimental conditions. Real-time PCR analysis of cell lysates showed that HCN-2 cells did not express GITRL mRNA constitutively, whereas the latter was potently induced by treatment of cultured cells with TRAIL. In addition, HCN-2 cells did not express the GITRL receptor GITR mRNA, neither in

control cultures, nor after treatment with TRAIL. Real-time PCR data were corroborated by western blot analysis, showing similar pattern of the correspondent proteins at the level of protein expression. The MTT cell viability assay showed that TRAIL, when combined with GITRL, was able to exert additive toxic, lethal effects on HCN-2 cells. A counterproof to the latter data was provided in experiments performed using a GITRL neutralizing antibody, resulting in significantly attenuated TRAIL-mediated neurotoxicity in HCN-2 cells. Results suggest that TRAIL/GITRL redundancy during neurodegenerative processes implies reciprocal potentiation of detrimental effects of both cytokines on neurons, eventually leading to larger cell damage and death. Finally, characterization of novel molecular targets within the TRAIL/GITRL interplay may represent a platform for innovative treatment of neurodegenerative diseases.

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Poster

807. Neuroinflammation

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Program#/Poster#: 807.25/Y14

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS T32NS007413

Supplemental support from the L. Morton Morley Funds of the Philadelphia Foundation

Title: Epitope-binding specificity and cloning of human anti-NMDA receptor antibodies

Authors: *J. A. PANZER[1], R. SHARMA[3], N. SIMOROWSKI[4], B. H. BAUMANN[5], A. J. GLEICHMAN[6], H. PRUSS[7], H. FURUKAWA[4], S. DESSAIN[3], D. R. LYNCH[2]; [1]Neurol., [2]Children's Hosp. of Philadelphia, Philadelphia, PA; [3]Lankenau Inst. for Med. Res., Wynnewood, PA; [4]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [5]Univ. of Pennsylvania, Philadelphia, PA; [6]UCLA, Los Angeles, CA; [7]Charité – Universitätsmedizin Berlin, Berlin, Germany

Abstract: Human auto-antibodies specific for NMDAR have been implicated in anti-NMDAR encephalitis and dementia. Anti-NMDAR encephalitis is one of the most commonly identified causes of encephalitis and results in psychosis, altered consciousness, seizures, dyskinesias, and autonomic dysfunction. IgG antibodies in this syndrome are thought to bind to the extracellular

amino-terminal domain (ATD) of the NMDAR's GluN1 subunit, resulting in transient stabilization of the receptor's open conformation followed by NMDAR hypofunction due to receptor cross-linking and internalization. In dementia, IgA antibodies to the NMDAR have been described that are distinct from the IgG antibodies seen in encephalitis. The pathophysiological significance of these antibodies is unclear, and comparison of antibodies that bind the same receptor, but with different pathophysiological effects, will enable detailed structure/function studies. Limited amounts of anti-NMDAR antibodies can be obtained from individual patients, and these supplies are not pure. Thus, it has been impossible to establish definitively if anti-NMDAR antibodies are disease-causing. Furthermore, the polyclonal nature of serum and CSF antibodies makes diagnostic testing and detailed structure:function studies difficult. We have characterized the binding and activity of patient-derived, polyclonal anti-NMDAR antibodies and correlated these activities with anti-NMDAR monoclonal antibodies (mAbs) cloned from patients. Using a cell-free assay that employs a construct that preserves the native GluN1-ATD conformation, we have shown that the receptor's ATD alone is sufficient for IgG binding in anti-NMDAR encephalitis. We created a novel, whole cell ELISA (WCE) by expressing this domain in 293T cells. We collected B-cells from patients with anti-NMDAR encephalitis, and cloned 4 distinct mAbs immunoreactive with the GluN1-ATD WCE. The antibodies are affinity-matured, suggesting that they arose through T-cell dependent processes. We are characterizing the binding and activity of these mAbs using cultured neurons and transfected HEK cells. Studying anti-NMDAR polyclonal IgA antibodies from dementia patients, we have observed that these antibodies have different epitope-binding specificities than the IgG antibodies in anti-NMDAR encephalitis. Deletion of the ATD does not fully eliminate antibody binding, and that the C-terminal domain may play an important role. Furthermore, within GluN1 transfected HEK cells, IgA-dementia and IgG-encephalitis anti-NMDAR antibodies recognize distinct GluN1 pools, indicating a role for GluN1 post-translational processing in antibody recognition.

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Poster

807. Neuroinflammation

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Program#/Poster#: 807.26/Y15

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DOE DE-SC0005251

Title: Fluoropalmitoylethanolamine biodistribution and its potential as a PET tracer

Authors: *S. J. GATLEY[1], M. PANDEY[2], R. I. DUCLOS[1], T. R. DEGRADO[2];
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Abstract: N-Acylethanolamines are found widely as lipid signaling messengers in both plants and animals. Palmitoylethanolamine (PEA) is available as a nutraceutical claimed to be a "natural painkiller", and as an antipruritic cream. Its status has recently been reviewed (PMID:23964161). It is reported to have anti-inflammatory, pain reducing, anti-convulsant and neuroprotective actions. Though structurally related to the endocannabinoid, N-arachidonoylethanolamine (anandamide), PEA is believed to act by means of non-cannabinoid mechanisms, including as an agonist at peroxisome proliferator-activated receptors. It is a substrate for hydrolytic enzymes including fatty acid amide hydrolase (FAAH) and N-acylethanolamine-hydrolyzing acid amidase (NAAA). We previously presented (Abstract#: 404.19, 2012) a brief description of the synthesis of omega-fluoropalmitoylethanolamine (N[16-fluorohexadecanoyl] ethanolamine; FHEA) labeled with Fluorine-18 and also some data on its distribution and metabolism in mouse brain. We showed that FHEA enters the brain and that radioactive lipids but no remaining labeled FHEA were present one hour after intravenous administration. In subsequent work we have evaluated the biodistribution of FHEA and its brain metabolism in animals given URB597, a specific inhibitor of FAAH. In controls, tissue concentrations were in the order: liver > kidney > lung > heart > fat > spleen > whole brain > muscle > bone > blood > skin > testis, and ranged from 15% injected activity/g to 1% injected activity/g. URB597 did not significantly alter tissue concentrations, except in bone. In contrast to the lack of effect on total tissue F-18, URB597 did alter the pattern of labeled lipids in brain tissue seen after Folch extraction and radio thin layer chromatography of the chloroform fraction. In controls euthanized at 5 min, phosphatidyl choline (PC) was the most abundant lipid species (40%), while unmetabolized FHEA represented 10% of the F-18. After URB597, PC and FHEA represented 20% and 50%, respectively. Mean levels of all other neutral and phospholipid species detected were also lower after URB597, consistent with reduced supply of labeled fluoropalmitate from FHEA, due to inhibition of FAAH. The failure of URB597 to change net tissue F-18 levels at 30 min is disappointing in terms of the potential of FHEA as a PET tracer for FAAH. Once FHEA has entered the brain, it is retained and does not clear quickly enough to reveal a signal from label trapped by incorporation into lipid pools. Understanding the reasons

for this retention is important for the goal of developing a FAAH radiotracer based on metabolic trapping.

Disclosures: **S.J. Gatley:** None. **R.I. Duclos:** None. **M. Pandey:** None. **T.R. DeGrado:** None.

Poster

807. Neuroinflammation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 807.27/Y16

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: S06GM051971

Title: Neonatal stress alters inflammatory responses in the adult mouse brain

Authors: G. ODEBODE[1], L. NAIDU[2], *C. F. HOHMANN[1];
[1]Biol., Morgan State Univ., BALTIMORE, MD; [2]Biol., Morgan State Univ., Baltimore, MD

Abstract: Early life stress and trauma are risk factors for mental health disorders including schizophrenia, depression and Autism Spectrum Disorder (ASD). Neuroinflammation and increased production of pro-inflammatory cytokines have also been observed in these disorders. Here, we investigate, if early postnatal stress exposure can alter inflammatory responses in the brain and serum of Balb/CByJ mice. Pups were separated from the dam for one hour/day between postnatal days (PND) 2 and PND 7 and simultaneously exposed to cold and hot temperatures on alternating days. Stressed (STR) pups were compared to litter mate controls (LMC) that remained with the dam and age matched controls (AMC) in separate litters. In experiment one, cortices of 8 male and female STR, LMC and AMC each, were harvested on PND 90 and processed for ELISA (ebioscience) detection of cytokines; trunk blood was collected for serum corticosterone measurements. In a second experiment 21 STR, 19 LMC and 18 AMC mice of both sexes were injected, as adults, with LPS (5ug/250 microl.) or saline (0.9%) and sacrificed 2 hours later for collection of brain tissue and blood/serum for cytokine measurements. Baseline cytokine levels in the cortex of non-injected STR, LMC and AMC mice did not differ significantly. Similarly, baseline serum levels of corticosterone were not significantly altered in the STR or LMC groups. However, following LPS injections, production of IL-6 and TNF-alpha was significantly elevated in STR and LMC compared to AMC mice. Serum levels of IL-6 were significantly increased in STR and LMC mice whereas serum levels of TNF-alpha decreased compared to AMC. Thus, early life adverse conditions predispose for an

altered neuroinflammatory and cytokine responses later in life, in Balb/CByJ mice. While increased serum levels of IL-6 were consistent with prior observations, decreased serum TNF-alpha, after LPS injections, was unexpected. In addition, comparison of experiments 1 & 2 revealed higher production of both TNF-alpha and IL-6 in saline injected STR mice, compared to non-injected mice, suggesting, that injection-stress may be sufficient to trigger cytokine production selectively in the STR mice. These data suggest, that increased inflammatory responses, seen in mental health disorders, may have been programmed by early life experience. Supported by S06GM051971.

Disclosures: G. Odebode: None. **L. Naidu:** None. **C.F. Hohmann:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.01/Y17

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH-R01-MH0811,

NARSAD

State of CT

Title: Enhancement of glutamate clearance via cortical glial glutamate transport (GLT-1) induced resiliency to stress

Authors: M. BANASR, A. LEPACK, J. MALDONADO-AVILES, C. KISELYCZNYK, V. DURIC, J. ZHANG, S. WILBER, K. TANAKA, R. DILEONE, R. DUMAN, *G. SANACORA; Dept Psychiatry, Yale Univ., NEW HAVEN, CT

Abstract: Glial pathology and impaired glial mediated glutamate uptake are implicated in the pathophysiology of several neuropsychiatric and substance abuse disorders. We previously demonstrated stress-induced effects on GLT-1 (EAAT2 in humans), the primary glutamate transporter, that are associated with decreased rates of glutamate/glutamine cycling in rodent models. In these studies we sought to examine if pharmacological and genetic manipulations of GLT-1 function and expression could modulate stress responsiveness in similar rodent models. Regional microinjections of dihydrokainate (DHK) were applied to frontal cortex to examine the effect of pharmacological blockade of GLT-1 function on stress response. We used transgenic

mice (GLT1+/- mice) along with a targeted viral GLT-1 overexpression to examine the effects of genetically determined GLT-1 expression on stress-response. Three weeks after surgery and infusion and exposure to chronic unpredictable stress (CUS), animals were tested for anhedonia-like or anxiety-like behaviors. Mice with impaired GLT-1 function, either secondary to local micro-infusion of DHK into the infra/pre-limbic regions or heterozygous GLT-1 knockout, exhibited an exacerbated response to CUS on sucrose consumption and other behavioral assays. Viral overexpression of GLT-1 had little effect on baseline measures but significantly attenuated the effects of stress on the behavior of both heterozygous GLT-1 knockout mice and wild type littermates. We also demonstrated that two drugs, riluzole and ceftriaxone known to increase GLT-1 expression, have antidepressant-like effects in chronically stressed animals and that increased cortical GLT-1 activity is necessary for antidepressant action of these drugs. Indeed, we showed, using transgenic mice (GLT1+/- mice) and PFC infusion of DHK (dihydrokainate, GLT-1 inhibitor) in rats, impaired response to both riluzole and ceftriaxone in GLT1+/- mice and blockade of the behavioral effects of these drugs in rats infused with DHK in the mPFC. Together our results demonstrate that impairment of glutamate uptake can exacerbate the deleterious effects of stress, but enhancement of glutamate clearance by astrocytes in the PFC is sufficient to induce resiliency to stress and can be targeted for antidepressant development.

Disclosures: **M. Banasr:** None. **G. Sanacora:** 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.; grant support from AstraZeneca, Bristol-Myers Squibb, Merck & Co., Roche, and Sepracor Inc.. F. Consulting Fees (e.g., advisory boards); consulting fees form Abbott Labs, AstraZeneca, Bristol-Myers Squibb, Evotec, Eli Lilly & Co., Johnson & Johnson, Novartis, Roche, and Sepracor Inc.. **A. Lepack:** None. **J. Maldonado-Aviles:** None. **C. Kiselycznyk:** None. **J. Zhang:** None. **S. Wilber:** None. **K. Tanaka:** None. **R. Dileone:** None. **R. Duman:** None. **V. Duric:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.02/Y18

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: FAPESP 11/17281-7

FAPESP 12/17626-7

CNPq 473569/2012-4

Title: Conventional antidepressant drugs and DNMT inhibitors effects on stress-induced behavior and DNA methylation changes in mice submitted to forced swimming test

Authors: *S. S. JOCA[1], A. J. SALES[2];
[1]Physics-Chemistry/Pharmacology, FCFRP-USP, Ribeirao Preto-SP, Brazil; [2]Pharmacol., Sch. of Med. of Ribeirão Preto, USP, Ribeirao Preto-SP, Brazil

Abstract: DNA methylation is an epigenetic mechanism that is thought to play an important role in the neurobiology of depression. While stress increases DNA methylation and decreases the expression of genes involved in neuronal plasticity, DNA methyltransferases inhibitors (DNMTi) increase gene expression and induce antidepressant-like effects in preclinical models. As an attempt to furhter explore the involvement of DNA methylation in stress-induced behavioral changes, we investigated in the present work if DNMTi administration would have synergistic effect with conventional antidepressant drugs in behavioral and DNA methylation changes in mice submitted to the forced swimming test (FST). Therefore, mice received systemic injections of DNMTi, 5-aza-2'-deoxycytidine (5-AzaD, 0.1 and 0.2mg/Kg) and RG108 (0.1, 0.2 and 0.4mg/kg), or antidepressants, desipramine (DES, 2.5, 5 and 10mg/Kg) and fluoxetine (FLX, 5, 10, 20 and 30mg/Kg) and were submitted to FST or to the open field test (OFT). Additional groups received a combination of subeffective doses of 5-AzaD or RG108 with subeffective doses of DES or FLX. Subeffective doses of RG108 (0.1mg/Kg) or 5-AzaD (0.1mg/Kg) followed by DES (2.5mg/Kg) or FLX (10mg/Kg) induced significant antidepressant-like effects (one-way ANOVA, p0,05; n=8-9). Effective doses of RG108 (0.2 mg/Kg), 5-AzaD (0.2 mg/Kg), DES (10 mg/Kg) and FLX (20 mg/Kg) induced antidepressant-like effects ($F_{4,35}=4,715$, $p<0,05$) associated to similar changes in DNA methylation levels in the hippocampus (HPC) and prefrontal cortex (PFC). FST induced increase and decrease in DNA methylation in the HPC and PFC, respectively, and these effects were attenuated by antidepressants and DNMTi (HPC: $F_{5,37}=6,527$, $p<0,05$; PFC: $F_{5,40}=28,67$, $p<0,05$). These results suggest DNMTi potentiate the behavioral effect of antidepressant drugs and that antidepressants, as well as DNMTi, are able to modulate stress-induced changes in DNA methylation in brain regions closely related to the neurobiology of depression.

Disclosures: S.S. Joca: None. **A.J. Sales:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.03/Y19

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

State of CT

Title: Deletion of fibroblastic growth factor receptor 1 from NG2 cells exerts anxiolytic properties

Authors: *A. BECKER, A. LEPACK, X.-Y. LI, R. DUMAN;
Dept. of Mol. Psychiatry, Yale Univ., New Haven, CT

Abstract: Major depression is the most debilitating mood disorder in the United States and is often comorbid with anxiety disorders. Reciprocally, antidepressants of the SSRI type are also effective for treating anxiety disorders. In search of new treatment strategies for depression and anxiety disorders, accumulating evidence indicates modulation of the fibroblastic growth factor (FGF) signaling as a promising novel treatment approach. The potent neurotrophic activity of FGFs has been implicated in proliferation control, specification and survival of progenitor cells, axon guidance and regulation of synaptic plasticity, mechanisms that are disturbed in depression and potentially contribute to anxiety disorders. Recent findings from our lab show that FGF-2/FGF receptor signaling is sufficient and necessary for the behavioral, as well as gliogenic, actions of antidepressants. We have now investigated the behavioral and molecular consequences of selective FGF receptor 1 (FGFR1) ablation on NG2 positive cells in the context of depression-like symptoms and anxiety. Knockout mice were generated by breeding of NG2-Cre mice with homozygous FGFR1 floxed mice. Adult NG2-FGFR-/- mice did not differ from non-ablated littermates (NG2-FGFR+/+) in bodyweight, water and sucrose consumption, or in the amount of locomotor activity. However, NG2-FGFR-/- mice displayed decreased anxious behavior in animal models of anxiety, including increased exploration in the center of an open-field arena, more time spent in the open arms of an elevated plus maze, and decreased latency to feed in the novelty suppressed feeding paradigm. This finding is consistent with a function of FGFs as modulators of emotional behaviors, albeit opposite to predicted results. In line with the reported

correlation of anxiolytic behavior with increasing levels of FGF2, NG2-FGFR-/- mice showed a significant increase in hippocampal FGF2 which could explain this discrepancy. We are currently investigating further molecular consequences of the specific FGFR1 knockdown in NG2 positive cells, as understanding of the mechanisms involved in the regulation of anxiety by FGFR1 and their role on NG2 positive cells would open a new avenue for the treatment of anxiety disorders in humans.

Disclosures: **A. Becker:** None. **X. Li:** None. **R. Duman:** None. **A. Lepack:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.04/Y20

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

State of CT

Title: Molecular and behavioral characterization of optogenetic stimulation of infralimbic prefrontal cortex circuitry

Authors: *A. M. THOMAS;
Yale Sch. of Med., New Haven, CT

Abstract: Ketamine is an antidepressant that promises to be more effective and faster-acting than any of the currently available depression pharmacotherapies, but its clinical use is limited by side effects. It is thus important to understand how ketamine acts in the brain in order to develop new therapies that utilize its mechanism of action. One key area of study is the circuitry underlying ketamine's effects. Ketamine is known to increase glutamate release in the rodent medial prefrontal cortex (mPFC) as well as mammalian target of rapamycin (mTOR) signaling, both of which are critical to its antidepressant action. In humans, the prefrontal brain area thought to correspond most closely to the rodent mPFC is the anterior cingulate cortex, and functional brain imaging has shown differences in its activity in depressed patients compared to controls. We hypothesized that we could reproduce ketamine's antidepressant effects by inducing glutamatergic activity in the mPFC. To test this, we used a rAAV2/CaMKIIα-

ChR2(H134R)-EYFP construct injected into the infralimbic prefrontal cortex (IL-PFC) of male rats. A single, one-hour laser stimulation of excitatory neurons in this area produces an antidepressant effect lasting up to 2 weeks, as measured by the forced-swim test (FST) and novelty-suppressed feeding test (NSFT), which are measures of depression and anxiety, respectively. Using the same optogenetic manipulations, we have sought to characterize the circuitry underlying this effect. Three brain areas in particular, the dorsal raphe (dR), lateral habenula (lHb), and nucleus accumbens (NAc), have been found to have robust connections to the IL-PFC and to be involved in the development of depressive features in rodents. The relevance of these brain areas to the observed antidepressant effect of IL-PFC stimulation will be assessed by optogenetically stimulating axon terminals from the IL-PFC in these three regions after injection of the viral channelrhodopsin construct into the IL-PFC. Molecular studies are being conducted to characterize changes in activity in these regions and determine which cell types are the target of these projections.

Disclosures: A.M. Thomas: None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.05/Y21

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Colgate University: Division of Natural Sciences-Mathematics; Dept of Psychology & Neuroscience

Title: Neonatal exposure to clomipramine is a behavioral rat model of Obsessive Compulsive Disorder offering face validity as well as predictive validity for the GABA agonist diazepam, the serotonin uptake inhibitor fluoxetine, and the norepinephrine uptake inhibitor desipramine

Authors: *D. S. KREISS, S. E. FRANK, K. M. CRAIG, H. S. MULDER, A. L. HYDE, L. S. LAIKS;
Psychology and Neurosci. Dept, Colgate Univ., Hamilton, NY

Abstract: Obsessive Compulsive Disorder (OCD) - a psychiatric disorder affecting 2-3% of the population - is characterized by persistent, anxiety producing thoughts often accompanied by overwhelming urges to perform repetitive ritualistic behaviors. Current pharmacological treatments for OCD are only effective in 40-60% of patients and have an 8-10 week delayed

onset. Animal models with both face and predictive validity are imperative in the field of OCD so that better treatment avenues can be explored. The current study aimed to further validate a novel rodent model of OCD: neonatal exposure to clomipramine. Male Sprague-Dawley rats were administered clomipramine (15 mg/kg x 2) during neonatal Days 9-16 (= neoClom). Hole-board head dipping and behaviors in a marble arena (including marble burying and a novel marble checking behavior) were assessed at adulthood. At postnatal Days 208-228, neoClom rats (n=14) buried significantly more marbles than neoSaline controls, but did not differ in other observed behaviors. Our 2013 studies demonstrated that at postnatal Days 70-72, a group of 26 neoClom rats significantly buried more marbles, checked more marbles, had increased repetitive head dips, and spent more time in closed arms of the Elevated Plus Maze -> suggesting that younger behavioral testing and a larger group of neoClom rats facilitate observation of more robust differences. The effects of acute diazepam (1, 8 mg/kg) upon the current group of neoClom rats (n=14) differed significantly from neoSalines. The GABA agonist diazepam did not alter behaviors of neoClom rats. However in the NeoSalines, the low dose of diazepam significantly increased exploratory behaviors while the high dose significantly decreased behaviors. These results are congruent with the ineffectiveness of diazepam at alleviating symptoms of OCD patients and the typical behavioral alterations induced by GABA agonists in humans without OCD. In 2013, our lab demonstrated that 14-day repeated administration of fluoxetine (10 mg/kg) normalized marble and hole-board behaviors of the neoCloms, whereas repeated administration with desipramine (5 mg/kg) and saline had no effect. The ability of repeated fluoxetine and the inabilities of acute diazepam and repeated desipramine to normalize the altered behaviors of the experimental neoClom rats mirror the clinical efficacy of these agents in the treatment of OCD. These data confirm the face validity of the neoClom model of OCD and, for the first time, demonstrate the predictive validity of the model. The neoClomipramine model offers a unique permanent, multi-symptom behavioral profile of OCD that distinguishes it from models of anxiety and depression.

Disclosures: **D.S. Kreiss:** None. **S.E. Frank:** None. **K.M. Craig:** None. **A.L. Hyde:** None. **L.S. Laiks:** None. **H.S. Mulder:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.06/Y22

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MH045481

MH093897

MH102004

NSF GRFP

State of CT

Title: S6 Kinase 1 signaling in prefrontal cortex controls depressive behavior

Authors: *J. M. DWYER, J. MALDONADO-AVILES, A. E. LEPACK, R. J. DILEONE, R. S. DUMAN;
Yale Univ., New Haven, CT

Abstract: Major Depressive Disorder (MDD) affects nearly one fifth of the population and is the second leading cause of disability worldwide. Although the precise molecular mechanisms underlying MDD remain largely unknown, our lab has recently demonstrated an important role for the mechanistic target of rapamycin complex 1 (mTORC1)/S6 Kinase 1 pathway in mediating stress-related behavior and rapid antidepressant responses. The mTORC1/S6K1 pathway is a critical regulator of protein synthesis, cell growth and cytoskeletal rearrangement. In the present series of experiments, we asked whether direct modulation of this pathway is sufficient to control depressive behavior. To address this question, we used an AAV2 viral-mediated approach to deliver either a constitutively-active or a kinase-inactive S6K1 mutant (Dr. John Blenis) to the rat mPFC to determine if modulation of S6K1 signaling is sufficient to control depressive behavior. Bilateral infusion of the active S6K1 mutant in the mPFC reduced immobility times in the forced swim test (40% reduction compared to control; $P < 0.002$), indicative of a robust antidepressant response. These effects are not due to differences in locomotor activity as control- and S6K1 mutant-infused rats did not differ. The observed antidepressant-like effect is specific to the mPFC as infusion of the active S6K1 mutant into the dorsal striatum did not produce an antidepressant effect. Conversely, rats infused with kinase-inactive S6K1 demonstrated increased immobility time, indicative of pro-depressive behavior (39% increase compared to control; $P<0.05$). In a separate set of experiments, infusion of active S6K1 into the mPFC rendered rats resilient to the behavioral deficits produced by chronic stress ($P<0.05$), whereas kinase-inactive S6K1 mimicked the effects of chronic stress in naïve rats ($P<0.05$). Studies are currently underway to assess alterations in neuronal morphology following enhancement or suppression of S6K1 activity. Preliminary data in primary cortical cultures demonstrate a significant increase in neuronal complexity due to activation of S6K1. Together, these data suggest that aberrant protein synthesis through S6K1 contributes to the pathology of MDD, and further studies of the molecular events involved may lead to much needed advancements in antidepressant therapy.

Disclosures: **J.M. Dwyer:** None. **J. Maldonado-Aviles:** None. **A.E. Lepack:** None. **R.J. DiLeone:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.07/Y23

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MIUR Prin 2008, 200894SYW2

Toscana Life Sciences Foundation Orphan_0108 program

Title: Generation of Pet1210-Cre transgenic mouse line reveals non-serotonergic expression domains of Pet1 both in CNS and periphery

Authors: *M. PASQUALETTI[1,2], B. PELOSI[1], S. MIGLIARINI[1], G. PACINI[1];
[1]Biology, Cell and Developmental Unit, Univ. of Pisa, Pisa, Italy; [2]Ctr. for Neurosci. and Cognitive Systems, Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: Neurons producing serotonin (5-hydroxytryptamine, 5-HT) constitute one of the most widely distributed neuronal networks in the mammalian central nervous system (CNS) and exhibit a profuse innervation throughout the CNS already at early stages of development. Serotonergic neuron specification is controlled by a combination of secreted molecules and transcription factors such as Shh, Fgf4/8, Nkx2.2, Lmx1b and Pet1. In the mouse, Pet1 mRNA expression appears between 10 and 11 days post coitum (dpc) in serotonergic post-mitotic precursors and persists in serotonergic neurons up to adulthood, where it promotes the expression of genes defining the mature serotonergic phenotype such as tryptophan hydroxylase 2 (Tph2) and serotonin transporter (SERT). Hence, the generation of genetic tools based on Pet1 specific expression represents a valuable approach to study the development and function of the serotonergic system. We have generated a Pet1210-Cre transgenic mouse line in which the Cre recombinase is expressed under the control of a 210 kb fragment from the Pet1 genetic locus to ensure a reliable and faithful control of somatic recombination in Pet1 cell lineage. Besides Cre-mediated recombination accurately occurred in the serotonergic system as expected, Pet1210-Cre transgenic mouse line allowed us to identify novel, so far uncharacterized, Pet1 expression domains. Indeed, we showed that in the raphe Pet1 is expressed also in a non-serotonergic neuronal population intermingled with Tph2-expressing cells and mostly localized in the B8 and B9 nuclei. Moreover, we detected Cre-mediated recombination also in the developing pancreas and in the ureteric bud derivatives of the kidney, where it reflected a specific Pet1 expression.

Thus, Pet1210-Cre transgenic mouse line faithfully drives Cre-mediated recombination in all Pet1 expression domains representing a valuable tool to genetically manipulate serotonergic and non-serotonergic Pet1 cell lineages.

Disclosures: **M. Pasqualetti:** None. **B. Pelosi:** None. **S. Migliarini:** None. **G. Pacini:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.08/Y24

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Colgate University: Division of Natural Sciences-Mathematics; Dept of Psychology & Neuroscience

Title: The serotonin 5HT2 antagonist mianserin, but not the serotonin agonist 5HT2 mCPP (meta-chlorophenylpiperazine), shows predictive validity in the behavioral rat model of Obsessive Compulsive Disorder induced via neonatal exposure to clomipramine

Authors: *A. L. HYDE, H. S. MULDER, K. M. CRAIG, S. E. FRANK, L. J. KASPARSON, D. S. KREISS;
Psychology/Neuroscience, Colgate Univ., Hamilton, NY

Abstract: Obsessive Compulsive Disorder (OCD) - a psychiatric disorder affecting 2-3% of the population - is characterized by persistent, anxiety producing thoughts often accompanied by overwhelming urges to perform repetitive ritualistic behaviors. Current pharmacological treatments for OCD are only effective in 40-60% of patients and have an 8-10 week delayed onset. Animal models with both face and predictive validity are imperative in the field of OCD so that better treatment avenues can be explored. The current study aimed to further validate a novel rodent model of OCD: neonatal exposure to clomipramine. Male Sprague-Dawley rats were administered clomipramine (15 mg/kg x 2) during neonatal Days 9-16 (= neoClom). Hole-board head dipping and behaviors in a marble arena (including marble burying and a novel marble checking behavior) were assessed at adulthood. At postnatal Days 80-106, neoClom rats (n=14) buried significantly more marbles than neoSaline controls and trended towards poking more holes (p=0.06), but did not differ in other observed behaviors. Our 2013 studies demonstrated that at postnatal Days 70-72, a group of 26 neoClom rats significantly buried more marbles, checked more marbles, and had increased repetitive head dips -> suggesting that a

larger group of neoClom rats facilitates observation of more robust differences. Acute administration of mCPP (0.4, 0.8 mg/kg) to the current rats significantly decreased marbles checked in both neonatal groups, but did not alter other behaviors. These results are incongruent with the ability of mCPP to exacerbate symptoms in OCD patients, although the effects of alternative doses of mCPP upon neoCloms should be explored. In contrast to mCPP, the effects of acute mianserin (3 mg/kg) upon the current group of neoClom rats differed significantly from neoSalines. In the neoCloms, mianserin significantly decreased the total number of pokes, holes, and repeats - in comparison to vehicle injection. In neoSaline Controls, mianserin had no effect upon the behaviors. These results are congruent with the ability of serotonin 5-HT2 antagonists to alleviate symptoms in OCD patients. In combination, our 2013 and 2014 studies have shown that acute mianserin and repeated fluoxetine (10 mg/kg X 14 days) normalize the altered behaviors of experimental neoClom rats, whereas acute diazepam (1, 8 mg/kg) and repeated desipramine (5 mg/kg) do not. Our lab's data confirm the face validity of the neoClom model of OCD and demonstrate the predictive validity of the model. The neoClomipramine model offers a unique permanent, multi-symptom behavioral profile of OCD that distinguishes it from models of anxiety and depression.

Disclosures: **A.L. Hyde:** None. **H.S. Mulder:** None. **K.M. Craig:** None. **S.E. Frank:** None. **L.J. Kasparson:** None. **D.S. Kreiss:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

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Program#/Poster#: 808.09/Y25

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

The State of CT

Title: Ketamine stimulates mTORC1 and BDNF release in primary cortical cultures

Authors: *A. LEPACK, M. FUCHIKAMI, J. M. DWYER, A. TROG, M. BANASR, R. S. DUMAN;
Psychiatry, Yale, New Haven, CT

Abstract: Clinical and preclinical studies have shown that ketamine, a non-competitive NMDA receptor antagonist, produces rapid antidepressant actions in severely depressed patients. Preclinical studies demonstrate that the antidepressant actions of ketamine are dependent on glutamate-AMPA receptor activation, BDNF-mTORC1 signaling and synaptogenesis. While studies *in vivo* have answered many questions about the mechanisms underlying the effects of ketamine, we sought to establish an *in vitro* model to allow higher throughput testing of rapid antidepressant drugs and to assess specific mechanisms that are difficult to measure *in vivo*. In the present studies, we performed a comprehensive analysis in cortical neuronal cell cultures of ketamine as well as two other putative rapid acting antidepressants: scopolamine, an acetylcholine muscarinic receptor antagonist, and LY 341495, an $mGluR_{2/3}$ antagonist. We first assessed the dose and time course for ketamine stimulation of mTORC1 signaling. The results demonstrate that stimulation of ketamine (500 nM) produced rapid activation of mTORC1 signaling within 15 min and this effect lasted up to 1 hr. We have recently demonstrated that blockade of BDNF release within the mPFC blocks the behavioral effects of ketamine in the FST. In addition, the behavioral actions of ketamine are blocked in Val66Met knock-in mice, which have impaired activity dependent release of BDNF (Liu et al., 2012), suggest that the actions of ketamine require BDNF release. To test this hypothesis, we treated cells with ketamine (500 nM) for 15, 60 min and 6 hr. The results show that ketamine increased BDNF release (~50%) into the culture media within 15 min and that this effect was sustained up to 6 hr. We have also recently shown that the behavioral actions of ketamine are dependent on activation of AMPA receptors and L-type calcium channels. Pretreatment with either NBQX (50 uM), an AMPA receptor antagonist, or verapamil (10 uM), an L-type calcium channel antagonist, blocked ketamine-induced BDNF release. We have also determined doses that activate mTORC1 signaling for scopolamine and LY 341495. Studies are underway to assess if these compounds also increase BDNF release similar to ketamine and if this release is dependent on activation of AMPA receptors and L-type calcium channels. Future studies will also include analysis of dendritic branching and morphology of neurons following stimulation of each compound. Taken together, the current findings demonstrate that primary neuronal culture may provide an alternative platform for screening antidepressant drugs and determining mechanisms of action.

Disclosures: **A. Lepack:** None. **M. Fuchikami:** None. **J.M. Dwyer:** None. **A. Trog:** None. **M. Banasr:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.10/Y26

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Development of stem-cells therapy for depressive disorders combined with nanotechnology based CT imaging

Authors: *O. BETZER[1,2], A. SHWARTZ[3], M. MOTIEI[4], G. KAZIMIRSKY[3], I. GISPAN[3], C. BRODIE[3,5], R. POPOVTZER[4], G. YADID[2];
[2]Gonda Brain Res. Ctr., [3]Everard and Mina Goodman Fac. of Life Sci., [4]Fac. of Engin. & Inst. of Nanotechnology & Advanced Material, [1]Bar Ilan Univ., Ramat Gan, Israel; [5]Hermelin Brain Tumor Center, Dept. of Neurosurg., Henry Ford Hosp., Detroit, MI

Abstract: Cell-based therapy using mesenchymal stem cells (MSCs) has emerged as a novel and successful approach for the treatment of various brain pathologies mainly due to their ability to migrate to sites of injury, secrete various vital soluble factors and promote neurogenesis. Depressive disorders are severe and debilitating medical illnesses affecting millions of individuals worldwide. Current psychotherapy & pharmacological agents address these disorders sub-optimally, causing patients to experience low remission rates and relapses and do not provide long term therapeutics, while DBS and ECT treatments are still under evaluation. In this study we have developed a novel MSC-based long term therapeutic application for treating these disorders, tested on the Flinders sensitive line (FSL) rat model. Another novel aspect of this research is the development of nanoparticle-based CT imaging technique for non-invasive longitudinal MSCs tracking. Tracking cells within deep brain structures is a key challenge, which cannot be thoroughly addressed using current techniques. This approach enabled us monitoring behavioral manifestation in parallel with longitudinal, real-time MSCs tracking by CT-based imaging. The results of this study demonstrate the therapeutic impact of MSCs on the depressive-like behavior displayed by the rat model. Cell's migration was traced up to one month post-transplantation, enabling sensitive detection and quantitative, longitudinal migration tracking of MSCs. Our data revealed that MSCs specifically navigated and homed to distinct depression-related brain regions. Furthermore we demonstrated that cell migration correlates with the therapeutic effect of MSCs on the depressive-like behavior displayed by the rat model (figure). Our new technique suggests future insight into the mechanisms underlying success, or failure of a potential cell therapy, which is currently poorly understood. .

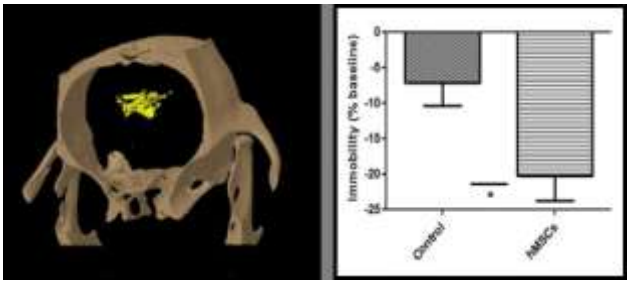

Figure: *In vivo* volume rendering CT scan one month post transplantation correlates with behavioral Fast swim test fold change differences after MSCs treatment.

Disclosures: O. Betzer: None. **A. Shwartz:** None. **G. Kazimirsky:** None. **I. Gispan:** None. **C. Brodie:** None. **R. Popovtzer:** None. **G. Yadid:** None. **M. Motiei:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.11/Y27

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Differential serotonergic mechanisms of the noncompetitive NMDA receptor antagonists ketamine, memantine, MK-801 in the Differential Reinforcement-of-Low-Rate (DRL) 72 s procedure in rats

Authors: *J. H. PORTER, C. R. MERRITT, T. M. HILLHOUSE;
Psychology, Virginia Commonwealth Univ., Richmond, VA

Abstract: The glutamatergic system has emerged as a therapeutic target for treatment of major depressive disorder (MDD). The noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist ketamine produces robust antidepressant effects in treatment-resistant patients. Other NMDA receptor antagonists such as memantine fail to produce reliable antidepressant effects in clinical research. The goals of the present experiment were to: 1) compare the antidepressant-like effect of the noncompetitive NMDA receptor antagonists ketamine, memantine, and MK-801 in the differential-reinforcement-of-low-rate (DRL) 72 s procedure with rats; 2) determine if the antidepressant-like and psychostimulant-like effects of ketamine and MK-801, respectively, in the DRL 72 s procedure are mediated by serotonin 5-HT2 receptors antagonism; 3) determine if the antidepressant-like effects of ketamine and memantine in the DRL 72 s procedure are mediated by inhibition of serotonin transporters. First, ketamine (5.6-10.0 mg/kg) and memantine (10.0 mg/kg) produced antidepressant-like effects in the DRL 72 s procedure; whereas, MK-801 (0.1-0.18 mg/kg) produced a psychostimulant-like effect. Second, the antidepressant-like effects of ketamine in the DRL 72 s procedure do not appear to be mediated by 5-HT2 receptors as co-administration of the 5-HT2 antagonist ritanserin (1.0 mg/kg) with a subeffective dose of ketamine (3.2 mg/kg) decreased responses, but failed to alter reinforcers or shift IRT distributions. Additionally, the 5-HT2 agonist quipazine failed to attenuate the antidepressant-like effects of ketamine in the DRL 72 s procedure. Conversely, the

psychostimulant-like effects of MK-801 in the DLR 72 s procedure are mediated, in part, by 5-HT2 receptors as 1.0 mg/kg ritanserin attenuated the increase in responses and leftward shift in IRT distributions produced by 0.18 mg/kg MK-801. Third, the antidepressant-like effects of ketamine in the DRL 72 s procedure do not appear to be mediated by inhibition of serotonin transporters as a subeffective dose of the SSRI fluoxetine (5.0 mg/kg) did not potentiate a subeffective dose of ketamine (3.2 mg/kg). In contrast, the antidepressant-like effects of memantine in the DRL 72 s procedure are mediated, in part, by inhibition of serotonin transporters as co-administration of subeffective doses of 5.0 mg/kg fluoxetine and 5.0 mg/kg memantine increased reinforcers and decreased responses, but failed to shift IRT distributions ($p = 0.58$). Taken together, these results indicate that the behavioral effects of these NMDA receptor antagonists are differentially mediated by serotonergic mechanisms.

Disclosures: J.H. Porter: None. **C.R. Merritt:** None. **T.M. Hillhouse:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.12/Y28

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Discriminative stimulus properties of the noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist ketamine in rats

Authors: *B. L. JOSEPH, H. S. POPAL, T. M. HILLHOUSE, J. H. PORTER;
Psychology, Virginia Commonwealth Univ., Richmond, VA

Abstract: The noncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist ketamine produces rapid and sustained antidepressant effects in treatment-resistant patients suffering from major depressive disorder. However, the abuse liability of ketamine is a concern. Drug discrimination procedures are used to evaluate the subjective effects of drugs, which may be associated with the abuse-related effects of drugs and with their therapeutic effects. Drug discrimination procedures also can be used to determine the underlying receptor mechanisms that mediate a drug's discriminative stimulus properties. In the present study, 17 male Sprague-Dawley rats were trained to discriminate 10.0 mg/kg ketamine from saline in a two-lever drug discrimination procedure for food reinforcement. Ketamine (7.5-20.0 mg/kg) produced full substitution for the training dose of 10.0 mg/kg ketamine, yielding an ED50 of 3.65 mg/kg. A time-course study found that the ketamine cue had a rapid onset, producing full substitution from

0-30 min injection times, but not at 60 and 100 min. The noncompetitive NMDA receptor antagonists MK-801 (0.1 mg/kg) and memantine (10.0 mg/kg) produced full substitution for the discriminative stimulus properties of 10.0 mg/kg ketamine, yielding ED50 values of 0.04 mg/kg and 2.83 mg/kg, respectively. The AMPA receptor antagonist NBQX (10.0 mg/kg) did not substitute for ketamine when administered alone and NBQX did not block or attenuate the discriminative stimulus properties of ketamine when administered in combination with 5.0 and 10.0 mg/kg ketamine. However, NBQX did produce a significant suppression of response rates when administered in combination with the 10.0 mg/kg dose of ketamine. The dopamine D2 agonist quinpirole (0.032-0.1 mg/kg) and selective serotonin reuptake inhibitor fluoxetine (2.5-20 mg/kg) did not substitute for the discriminative stimulus properties of ketamine, but both drugs produced rate suppression at the highest doses tested. These results suggest that the discriminative stimulus properties of ketamine are mediated by noncompetitive NMDA antagonism as only MK-801 and memantine produced full substitution for ketamine. Future studies will need to test selective ligands alone or in combination with ketamine to elucidate the underlying receptor mechanisms that mediate the discriminative stimulus properties of ketamine.

Disclosures: B.L. Joseph: None. **H.S. Popal:** None. **T.M. Hillhouse:** None. **J.H. Porter:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.13/Y29

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Behavioral and neurobiological correlates for predicting resilience and susceptibility to social defeat

Authors: *Y. GROSSMAN[1], R. E. WALDMAN[2], G. PANDEY[3], W. G. JANSSEN[3], D. DUMITRIU[3];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Vassar Col., Poughkeepsie, NY; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Depression is a neuropsychological disorder that affects millions of people. There are currently no predictors for susceptibility to depression and a large proportion of afflicted individuals are resistant to available treatments. Therefore, the ability to predict selective psychosocial vulnerability and resilience to stress holds great promise in preventing this

debilitating disorder. Social defeat (SD) is a highly validated mouse model of depression. We used acute and chronic social defeat stress for elucidating the neurocircuitry involved in establishment and maintenance of divergent behavioral phenotypes. We developed non-invasive behavioral paradigms to serve as a proxy for cortical and limbic function, and found that performance on a prefrontal-dependent task prior to SD was correlated with resilience, while performance on a hippocampal-dependent task was correlated with susceptibility. Furthermore, using a classifier algorithm that considers performance on multiple behavioral tests, we were able to strengthen the absolute predictive power of our behavioral battery. To probe the neuroactivation correlate of the above findings, we analyzed cFos expression in various cortical and subcortical regions following SD and found that resilient animals have higher correlative activation between prefrontal and temporal regions while susceptible animals exhibit anti-correlative activation patterns within the same circuits. To investigate the synaptic mechanisms underlying these activation differences, we used a GFP virus to isolate amygdala-projecting prefrontal neurons that are active during the establishment of the behavioral response to SD. We found that in this select subpopulation of neurons, resilient animals have higher densities of mushroom spines compared to susceptible animals. Together, these results suggest that resilient animals have pre-existing higher prefronto-temporal connectivity that can be predicted via non-stressful behavioral tests.

Disclosures: Y. Grossman: None. **R.E. Waldman:** None. **G. Pandey:** None. **W.G. Janssen:** None. **D. Dumitriu:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.14/Y30

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: KSU GSS Research Award

Title: Effects of minocycline on preventing a second depressive-like episode following stressor exposure

Authors: *J. L. REMUS[1], D. JAMISON[2], J. JOHNSON[2];
[1]Biol. Sci., [2]Kent State Univ., Kent, OH

Abstract: The biological mechanisms that contribute to recurrent depression are not well understood. Our lab has recently developed a rodent model of recurrent depression. Here animals are exposed to chronic mild stress for 35 days during which we see a suppression of sucrose intake. Stressors are then removed for 20 days and animal's sucrose intake increases. Animals are re-exposed to the chronic mild stress paradigm over 15 days and we see a steeper (more rapid) decrease in sucrose intake during their second exposure than during their first. This suggests that animals are sensitized to stress following their first episode of chronic mild stress. In the current study, we evaluate the role of microglia activation in regards to preventing the sensitized behavioral response during reexposure to stress. Adult male fischer rats were exposed to our animal model of recurrent depression. During the recovery phase half of the animals receive minocycline in their drinking water. We expect to see that stressed animals that received minocycline will not show a sensitized response when re-exposed to the chronic mild stress paradigm.

Disclosures: J.L. Remus: None. **D. Jamison:** None. **J. Johnson:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.15/Y31

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MH045481

MH093897

State of Connecticut

Title: Depression and diabetes interplay: First steps towards identifying the common neural pathological mechanisms and treatment strategies in rodent models

Authors: *S. DUTHEIL, K. T. OTA, A. E. LEPACK, R. S. DUMAN;
Yale Univ., New Haven, CT

Abstract: Numerous reports show that high fat diet (HFD) and sedentary lifestyle increase the risk of developing obesity, cardiac disease, hypertension, type 2 diabetes and cognitive impairments. According to the World Health Organization, diabetes and depression will have the greatest health care burdens worldwide by 2025, and increasing evidence suggests that a

bidirectional relationship exists between these two disorders. However, the molecular and cellular pathways underlying the increased risk of developing both diabetes and depression remain unknown. In this regard, the current study seeks to identify potential common pathological mechanisms using a rat HFD-induced diabetes model, and to determine if HFD causes brain alterations that contribute to anxiety and depressive behaviors. The results show that rats fed a HFD during 16 weeks develop glucose intolerance, increased neuronal and peripheral corticosterone levels and enhanced brain inflammation in the hippocampus sustained by activation of genes involved in cytokine production. In addition, HFD induces anxiety-like behavior and cognitive deficits accompanied by alterations of p70S6 kinase, a downstream substrate of the mammalian target of rapamycin complex 1 (mTORC1) which plays a major role in the pathophysiology and treatment of stress-induced anxiety and depressive behaviors. Hence, our data show that a long-term diet rich in fat not only induces peripheral changes and metabolic stress, but also affects neuronal signaling pathways with increased inflammation in hippocampus, as well as anxiety and cognitive behavior in some ways similar to what is observed in chronic stress models. Studies are currently underway to determine if anti-inflammatory treatment strategies can reverse both behavioral and biochemical impairments induced by HFD. This study is a first step towards understanding the mechanisms linking diet-induced diabetes to depression. In the future, early interventions targeting the disrupted pathways could help to decrease the prevalence of mood disorders in diabetic patients and reciprocally, minimize the burden associated with these two conditions.

Disclosures: **S. Dutheil:** None. **K.T. Ota:** None. **A.E. Lepack:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.16/Y32

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

State of Connecticut

Title: The role of the gabaergic interneurons of the prefrontal cortex in the rapid-acting antidepressant effects of ketamine

Authors: *D. M. GERHARD[1], K. T. OTA[2], E. S. WOHLEB[2], B. B. LAND[2], R. J. DILEONE[2], R. S. DUMAN[2];
[1]Psychology, [2]Mol. Psychiatry, Yale Univ., New Haven, CT

Abstract: Major depressive disorder (MDD) is a growing public health concern with widespread effects on personal welfare, the economy and society. Selective serotonin reuptake inhibitors are the first-line treatment for MDD despite their weak efficacy and required long-term treatment plan. Recent efforts have focused on the therapeutic benefits of ketamine, an NMDA receptor antagonist that produces rapid and sustained antidepressant effects in patients that have failed to respond to other forms of treatment. Rodent models of depression have demonstrated that ketamine rapidly increases synaptic connections and glutamate release in the prefrontal cortex (PFC) shortly following acute treatment. Furthermore, optogenetic activation of the mPFC has an immediate antidepressant-like behavioral effect and unpublished data from our lab demonstrates that, like ketamine, these effects are long-lasting. However, a new hypothesis to emerge posits that the increased spiking of excitatory pyramidal neurons occurs via ketamine deactivation of inhibitory GABAergic interneurons that have direct connections with excitatory principal neurons. Microinfusion of muscimol, a $GABA_A$ agonist, into the infralimbic cortex (IL) of the mPFC blocks the effects of ketamine, giving insight into a potential route of action of ketamine through the GABAergic interneuron system of the mPFC. To examine the role of the GABAergic interneurons, we are using a GAD67-Cre transgenic mouse line in combination with optogenetic inhibition (floxed halorhodopsin virus) or stimulation (floxed channelrhodopsin virus). We hypothesize that optogenetic inhibition of GABA neurons will produce an antidepressant response and/or occlude the effects of ketamine, while optogenetic excitation will block the effects of systemic ketamine. In addition, we are developing an approach to examine the specific NMDA receptor subtype(s) on GABA neurons that mediates the antidepressant-like effects of ketamine. NR2B antagonists have been shown to produce antidepressant effects similar to ketamine in both rodent models and clinical trials. To address this question we are using the GAD-67 transgenic mouse line along with a virus to express floxed shRNA for NR2B. Behavioral, histological and immunohistochemical results are currently being collected for this experiment and will be discussed.

Disclosures: D.M. Gerhard: None. **K.T. Ota:** None. **E.S. Wohleb:** None. **B.B. Land:** None. **R.J. DiLeone:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.17/Z1

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R01 MH080116

P50 MH90966

Title: Infralimbic and prelimbic contributions to anxiety-like behaviors in mice following developmental SSRI exposure

Authors: *M. K. CAFFREY[1], T. J. REBELLO[2], M. S. ANSORGE[1];
[1]Developmental Neurosci., Columbia Univ. and NYSPI, New York, NY; [2]Psychiatry, Columbia Univ., New York, NY

Abstract: Early-life serotonin (5-HT) signaling modulates brain development to impact adult behavior, but 5-HT sensitive periods, neural substrates, and behavioral consequences remain poorly understood. We have identified the period ranging from postnatal day 2-11 (P2-11) as 5-HT sensitive, with 5-HT transporter (5-HTT) blockade increasing anxiety-like behavior, and impairing fear extinction learning and memory in adult mice. Furthermore, P2-11 5-HTT blockade causes reduced excitability of infralimbic (IL) cortex pyramidal neurons that normally promote fear extinction, but increased excitability of prelimbic (PL) pyramidal neurons, which normally inhibit fear extinction. In order to test the effect of this altered medial prefrontal cortex (mPFC) balance on behavior, excitotoxic lesions of the IL and PL were performed, revealing that IL, but not PL lesions reproduce anxiety-related phenotypes. The relationship between IL, PL and anxiety/depressive-like behaviors was further explored using site-specific viral injections of activating cre-dependent hM3Dq DREADDs (designer receptors exclusively activated by designer drugs), which induce burst firing when activated by the otherwise inert drug CNO. This method allows for more precise probing of specific mPFC subregions to assess their contributions to affective alterations, without the confounding effects introduced by lesions. Together, our results suggest that increased 5-HT signaling during P2-11 alters adult mPFC function to increase anxiety and impair fear extinction, while also suggesting a dissociation between the roles of IL and PL in conditioned fear and unconditioned anxiety.

Disclosures: M.K. Caffrey: None. **T.J. Rebello:** None. **M.S. Ansorge:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.18/Z2

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

State of CT

Title: Acute ketamine administration alters dendritic spines in rat medial prefrontal cortex

Authors: *C. H. DUMAN, R. TERWILLIGER, R. S. DUMAN;
Dept. of Psychiatry, Yale Univ., New Haven, CT

Abstract: Major depression is a significant cause of disability, suffering and economic burden worldwide. While the utility of currently available antidepressant drug treatments is limited by the length of time required for therapeutic effectiveness and by treatment resistance in a significant proportion of patients, recent studies have shown that the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine can produce a rapid antidepressant response following acute treatment in humans and in animal models (1,4). The induction of synaptic spines after acute treatment is thought to be critical to the rapid antidepressant action of ketamine. This lab has documented increased synaptic protein levels and spine densities in rat prefrontal cortex following acute ketamine treatment, suggesting a synaptogenic action (3). The previous studies have focused on spines in dendritic fields of layer V pyramidal neurons, and the present study extends these findings and documents the effects of ketamine on dendritic spines of pyramidal neurons in layers II/II of rat prefrontal cortex. Pyramidal cells in fixed brain sections were intracellularly injected with Lucifer yellow under visual guidance following the method of Dumitriu et al., 2011 (2). Following dye filling, dendritic segments were imaged with confocal laser scanning microscopy and spine morphology was analyzed using NeuronStudio. Twenty four hours following acute, low-dose ketamine treatment, spine density was increased in apical dendrites of layer II/III pyramidal neurons in prefrontal cortex. This effect appears to be specific to the prelimbic region and was not apparent in neurons in the infralimbic cortex. We also found a region-specific increase in spine head diameters of layer II/III cells following ketamine treatment. These findings extend the evidence for cortical dendritic spines as important loci for structural and functional alterations associated with rapid antidepressant action. 1. Berman et al., 2000. Biol. Psychiat. 47:351. 2. Dumitriu et al., 2011. Nature Protocols. 6(9): 1391-1411. 3. Li et al., 2010. Science. 329:959-964. 4. Maeng et al., 2008. Biol. Psychait. 63(4):349-352.

Disclosures: C.H. Duman: None. **R. Terwilliger:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.19/Z3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant 1R21MH099562

NIMH Grant 5T32MH87004-4

NIMH Grant 5T32MH096678-02

Title: Sex differences in stress regulation of genome-wide transcriptional profiles in the mouse nucleus accumbens

Authors: *M. L. PFAU, I. PURUSHOTHAMAN, G. E. HODES, J. FENG, S. A. GOLDEN, H. M. CATES, M. FLANIGAN, L. SHEN, S. J. RUSSO;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Adult women are twice as likely as men to develop major depression, although the precise molecular mechanisms underlying sexual dimorphism in depression susceptibility are unknown. We have developed a stress paradigm--subchronic variable stress (SCVS)--to mimic these sex differences in mice. Female mice subjected to SCVS exhibit depression-like behavior after 6 days of stress exposure, whereas male mice exhibit this behavior only after chronic stress exposure (21-28 days, but not 6 days, of SCVS). Thus, this model is a valid recapitulation of the sexual dimorphism characteristic of the human population. We profiled sex differences in gene expression and transcriptional regulation in the mouse nucleus accumbens (NAc), an essential structure in the processing of reward and motivation, using next generation mRNA and small RNA sequencing. Both types of sequencing were performed on the same NAc tissue from intact male and female mice subjected to SCVS. Bioinformatic analysis was used to determine differential mRNA and microRNA (miR) expression patterns, predict miR targets, and identify biological pathway enrichment. We find very little overlap in stress-regulated mRNA and miR profiles between male and female mice. miR target and pathway analyses revealed evidence for sex differences in regulation of molecular processes related to stress vulnerability in males and females. Our results demonstrate that male and female mice initiate fundamentally different transcriptional responses to stress. These transcriptional profiles correlate with behavioral susceptibility or resilience to stress. Our findings provide insight into the molecular underpinnings of enhanced female susceptibility to stress.

Disclosures: **M.L. Pfau:** None. **I. Purushothaman:** None. **G.E. Hodes:** None. **J. Feng:** None. **S.A. Golden:** None. **H.M. Cates:** None. **M. Flanigan:** None. **L. Shen:** None. **S.J. Russo:** 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.; Janssen Pharmaceuticals.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.20/Z4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: The effects of Zoloft and Desyrel on crayfish behavior

Authors: G. M. PREZKOP, *R. F. WALDECK;
Biology/ Neurosci. Program, Univ. of Scranton, Scranton, PA

Abstract: Antidepressants are taken by millions of Americans. Among the most common antidepressants are selective serotonin reuptake inhibitors (SSRIs) such as Prozac, Zoloft, and Celexa. The effect of two antidepressants, Zoloft (an SSRI), and less common antidepressant, Desyrel (a serotonin antagonist and reuptake inhibitor-SARI), was tested on fight behavior in crayfish. Behavioral scores were obtained from fights to observe if there was an effect on the aggression of crayfish from pre-treatment to post-treatment and if one antidepressant was more effective than the other. Eleven female crayfish were used to collect behavioral data pre- and post-treatment with antidepressants. A modified behavioral scoring sheet (Mead, 2008) was used to score fighting (i.e. tail flip, retreat, boxing, etc.) and behavioral measurements were taken every ten seconds. Crayfish were given ventral injections over a period of ten days of the appropriate antidepressant and the control crayfish received saline injections. Doses given were 0.02 mL injections for each drug (Zoloft, Desyrel, and saline). Scores before treatment to after treatment showed a significant increase ($p=0.05$) in aggression of crayfish. The increase in aggression post-treatment can show that antidepressants have a risk of increasing aggression that can have an effect on humans currently taking these medications for treatment of depression symptoms. An increase in aggression in humans after taking these drugs can lead to not only an increase in aggression, but also thoughts or risk of suicide. These findings, through further research, can lead to a more effective drug for combating the symptoms of depression.

Disclosures: G.M. Prezkop: None. **R.F. Waldeck:** None.

Poster

808. Mood Disorders in Animal Models IV

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Program#/Poster#: 808.21/Z5

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH045481

NIH Grant MH093897

State of Connecticut

Title: Targeted knockdown of muscarinic acetylcholine receptors in the pre-frontal cortex to examine the rapid anti-depressant effects of scopolamine

Authors: *E. S. WOHLEB, K. T. OTA, A. NAVARRIA, A. E. LEPACK, J. M. DWYER, S. R. TAYLOR, M. R. PICCIOTTO, R. S. DUMAN;
Yale Univ., New Haven, CT

Abstract: Major depressive disorder (MDD) is a recurring psychiatric disorder that causes significant health and socioeconomic burdens. Although therapeutics to relieve depressive symptoms exist, they are limited in efficacy and many "treatment-resistant" patients receive no benefit from these drugs. Recent clinical reports revealed that the muscarinic acetylcholine receptor antagonist, scopolamine, caused rapid and persistent anti-depressant effects in individuals with MDD. Moreover, studies in our lab show that the anti-depressant behavioral effects of scopolamine were due to increased glutamate release, activation of mTOR signaling, and enhanced dendritic spine density in the medial pre-frontal cortex (mPFC). In fact, direct administration of scopolamine into the mPFC was sufficient to produce a rapid anti-depressant effect. These findings indicate that rapid anti-depressant effects caused by scopolamine are initiated through antagonism of muscarinic acetylcholine receptors in the mPFC. In addition, preliminary studies in the lab suggest that scopolamine exerts behavioral effects through blockade of M1-type muscarinic acetylcholine receptors (M1-AchR) in the mPFC. For instance, antagonists selective for M1-AchR activate mTOR signaling and promote anti-depressant effects comparable to scopolamine. This is pertinent because recent evidence suggests that M1-AchR are expressed primarily by glutamate-expressing (CamKII+) pyramidal neurons in the cortex. However, subsets of GABA-expressing (GAD67+) inhibitory neurons also express M1-AchR. We confirmed these results through immunohistology showing that M1-AchR co-localized with CamKII+ and GAD67+ neurons in the mPFC. Thus, the primary objective of these studies was to determine if the rapid anti-depressant effects of scopolamine can be modulated through

targeted knockdown of M1-AchR in CamKII+ or GAD67+ neurons in the mPFC. To examine the role of these different neuronal subsets we developed a viral construct that expresses a short-hairpin RNA targeting M1-AchR in a Cre-dependent manner. Using CamKII-Cre or GAD67-Cre mice we can effectively knockdown M1-AchR in these neuronal subsets. Further studies are aimed to examine the behavioral effects of M1-AchR knockdown in CamKII+ or GAD67+ neurons in the mPFC and will further define the cellular mechanisms underlying the rapid antidepressant actions of scopolamine. Supported by NIMH Grants MH045481 and MH093897, and the State of CT.

Disclosures: **E.S. Wohleb:** None. **K.T. Ota:** None. **A. Navarria:** None. **A.E. Lepack:** None. **J.M. Dwyer:** None. **S.R. Taylor:** None. **M.R. Picciotto:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 808.22/Z6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH grant P-50 MH-10028395

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unrestricted funds from the Children's Hospital of Philadelphia

Title: Capturing the complexity of depression from mood through molecules: Bioinformatic integration of behavioral, physiological, proteomic, morphometric and intracellular signaling datasets in a female monkey model of depression

Authors: *S. L. WILLARD[1], K. E. BORGMANN-WINTER[1,2], P. M. SLEIMAN[3], H. Y. WANG[4], C. A. SHIVELY[5], C. G. HAHN[1];
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Abstract: Depressive disorders are complex trait disorders with vastly heterogeneous manifestation as well as multi-faceted origins. One approach to addressing this is modeling depression in non-human primates, which may bring us closer to capturing and understanding the complexity of depression. Thus, we examined a matched set of adult female monkeys characterized for behavioral depression (N=8/grp) and evaluated the relationships between molecular and cellular perturbations of synapses and morphological, behavioral and endocrinological phenotypes. Depressive behavior and social interactions were observed over four years, during which numerous physiological assessments were conducted, including HPA-axis and ovarian function. The hippocampus was dissected at necropsy, and several aspects of depression neurobiology were investigated, from the whole structure down to the molecules. Specifically, we reported alterations in regional and layer volume, neuronal and glial cell counts, and synaptosomal protein and gene expression. Recently, we found altered composition of the synaptic proteome as measured via LC-SRM/MS of 200+ proteins in fractionated synaptic microdomains. In addition, we observed attenuated NMDA and heightened TrkB signaling in postmortem hippocampal tissue dissections from behaviorally depressed monkeys using an *ex vivo* stimulation method. For the bioinformatic integration of datasets, we generated correlation matrices of the proteomic data and individual phenotypic endpoints. This was done for each synaptic microdomain assessed, and fraction-specific proteomic patterns were visualized in heat maps following cluster analysis with dendogram-reordering based on Euclidean distance functions. Numerous correlations were observed between the proteomic data and 1) the morphometric and volume analyses, 2) depression, 3) social status, and 4) physiological characteristics of the monkey model. Cluster analyses revealed that functional groups of proteins clustered separately with morphometry and depression, and these clusters varied among synaptic microdomains. Preliminary correlational analyses between NMDA and TrkB signaling data and hippocampal structural measures suggest a very high degree of association between most variables. The relationships that emerged from this mood-to-molecule bioinformatic approach were not apparent prior to this integration of data. These complex relationships as observed in a nonhuman primate model of depression will enable us to unravel the complexity of depression in ways that have not been done before.

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Poster

808. Mood Disorders in Animal Models IV

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Program#/Poster#: 808.23/Z7

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: R01 MH080116

Title: Effects of altered developmental monoamine signaling on adult aggressive behaviors

Authors: *D. MAHADEVIA[1], C. M. TEIXEIRA[1], Q. YU[2], D. SURI[1], M. ANSORGE[1]; [1]Psychiatry, Columbia Univ., New York, NY; [2]Ctr. for Human Genet. Res., Massachusetts Gen. Hosp., Boston, MA

Abstract: Early-life alterations in the strength of monoamine signaling can impact adult behavior. We have identified a peri-adolescent (P22-P41) sensitive time window during which altered dopamine (DA) and serotonin (5-HT) signaling affect the development of circuitries underlying adult aggression in mice. Monoamine oxidase A (MAOA) and DA transporter (DAT) blockade during peri-adolescence increases adult aggressive behavior, and 5-HT transporter (5-HTT) blockade during the same time period reduces adult aggression. To refine this sensitive period, we tested the consequence of monoaminergic interference during narrower time-windows and found that increased DA signaling during P32-P41 is sufficient to increase adult aggression. Initial insight into the underlying circuit-based mechanism was provided by our finding that levels of aggression following peri-adolescent blockade of DAT, 5-HTT or MAOA correlate positively with locomotor response to an amphetamine challenge in adulthood. These data suggest that DAergic function is permanently altered, which is congruent with findings implicating DA hyperactivity in pathological adult aggression. Together these data indicate that increased P32-P41 DAergic signaling permanently alters DAergic function to enhance adult aggressive behavior. To directly assess the causal role of DA function in aggression we used *in vivo* optogenetics. We found that ChR2-based activation of VTA but not SNc DAergic neurons increases aggressive behavior, supporting the hypothesis that altered mesolimbic/mesocortical DAergic function underlies altered aggression. To further dissect the DA-associated circuitry underlying adult aggression, we optogenetically probe VTA-target regions. Together our data provide insight into a monoamine-sensitive period that determines the developmental trajectory of aggression and behavioral sensitivity to psychostimulants, which might ultimately aid improving prevention and treatment approaches for neuropsychiatric disorders.

Disclosures: D. Mahadevia: None. **C.M. Teixeira:** None. **Q. Yu:** None. **D. Suri:** None. **M. Ansorge:** None.

Poster

808. Mood Disorders in Animal Models IV

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH R37MH45481

NIMH R01MH93897

NIMH F32MH98513

NIGMS P30GM103328

State of Connecticut

Yale University

Title: BICC1 expression is elevated in depressed subjects and contributes to depressive behavior in rodents

Authors: *K. T. OTA[1], W. ANDRES[1], D. A. LEWIS[2], C. A. STOCKMEIER[3], R. S. DUMAN[1]; [1]Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; [2]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [3]Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Major depressive disorder (MDD) is a recurring psychiatric illness that affects up to 17% of the American population and can be severely debilitating by affecting sleep, work, social relationships, and appetite (Kessler et al., 2005). Studies to elucidate the pathophysiology of depression have recently included analysis of the genetic basis of MDD, focusing on single nucleotide polymorphisms (SNPs) that are associated with susceptibility to mood disorders. Consistent with this, a recent genome-wide association study (GWAS) identified two SNPs in the Bicaudal C homologue 1 (BICC1) gene showing suggestive significance for association in depressed patients compared to psychiatrically healthy controls (Lewis et al., 2010). BICC1 is an RNA-binding protein that plays a role in cytoskeletal organization and cell-to-cell communication (Mahone et al., 1995; Chicoine et al., 2007; Snee and Macdonald, 2009). Further, BICC1 can block Wnt signaling, a pathway implicated in the pathophysiology of depression (Voleti et al., 2011), via the uncoupling of disheveled-2 signaling from the canonical Wnt pathway (Maisonneuve et al., 2009). BICC1 is well-studied in the context of polycystic kidney disease (Guay-Woodford, 2003), but there have been few studies of BICC1 in brain. Here, we show that BICC1 mRNA is up regulated in the dorsolateral prefrontal cortex (dlPFC) and dentate gyrus (DG) of human postmortem MDD patients. We also show that BICC1 is increased in prefrontal cortex (PFC) and hippocampus in the rat chronic unpredictable stress (CUS) model of depression. Additionally, we show *in vivo* that a single acute anti-depressant

dose injection of ketamine leads to a rapid decrease of BICC1 mRNA, while *in vitro*, we show that this is likely due to activity-induced down-regulation of BICC1. Finally, we show that BICC1 knockdown in the hippocampus protects rats from CUS-induced anhedonia. Taken together, these findings identify a role for increased levels of BICC1 in the pathophysiology of depressive behavior.

Disclosures: **K.T. Ota:** None. **W. Andres:** None. **D.A. Lewis:** 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.; Bristol-Myers Squibb, Pfizer. F. Consulting Fees (e.g., advisory boards); Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, Sunovion. **C.A. Stockmeier:** None. **R.S. Duman:** None.

Poster

808. Mood Disorders in Animal Models IV

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Program#/Poster#: 808.25/Z9

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Antidepressant-like effects of ketamine in the Differential Reinforcement-of-Low-Rate (DRL) 72 sec operant procedure in mice

Authors: *M. A. FRIAR[1], T. M. HILLHOUSE[2], K. A. WEBSTER[2], J. H. PORTER[2]; [2]Psychology, [1]Virginia Commonwealth Univ., Richmond, VA

Abstract: Major depressive disorder (MDD) is the most common mood disorder in the United States affecting approximately 14% of Americans. Most preclinical procedures (e.g. forced swim test, chronic unpredictable stress, etc.) used for assessing antidepressant drugs utilize both rats and mice; however, the differential-reinforcement-of-low-rate (DRL) 72 sec operant procedure, which was established in the 1980s to selectively screen antidepressant drugs, has been used almost exclusively with rats. Recently, we reported that ketamine produces an antidepressant-like profile in the DRL 72 sec task with rats (Hillhouse & Porter 2014). However, the DRL 72 sec task has not been used in mice to evaluate the antidepressant-like effects of ketamine. In the DRL procedure animals are required to wait 72 sec between operant responses in order to receive a reinforcer. A drug demonstrates an antidepressant-like profile when the number of reinforcers is increased and the number of responses is decreased. The present study first established the DRL 72 sec task in 2 groups of C57BL/6 mice. In one group the response operandum was a nose

poke response and in a second group the response operandum was a lever press response (as used in DRL studies with rats). In the lever press group, ketamine (32 mg/kg), the tricyclic antidepressant imipramine (32 mg/kg), and the SSRI fluoxetine (32 mg/kg) significantly increased the number of reinforcers and decreased responses, producing an antidepressant-like profile similar to that previously reported in rats. In contrast, ketamine did not produce any significant changes in the number of reinforcers or responses in the nose poke group. While imipramine and fluoxetine at 32 mg/kg did significantly increase reinforcers in the nose poke group, there was no significant change in the number of responses. The higher affinity noncompetitive NMDA antagonist MK-801 (0.1 mg/kg) produced a significant decrease in the number of reinforcers and a significant increase in responses for lever press and nose poke - a psychostimulant-like profile that is consistent with DRL studies using rats. These results demonstrated that the DRL 72 sec operant schedule is a valid procedure for assessing antidepressant-like effects of drugs in mice, but only when a lever press response is used as the operandum. The antidepressant-like effects of ketamine may involve non-NMDA mechanisms as MK-801 did not produce an antidepressant-like profile.

Disclosures: **M.A. Friar:** None. **T.M. Hillhouse:** None. **K.A. Webster:** None. **J.H. Porter:** None.

Poster

808. Mood Disorders in Animal Models IV

Location: Halls A-C

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Program#/Poster#: 808.26/Z10

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CAPES-MEC-Brazil / Grant AUX-PE-PROCAD 657/2008

Title: Mood stabilizers regulate cytokine levels but have no effect on memory impairment and BDNF reduction induced by maternal deprivation in rats

Authors: *M. MARTINS DE LIMA[1], R. M. C. PINHEIRO[1], B. C. D. PORTAL[1], S. B. BUSATO[1], L. FALAVIGNA[1], R. D. P. FERREIRA[1], A. C. PAZ[2], B. W. AGUIAR[2], F. P. KAPCZINSKI[2], N. SCHRÖDER[1];
[1]Pontifical Catholic Univ. of Rio Grande do Su, Porto Alegre, Brazil; [2]Federal Univ. of Rio Grande do Sul, Porto Alegre, Brazil

Abstract: Exposure to stressful events early in life may increase the susceptibility to the onset of psychiatric disorders that often share cognitive impairments as a common component in adult life. Brain-derived neurotrophic factor (BDNF) levels have been shown to be decreased in the serum of patients with psychiatric disorders. On the other hand, inflammatory cytokines, such as interleukins (IL) and tumor necrosis factor-α (TNF-α), have been shown to be increased in the serum of these patients. Thus, the aim of the present study was to investigate the effects of maternal deprivation on cognition and on the levels of BDNF and IL-10, and TNF-α in the hippocampus and prefrontal cortex of adult rats. We also evaluated the potential ameliorating properties of valproic acid and topiramate (two anticonvulsants that also act as mood stabilizers) on memory impairment and BDNF and cytokines changes associated with maternal deprivation. The results indicated that, in addition to inducing memory impairment, maternal deprivation decreased hippocampal levels of BDNF and increased the levels of IL-10 in the hippocampus, and TNF-α in the hippocampus and in the cortex, in adult life. Neither valproic acid nor topiramate were able to ameliorate memory impairment or the reduction in BDNF levels induced by maternal separation. The highest dose of topiramate was able to reduce IL-10 in the hippocampus and TNF-α in the prefrontal cortex, while valproate only reduced IL-10 levels in the hippocampus. These findings may help to clarify the underlying mechanisms associated with alterations observed in adult life induced by early stressful events, and for the development of novel therapeutic strategies.

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Poster

808. Mood Disorders in Animal Models IV

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Program#/Poster#: 808.27/Z11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH RO1 MH081211

Title: Diffusion Tensor Imaging (DTI) in rats suggests that the posterior thalamus and prefrontal cortex are targets of Chronic Unpredictable Stress (CUS)

Authors: *C. L. KISELYCZNYK, M. BANASR, C. ABDALLAH, D. COMEN, F. HYDER, G. SANACORA;
Dept. of Psychiatry, Yale Univ., New Haven, CT

Abstract: In the investigation of stress-sensitive disorders such as depression, the rodent chronic unpredictable stress (CUS) model is useful in determining stress-induced molecular changes potentially underlying psychiatric disorders. CUS studies have uncovered stress-induced reductions in dendritic complexity and synaptic- and glial-related proteins in regions implicated in depression such as the prefrontal cortex (PFC). As changes in cellular morphology could reflect alterations in cell density and extracellular space that potentially affect water diffusion, measuring the ability of water to diffuse in these regions through Diffusion Tensor Imaging (DTI) is one potential method to observe stress-induced changes in both preclinical and clinical settings. Here we used DTI after CUS in rats to measure the functional anisotropy (FA) and average diffusion coefficient (ADC) throughout the brain. In a separate cohort of animals, we performed western blot analysis in the regions implicated by DTI to measure alterations in markers previously associated with CUS and stress, such as synaptic proteins, glial markers, and markers of the extracellular matrix. The most striking changes were observed in measurements of ADC, with stress-induced increases in water diffusion observed in the ventral medial prefrontal cortex (PFC), but also in the posterior thalamus, a region not typically associated with chronic stress. Subsequent western blot analysis of the thalamus replicated stress-induced alterations previously observed in the PFC, including reductions in the glial-related marker GFAP, and the synaptic marker PSD95, with a trend for reduction of GluR1. It is unclear if these stress-induced molecular changes are selective to regions indicated by our DTI findings and more work is needed to directly tie molecular changes to the alterations in ADC observed after stress. Ultimately, our current work demonstrates the use of DTI to discover new regions previously not known to be involved in chronic stress, and produces the novel finding that the posterior thalamus shows stress-induced alterations in glial- and synaptic-related proteins.

Disclosures: C.L. Kiselycznyk: None. **M. Banasr:** None. **C. Abdallah:** None. **D. Comen:** None. **F. Hyder:** None. **G. Sanacora:** None.

Poster

808. Mood Disorders in Animal Models IV

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH-R01-MH0811,

NARSAD

State of CT

Title: Cortical GFAP astrocyte-specific ablation induces depressive-like and anxiety-like behaviors similar to chronic stressCortical GFAP astrocyte-specific ablation induces depressive-like and anxiety-like behaviors similar to chronic stress

Authors: *M. BANASR, M. XU, A. LEPACK, C. KISELYCZNYK, J. ZHANG, S. WILBER, G. SANACORA, C. PITTENGER, R. DUMAN;
Dept Psychiatry, Yale Univ., NEW HAVEN, CT

Abstract: Growing evidence implicates glial dysfunction in the pathophysiology of depression. Astrocyte number is reduced in the cortex of patients with major depression and in rodent models of depression. We previously reported that glial ablation in the medial prefrontal cortex (mPFC) using local infusion of a gliotoxin induces behavioral deficits similar to those produced by chronic stress in rat, including anhedonia, anxiety and helplessness. However, the contribution of glial subtypes in the development of depressive-like symptoms remains to be characterized. To address this question, we engineered targeted ablation of GFAP+ glial cells in the mPFC in mice. We used the cre/loxP system and a cre-activated virus to express the diphtheria toxin (DT) receptor (DTR) specifically in GFAP+ cells of the mPF, making these cells sensitive to DT. Three weeks after infusion of the virus (iDTR) into the mPFC, animals were injected with saline or DT at 3 different doses (0.1, 5, 20 ug/kg) i.p. once daily for 4 days. Sucrose consumption, which is reduced in depression rodent models, was measured on each day. GFAPcre+iDTR mice injected with 20ug/kg of DT showed a significant decrease in sucrose consumption on both days 2 and 3; GFAPcre+iDTR injected with 5 µg/kg DT also showed reduced sucrose consumption on day 3. Both groups still showed decreased sucrose consumption on D8 (4 days after the last injection of DT), but sucrose consumption levels returned to baseline by day 14. Similar effects were observed in the forced swim test: administration of DT (20ug/kg) to GFAPcre+iDTR mice showed significantly increased immobility on day 8, but not on day 14. GFAPcre+iDTR animals treated with DT also exhibited enhanced novelty-induced hypophagia test and a trend towards decreased time in the center in an open field, both of which are indicative of increased anxiety. We also demonstrated that GFAP cell ablation in striatum has no effect in these behavioral tests. Our results demonstrate that selective ablation of GFAP+ cells in the mPFC rapidly induces anhedonia-like and anxiety-like deficits that persist for at least 8 days. Our results suggest that the astrocytic loss observed in human depression is not just a consequence of depression but contributes to core symptoms. These studies also demonstrate the feasibility of our targeted ablation approach for the study of the contribution of specific glial (or neuronal) subtypes in PFC function.

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Poster

808. Mood Disorders in Animal Models IV

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH045481

NIMH Grant MH093897

State of CT

Title: Differential sensitization of microglia and peripheral blood mononuclear cells following short-term and chronic unpredictable stress

Authors: *T. C. FRANKLIN, E. S. WOHLEB, R. S. DUMAN;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Exposure to acute and chronic stress has been shown to alter innate immune function leading to enhanced responses to subsequent stimuli. It has recently been demonstrated that sub-chronic and chronic unpredictable stress have contrasting effects on innate immune cells, specifically microglia, resulting in polar cell number and morphological changes. However, the sensitivity of innate immune cells to subsequent stimuli has not yet been investigated following short-term (3 d) and chronic (28 d) unpredictable stress (SUS and CUS, respectively). In this study, we identified stress induced alterations in the expression of inflammatory factors that may contribute to priming of peripheral blood mononuclear cells (PBMC) and microglial cells isolated from the prefrontal cortex (PFC) or hippocampus (HPC), two regions known to be susceptible to stress induced alterations. We demonstrate for the first time a differential effect of SUS and CUS on the function of PBMC and microglial cells following an immunological challenge. The mRNA expression level of several inflammatory factors including IL1b, TNFa, HMGB1, TLR2, TLR4 and RAGE were significantly increased in PBMC and PFC microglial cells following SUS compared to control. Moreover, LPS administration (100ug/kg i.p.) significantly enhanced the expression of these factors revealing a robust priming effect of SUS. In contrast, the mRNA levels of many of these factors in PBMC cells were significantly reduced

by CUS. LPS exposure did not increase the expression of these factors in PFC microglial cells suggesting an immunosuppressive effect of CUS. SUS and CUS did not significantly alter the expression of these genes in HPC microglial cells. Our data suggest that stress-induced sensitization of PBMC and microglial cells following unpredictable stress occur in a differential manner depending on the duration of the stress paradigm. Supported by NIMH Grants MH045481 and MH093897, and the State of CT.

Disclosures: T.C. Franklin: None. **E.S. Wohleb:** None. **R.S. Duman:** None.

Poster

809. Amphetamine Reinforcement II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 809.01/Z14

Topic: C.17. Drugs of Abuse and Addiction

Support: This work is supported Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

Title: Methamphetamine produces increased neuropeptide gene expression by TET-dependent DNA methylation/hydroxymethylation events in the rat nucleus accumbens

Authors: *S. JAYANTHI[1], B. GONZALEZ[2], P. WONGPRAYOON[3], M. T. MCCOY[1], B. LADENHEIM[1], J. L. CADET[1];
[1]Mol. Neuropsychiatry Br., NIDA-IRP, BALTIMORE, MD; [2]Inst. de Investigaciones Farmacológicas, Buenos Aires, Argentina; [3]Res. Ctr. for Neurosci., Inst. of Mol. Biosciences, Mahidol Univ., Nakornpathom, Thailand

Abstract: Methamphetamine (METH) addiction is a relapsing neuropsychiatric disorder that is secondary, in part, to neuroadaptations within neurocircuitries that mediate stress responses. Our recent microarray and quantitative PCR (qPCR) analyses had revealed that an injection of METH (10mg/kg) upregulated several stress-related gene markers measured one month later using tissues from the rat nucleus accumbens. These genes included corticotropin-releasing hormone (*Crh/ Crf*), vasopressin (*Avp*) and cocaine-amphetamine regulated transcription factor (*Cart*). The long-lasting increases in *Crh*, *Cart*, and *Avp* mRNA expression had suggested that METH exposure might produce prolonged activation of the endogenous stress system via epigenetic modifications that have been identified as important mediators of long-lasting memories. For example, methylation of cytosine (5mC) bases in DNA is known to cause gene

silencing whereas reversible conversion of 5mC to its hydroxylated form, 5hmC, by the Ten-eleven translocation (Tet) family of enzymes can increase gene transcription. To assess if METH treatment could cause changes in DNA methylation, we used methylated DNA immunoprecipitation (MeDIP) followed by qPCR to measure DNA methylation at CpG sites around the TSSs of *Crh, Avp,* and *Car* genes. We found that METH significantly decreased cytosine methylation at CpG-rich sites near *Crh* and *Avp* TSSs but caused no significant changes near the *Cart* TSS. In addition, we used hydroxymethylated DNA immunoprecipitation (hMeDIP)-qPCR to test potential roles of DNA hydroxymethylation in METH-induced neuropeptide upregulation. There was significant increased DNA hydroxymethylation at the CpG-rich sequences located near *Crh, Avp,* and *Cart* TSSs. Because TET enzymes catalyze DNA hydroxymethylation, we conducted chromatin immunoprecipitation (ChIP) assay using TET1 and TET2 antibodies and found significant METH-induced increase in TET1 binding on the *Crh* promoter sequence. In contrast, we found no significant changes in TET1 recruitment on *Avp* and *Cart* promoter sequences. There were also no changes in TET2 binding on either *Crh*, *Avp* or *Cart* promoter sequences. Together, these results show a potential role of Tet1 in mediating METH-induced up-regulation of *Crh* mRNA expression in the nucleus accumbens. These observations hint to the possibility of using TET1 inhibitors to alleviate stress-induced neurobiological alterations in METH addicts. Acknowledgement: This work is supported Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

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Poster

809. Amphetamine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: National Health Research Institutes, Taiwan

Title: Methamphetamine induces a rapid increase of intracellular Ca++ levels in neurons overexpressing GCaMP5

Authors: *Y. WANG[1], K.-J. WU[1], E. K. BAE[1], M.-J. HSU[1], C. T. RICHIE[2], B. K. HARVEY[2], S.-J. YU[1];

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Abstract: In this study, methamphetamine (Meth) and glutamate (Glu) -mediated intracellular Ca++ (Ca++i) signals were examined in real time in primary cortical neurons overexpressing an intracellular Ca++ probe, GCaMP5, by AAV serotype 1. Binding of Ca++ to gCaMP increased green fluorescence intensity in cells. Both Meth and Glu induced a rapid increase in Ca++i, which was blocked by MK801, suggesting Meth enhanced Ca++i through Glu receptor in neurons. The Meth-mediated Ca++ signal was also blocked by Mg++, low Ca++, or the L-type Ca++ channel inhibitor nifedipine. The ryanodine receptor (RyR) inhibitor dantrolene did not alter the initial Ca++ influx but partially reduced the peak of Ca++i. These data suggest Meth enhanced Ca++ influx through membrane Ca++ channels, which then triggered release of Ca++ from the endoplasmic reticulum in the cytosol. AAV-GCaMP5 was also injected to the parietal cortex of adult rats. Administration of Meth enhanced fluorescence in the ipsilateral cortex. Using immunohistochemistry, Meth -induced green fluorescence was found in the NeuN containing cells in cortex, suggesting Meth increased Ca++ in neurons *in vivo*. In conclusion, we have used *in vitro* and *in vivo* techniques to demonstrate a rapid increase of Ca++i by Meth in cortical neurons through overexpression of GCaMP5. Since Meth induces behavioral responses and neurotoxicity through Ca++i, modulation of Ca++i may be useful to reduce Meth related reactions.

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Poster

809. Amphetamine Reinforcement II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 809.03/Z16

Topic: C.17. Drugs of Abuse and Addiction

Support: KFDA Grant 13182KFDA656

KFDA Grant 14182MFDS979

Title: Pentedrone leads to psychological dependence via dopaminergic system

Authors: J.-Y. HWANG, S.-H. KWON, S.-I. HONG, Y.-H. JUNG, S.-X. MA, J.-S. KIM, J.-Y. SEO, J.-H. LEE, S.-Y. LEE, *C.-G. JANG;
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Abstract: Cathinone derivatives are new recreational drugs known to produce psychostimulant effects. Addictive potential of cathinone derivatives has not been widely studied. Here, we investigated the addictive potential of pentedrone, a kind of cathinone derivatives, in conditioned place preference (CPP). As a result, pentedrone 3 and 10 mg/kg showed significant increase of CPP in mice. Also, acute administration of pentedrone enhanced locomotor activity and apomorphine-induced climbing behavior in a dose-dependent manner. However, chronic administration of pentedrone did not induce behavioral sensitization. Furthermore, in order to confirm the involvement of dopamine in the addictive property of pentedrone, we also carried out RT-PCR and Western blot. We revealed that pentedrone decreased tyrosine hydroxylase mRNA level and increased dopamine transporter, D1 receptor, D2 receptor mRNA levels and phosphorylation of CREB in PC-12 cells. These data suggest that the psychological dependence induced by pentedrone may be due to the effect of pentedrone on the dopaminergic system.

Disclosures: J. Hwang: None. **S. Kwon:** None. **S. Hong:** None. **Y. Jung:** None. **S. Ma:** None. **J. Kim:** None. **J. Seo:** None. **J. Lee:** None. **C. Jang:** None. **S. Lee:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA036328

Title: Decrease in dopamine neuron activity following acute amphetamine withdrawal is mediated by the BLA and reversed by ketamine

Authors: *P. BELUJON, A. A. GRACE;
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Abstract: The negative emotional state described after drug use often drives an individual to subsequent use, leading to abuse. For drugs such as amphetamine, the increase of dopamine (DA) release from neurons in the ventral tegmental area (VTA) facilitates reward-driven

behaviors and therefore is critical for their acute reinforcing actions. However, this positive affect is followed by a negative emotional state each time a drug is taken, including the first time. Anhedonia has been repetitively associated with dysfunction in the DA system; this negative state drives additional drug intake which we propose is due to a compensatory decrease in DA neuron activity. In the chronic mild stress and the learned helplessness models of depression, the decrease in DA activity can be reversed by prior inactivation of the basolateral amygdala (BLA), and prior injection of ketamine, respectively. Using *in vivo* extracellular single-unit recordings from identified DA neurons from the VTA in chloral hydrate-anaesthetized male rats, we found that an acute dose of amphetamine (2 mg/kg) had no effect on DA activity measured one hour after amphetamine injection; however there was a decrease specifically in tonic DA neuron activity 18 hours following an acute dose of amphetamine. This response partially reverses after 48 hours, meaning that the impact of acute amphetamine withdrawal on dopamine neuron activity is transient. Moreover, either ketamine administration or BLA inactivation one hour prior to recording circumvents the decrease in DA neuron tonic firing that we propose underlies this negative withdrawal state. These data suggest that the negative state, due to a DA deficit, could be reversed by ketamine administration, which would circumvent the seeking and taking of more drugs after a single use of amphetamine.

Disclosures: P. Belujon: None. **A.A. Grace:** None.

Poster

809. Amphetamine Reinforcement II

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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: Long-term effects of a single use of methamphetamine administration on the expression of histone deacetylases (HDACs) in the rat nucleus accumbens

Authors: *M. T. MCCOY, S. JAYANTHI, B. LADENHEIM, J. CADET;
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Abstract: Methamphetamine (METH) is an illicit psychostimulant that is abused worldwide. The cellular and molecular mechanisms involved in the long-term effects of taking a single dose

of the drug are not known. In fact, this pattern of drug injection might be associated with plastic changes in the transcriptional machinery in the brain. These might occur via epigenetic alterations that might include changes in the expression of histone deacetylases (HDACs) that catalyze the removal of acetyl moieties from histones. To test this idea, rats were given a single injection of METH (10 mg/kg) or saline and the nucleus accumbens (NAc) was dissected one month later. HDAC expression was measured by quantitative PCR analysis. We found that the METH injection caused decreased mRNA levels of HDAC2, HDAC3, HDAC7, Sirt1, Sirt2, Sirt3, Sirt4, and Sirt7. These changes in HDAC expression may be responsible for the large-scale changes in gene expression observed in animals similarly treated and for METH-induced molecular neuroadaptations in the rat brain. 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. **S. Jayanthi:** None. **B. Ladenheim:** None. **J. Cadet:** None.

Poster

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Program#/Poster#: 809.06/Z19

Topic: C.17. Drugs of Abuse and Addiction

Support: Startup

Title: Egr3 regulates mitochondrial biogenesis genes in nucleus accumbens neuron subtypes in cocaine abuse

Authors: *R. CHANDRA[1], T. C. FRANCIS[1], A. AMGALAN[1], L. JENSEN[1], P. KONKALMATT[2], A. GANCARZ[3], S. A. GOLDEN[4], G. TURECKI[5], S. J. RUSSO[4], D. M. DIETZ[3], M. K. LOBO[1];
[1]Anat. and Neurobio., [2]Div. of Nephrology, Univ. of Maryland, Baltimore, Baltimore, MD; [3]Dept. of Pharmacol. and Toxicology and Inst. on Addictions, Univ. of Buffalo, Buffalo, NY; [4]Fishberg Dept. of Neurosci., Mount Sinai Sch. of Med., New York, NY; [5]Depressive Disorders Program, Douglas Mental Hlth. Univ. Inst. and McGill Univ., Montréal, QC, Canada

Abstract: Previous studies demonstrate that the two projection neuron subtypes in nucleus accumbens (NAc), the medium spiny neuron (MSN) subtypes have opposite rewarding and reinforcing roles in drugs abuse. The two MSN subtypes are differentiated based on their

enrichment of genes including dopamine 1 (D1) receptors or dopamine 2 (D2) receptors. To provide insight into the molecular mechanisms occurring in the D1-MSN vs. D2-MSN subtypes, after chronic cocaine exposure, we examine the early growth response 3 (Egr3), a transcription factor. Egr3 is a known target of BDNF and dopamine signaling, two critical signaling pathways in drug abuse. Using Cre-inducible Ribotag mice combined with D1-Cre and D2-Cre mouse lines, we are able to isolate ribosome-associated RNA from both MSN subtypes after 7 days of cocaine (20mg/kg, i.p.). We find that that Egr3 is increased in D1-MSNs and decreased in D2-MSNs in this condition. We used a Cre-inducible adenoassociated virus (AAV) to overexpress Egr3 in NAc MSN subtypes in D1-Cre and D2-Cre mice. We observe that Egr3 overexpression in D1-MSNs enhances behavioral responses in cocaine conditioned place preference and cocaine-induced locomotion while overexpression in D2-MSNs blunts these behaviors. We next investigated target genes of Egr3 and demonstrate, with chromatin immunoprecipitation (ChIP), that Egr3 binding is increased on promoters of mitochondrial biogenesis genes after chronic cocaine. We find many of these mitochondrial biogenesis genes upregulated in the NAc after chronic cocaine conditions including cocaine self-administration (10 days, 20mg/kg, i.v.) and in postmortem NAc of human cocaine dependent individuals. When we examine ribosome-associated RNA in MSN subtypes, we find that the increase in mitochondrial biogenesis genes occurs in D1-MSNs while these genes are decreased in D2-MSNs after chronic cocaine. We are currently exploring the role of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), an Egr3 regulated mitochondrial biogenesis transcriptional activator, in MSN subtypes in cocaine-mediated behaviors. Our studies implicate a novel role for Egr3 in mediating energy homeostasis, through mitochondrial biogenesis, in MSN subtypes in cocaine abuse.

Disclosures: **R. Chandra:** None. **T.C. Francis:** None. **A. Amgalan:** None. **L. Jensen:** None. **P. Konkalmatt:** None. **A. Gancarz:** None. **S.A. Golden:** None. **G. Turecki:** None. **S.J. Russo:** None. **D.M. Dietz:** None. **M.K. Lobo:** None.

Poster

809. Amphetamine Reinforcement II

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Program#/Poster#: 809.07/Z20

Topic: C.17. Drugs of Abuse and Addiction

Title: Cocaine-mediated epigenetic adaptations in the ventral pallidum

Authors: M. ENGELN[1], J. LENZ[1], R. CHANDRA[1], A. M. GANCARZ[2], T. FRANCIS[1], D. M. DIETZ[2], *M. LOBO[1];
[1]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Dept. of Pharmacol. & Toxicology, State Univ. of New York at Buffalo, Buffalo, NY

Abstract: Cocaine exposure has profound effects on chromatin remodeling in the nucleus accumbens (NAc). However, there is no information about cocaine-induced epigenetic alterations in the ventral pallidum (VP), a target of both NAc projection medium spiny neuron (MSN) subtypes. In this study we investigate gene expression changes in chromatin remodeling enzymes in the VP in two models of chronic exposure to cocaine. First, we collected VP tissue in mice that received 7 days of cocaine (i.p., 20mg/kg) or in rats after 10 days of cocaine (i.v., 20mg/kg) self-administration. We performed quantitative RT-PCR on cDNA from VP for chromatin remodeling enzymes, including the histone-lysine N-methyltransferase Set domain (Setd) proteins and histone deacetylases (HDACs) to gain insight into cocaine-induced molecular adaptations in the VP. Both repeated cocaine exposure and cocaine self-administration induced alterations in Setd genes such as Setd2, Setd3, and Setd7 in the VP. Interestingly, in contrast to the NAc literature, HDAC5 expression was increased in the VP after both 7 days of cocaine exposure and 10 days of cocaine self-administration. Finally, we are exploring the effects of NAc MSN inputs on these molecular adaptations in the VP by using bidirectional optogenetic control of dopamine receptor 1 or 2 MSN subtypes. Using Cre-inducible viruses combined with D1-Cre and D2-Cre mice we can inhibit eNpHR3.0 expressing D1-MSNs with green (532nm) light or activate ChR2(H134) expressing D2-MSNs with blue (473nm) light to blunt cocaine-induced locomotion. Preliminary studies demonstrate that D1-MSN inhibition abolishes the cocaine-mediated induction of Setd2, Setd3 and Setd7 in the VP. Our findings explore the currently unknown molecular role of the VP in cocaine abuse and provide information on the NAc-VP circuit specific regulation of epigenetic markers in VP by cocaine.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: KAKENHI (20375489)

KAKENHI (20659177)

KAKENHI (23591671)

KAKENHI (26461716)

Title: NrCAM regulating neural systems in addiction vulnerability

Authors: *H. ISHIGURO[1,2], K. TABATA[1], T. SAKURAI[3], M. GRUMET[4], E. ONAIVI[2], G. UHL[5], N. MOTOHASHI[1];
[1]Univ. of Yamanashi, Chuo, Yamanashi, Japan; [2]William Paterson Univ., Wayne, NJ; [3]Kyoto University, Med. Innovation Ctr., Kyoto, Japan; [4]W.M. Keck Ctr. for Collaborative Neuroscience, Rutgers Univ., Piscataway, NJ; [5]NIDA-IRP/NIH, Baltimore, MD

Abstract: We have previously shown that genetic variations associated with decreased NrCAM expression in brain is protective against addiction vulnerability in humans and that Nrcam knockout mice do not develop conditioned place preferences for illegal drugs or alcohol. In order to gain insight into NrCAM involvement in addiction vulnerability, which may involve specific neural circuits underlying behavioral characteristics relevant to addiction, we evaluated several behavioral phenotypes in Nrcam knockout mice. NrCAM works in substance abuse including alcoholism vulnerability, possibly through its effects on behavioral traits that may affect addiction vulnerability, including novelty seeking, obsessive compulsion and responses to aversive or anxiety-provoking stimuli. In order to prove glutamate and GABAergic homeostasis hypothesis of addiction specific neural circuits, we analyzed expression patterns of glutamatergic and GABAergic molecules regulated by Nrcam and methamphetamine (MAP) treatment in midbrain of Nrcam knockout mice, using micro-array gene expression analysis in comparison between wild and heterozygote genotypes, and between treatment with saline and MAP. Glutaminase appears to be reduced in low expression of NrCAM tissue in human and mouse. An inhibitor of the enzyme, PLG, treatment produced, at least, some of the phenotypes of mice shown in alcohol preference and in anxiety-like behavior. Further, metabotropic glutamate receptor 2 appears to be reduced in MAP treated Nrcam heterozygote mice, although any difference of its expression was found between genotypes with saline treatment. GABAergic molecules are also detected differences in their expression. Thus, NrCAM could affect addiction-related behaviors via at least partially modulation of some glutamatargic and GABAergic pathways and neural function in brain.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NiFDS14182마약안979

Title: Transcriptional changes induced by amphetamine addiction in the striatum of spontaneously hypertensive rats (SHR), an animal model of attention-deficit/hyperactivity disorder (ADHD)

Authors: *J. CHEONG[1], I. DELA PEÑA[1], J. DE LA PEÑA[1], I. DELA PEÑA[1], M. NOH[2]; [1]Dept Pharm., Sahmyook Univ., Seoul, Korea, Republic of; [2]Col. of Pharmacy, Seoul Natl. Univ., Natural Products Res. Inst., Seoul, Korea, Republic of

Abstract: Attention-deficit/hyperactivity disorder (ADHD), the most commonly diagnosed neurobehavioral disorder of childhood, is usually treated with psychostimulants (e.g. amphetamine). Little is known about the neuronal correlates associated with long-term amphetamine use or abuse in individuals with ADHD. Of all ADHD animal models, the spontaneously hypertensive rat (SHR) is the most validated and widely used. In this study, we evaluated striatal transcriptomes in amphetamine-addicted SHR. Addiction was induced by repeated amphetamine pretreatment (5 mg/kg, i.p. for 7 days [twice daily]). The striatum of rats which showed conditioned place preference to and self-administration of amphetamine were analyzed. Genome-wide transcriptional analysis revealed an mRNA increase in 55 genes (>1.65-fold increase) while 17 genes were downregulated (<0.6-fold) in the striatum of SHR in response to amphetamine reinforcement. These differentially expressed genes included transcription factor-encoding genes (e.g. Cebpb, Per2), genes associated with angiogenesis (e.g. Kdr, Klf5), cell adhesion (e.g. Col11a1, Ctgf), apoptosis (e.g. Nfkbia, Perp) and neuronal development (e.g. Egr2, Nr4a3). Therefore, amphetamine regulates the expression of several genes in the striatum of SHR. These transcripts may mediate amphetamine-induced behavioral outcomes and could serve as candidate genes for amphetamine-abuse alterations in the brains of individuals with ADHD.

Disclosures: J. Cheong: None. **I. dela Peña:** None. **J. de la Peña:** None. **I. dela Peña:** None. **M. Noh:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA031883

NIH Grant DA19447

Title: Mephedrone alters basal ganglia and limbic dynorphin systems

Authors: *A. E. FLECKENSTEIN[1], A. J. HOONAKKER[2,3], G. R. HANSON[2,3], C. L. GERMAN[2];
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Abstract: Mephedrone (4-methymethcathinone) is a synthetic cathinone designer drug that disrupts central nervous system (CNS) dopamine (DA) signaling. Numerous neuropeptide systems reciprocally interact with dopaminergic neurons to provide regulatory counterbalance and are likely dysregulated by mephedrone and aberrant DA activity. Endogenous opioid neuropeptides are highly concentrated within dopaminergic CNS regions and involved in facilitating the rewarding and aversive properties associated with drug consumption. Dynorphin, an opioid neuropeptide and kappa receptor agonist, signals within the basal ganglia and limbic systems to cause dysphoria and aversion to drug consumption, and is affected by numerous psychostimulants. This work evaluated how mephedrone altered basal ganglia and limbic system dynorphin content, and the potential role of DA signaling in these changes. Repeated mephedrone administrations (4 x 25 mg/kg/injection, 2-h intervals) increased dynorphin content within various regions of the striatum and globus pallidus, while decreasing dynorphin content within the frontal cortex. Pre-treatment with D1-like (SCH-23380) or D2-like (eticlopride) antagonists blocked mephedrone-induced changes in dynorphin content, indicating increased dynorphin is a due to excess DA signaling at these receptors. The potential consequences of mephedrone-induced dynorphin changes in behavior and drug consumption will be discussed.

Disclosures: A.E. Fleckenstein: None. **A.J. Hoonakker:** None. **G.R. Hanson:** None. **C.L. German:** None.

Poster

809. Amphetamine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: GM64783

Title: Methylphenidate-induced ultrasonic vocalizations in adolescent and adult rats

Authors: *A. BINETTE, A. ROOS, C. G. GONZALEZ, K. A. TRUJILLO;
California State Univ. San Marcos, San Marcos, CA

Abstract: Methylphenidate (Ritalin) is a common treatment for children, adolescents and adults afflicted with Attention Deficit Hyperactivity Disorder (ADHD). There is growing concern about how methylphenidate affects the developing adolescent brain, and if there are any differences in response to the drug between adolescents and adults. The present study examined behavioral differences between adolescent and adult rats in response to methylphenidate. To assess behavior, ultrasonic vocalizations (USVs) of adolescent rats (30 days of age) and adult rats (60 days of age) were recorded following treatment with saline or methylphenidate (5 mg/kg). In addition, the animals were tested 10 and 20 days later. Based on previous work in our laboratory with other psychostimulants, it was hypothesized that methylphenidate would induce increases in complex 50 kHz USVs in rats and that methylphenidate-treated adult rats would emit a greater number of complex 50 kHz calls than methylphenidate-treated adolescent rats. Although we did not find any significant differences in the overall number of complex 50 kHz calls emitted between the methylphenidate-treated adolescent and adult groups, there were very interesting trends. Methylphenidate-treated adolescents showed a greater number of other 50 kHz and complex 50 kHz USVs during the first half the session. Later in the session these trends reversed, and adults showed greater methylphenidate-induced increases in complex 50 kHz and other 50 kHz USVs. As the adolescent rats increased in age, they transitioned to the adult pattern of response. Although the lack of statistical differences does not allow us to arrive at strong conclusions, the results point toward differences between adolescents and adults in response to methylphenidate. We are currently examining a higher dose of methylphenidate to determine if significant differences will emerge, and to explore the dose-dependency of these effects.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NSF Grant HRD1302873

Title: Adolescents show a reduced behavioral response to methamphetamine and dextroamphetamine when compared to adults

Authors: *V. ESPINOZA, C. Y. HELLER, A. ROCHA, K. A. TRUJILLO;
California State Univ. San Marcos, San Marcos, CA

Abstract: Methamphetamine (METH) and Dextroamphetamine (D-AMPH) are psychomotor stimulants and common drugs of abuse. According to the Monitoring the Future Survey, amphetamines are second to marijuana in use by 12th graders, and among the highest used in 8th and 10th graders; thus, they are very significantly abused by adolescents. This is important because there is significant evidence linking initiation of drug use during adolescence to the formation of drug addiction. Despite this, relatively little is known about the effects of METH and D-AMPH in adolescents when compared to adults. Research to date suggests that adolescents show a reduced response when compared to adults. In this study, the locomotor response to METH and D-AMPH was examined in adolescent (30 days of age at the start of the study) and adult (60 days of age at the start of the study) male Sprague Dawley rats. Animals were administered METH (0.3 mg/kg or 1.0 mg/kg s.c.) or D-AMPH (1.0 mg/kg s.c.) at three developmentally relevant time points. A Kinder Scientific Motor Monitor was used to assess locomotor behavior. METH and D-AMPH produced an increase in activity in adolescents and adults. The results show that adolescents and adults exhibit similar locomotor effects for these two types of amphetamines. Differences between adolescents and adults depended on the specific type of locomotor behavior examined. For example, adults showed a greater response when examining fine movements (indicative of stereotypy), but not when examining horizontal activity (indicative of forward locomotion). As the adolescents approached adulthood (60 days of age), differences diminished. These results show that adults are more sensitive to both drugs when compared to adolescents, but only for specific aspects of locomotor behavior. To the extent that the differences indicate a reduced subjective effect of the drugs in adolescence, the consequence may be an increased intake of the drugs. Further studies will explore other behaviors related to METH and D-AMPH in adolescents and adults.

Disclosures: **V. Espinoza:** None. **C.Y. Heller:** None. **A. Rocha:** None. **K.A. Trujillo:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH GM08807

NIH GM81069

Title: Methamphetamine-induced ultrasonic vocalizations differ in adolescent and adult rats

Authors: ***C. G. GONZALEZ**, T. TOWNER, F. LOPEZ, A. ROCHA, K. A. TRUJILLO; Dept. of Psychology, California State Univ. San Marcos, San Marcos, CA

Abstract: A growing body of work has demonstrated behavioral differences between adolescents and adults in response to drugs of abuse. It has also been suggested that rat ultrasonic vocalizations (USVs) may reflect emotional states, with 50 kHz frequency-modulated USVs associated with positive states (such as reward) and flat 22 kHz USVs associated with negative states (such as aversion). Previous studies have shown that psychomotor stimulants, including cocaine and amphetamine, elicit 50 kHz USVs in adult rats. However, relatively little is known about the response of adolescent rats, and whether they emit these calls to the same degree as adults in response to drugs of abuse. In addition, 14 call variations in the 50 kHz range have been reported (Wright et al. 2010). These calls remain poorly understood in the context of affective status, developmental period, or drugs of abuse. The purpose of this study was to explore these 14 calls in adolescents (30 days of age) and adults (60 days of age) after administration methamphetamine (METH, 1 mg/kg). 12 adults and 12 adolescent rats were given 15 minutes to habituate to a sound-attenuating recording chamber, injected with METH or saline, and placed back in the chamber for an additional 45 minutes of recording. As expected, METH administration dramatically increased the number of 50 kHz USVs in both adults and adolescents. A subtype known as complex 50 kHz USVs increased robustly in adults while only a moderate increase was seen in adolescents. Interestingly, age dependent differences in several other USV types were also noted. For example, adults emitted a significantly greater number of "step-up" calls, while a strong trend toward increased "step-down" calls was evident in adolescents; these patterns were not present in control animals. Taken together, the results

demonstrate age-related differences in response to METH administration. Further work establishing the salience of these calls will give us greater insight into rat models of affective states of drug abuse and addiction.

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Poster

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Support: GAUK 545612

GACR 14-03708S

GAČR P303/10/0580

Title: The influence of methamphetamine on maternal behavior and development of the pups during the neonatal period

Authors: *M. MALINOVA, I. HREBICKOVA, E. MACUCHOVA, R. SLAMBEROVA; Dept. of Normal, Pathological and Clin. Physiol., Charles Univ. In Prague, Third Fac. of Med., Prague, Czech Republic

Abstract: Methamphetamine (MA) as a psychostimulant affects maternal behavior. During lactation period, MA can penetrate into the breast milk and influence the postnatal development of the pups. The present study examined the effect of MA on maternal behavior when MA is abused during 11 days after parturition and the differences in postnatal development of the pups when MA is injected to the pups directly or is received by breast milk of mother during postnatal days (PD) 1-11. The development of nervous system of the rat pups during PD 1-11 should correspond to the third trimester of humans. Maternal behavior was examined by observation test (PD 1-22) and retrieval test (PD 1-12). Development of the pups was observed in the following tests: righting reflex on surface (PD 1-12), negative geotaxis (PD 9), righting reflex on mid-air (PD 17), rotarod and beam-balance test (PD 23). The weight of the pups was examined for the whole testing period and the day of eyes opening was recorded. Mothers who received MA groomed their pups less than controls although MA-treated mothers nursed actively their pups

more than control dams. In retrieval test, MA-treated mothers showed increased latency to return all the pups into the nest relative to controls. Due to anorectic effect of MA, the weight gain of the pups was significantly lower during the injection period relative to saline-exposed. In addition, when receiving drug via the breast milk all pups weighted less than the pups who received the drug directly. Pups who received the drug directly were faster in righting reflex on surface in first 3 PDs compared to pups who received drug via the breast milk. Females who received MA via the breast milk were faster in righting in first 2 PD´s compared to control females. However, the gender did not affect the righting reflex. In the test of negative geotaxis, pups receiving the drug via the breast milk were slower in turning upward in comparison to pups who received the drug directly. Neither drug nor gender influenced the performance. MA caused the earlier eyes opening when the pups received drug via the breast milk compared to controls. No differences in eyes opening were observed when the drug was administered directly to the pups. At the end of lactation period, males who received MA via the breast milk were less able to balance on a beam compared to males who received MA directly. The performance of males was worst compared to performance of females on a beam. In addition, the neonatal treatment of MA improved the performance of rotarod test relative to saline-exposed pups. Thus it seems that MA exposure in neonatal period affects early and also late sensorimotor development of the pups.

Disclosures: **M. Malinova:** None. **I. Hrebickova:** None. **E. Macuchova:** None. **R. Slamberova:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: 260045/SVV/2014

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GACR 14-03708S

Title: Gender differences in behavioral changes caused by early neonatal methamphetamine exposure and application of the same drug in adult rats

Authors: *I. HREBÍCKOVÁ, M. MALINOVA, E. MACUCHOVA, R. SLAMBEROVA; Dept. of Normal, Pathological and Clin. Physiol., Charles University, Third Fac. of Med., Prague 2, Czech Republic

Abstract: Methamphetamine (MA) is a street drug causing potent psychomotor activation. Our previous works demonstrated that effect of MA exposure on behavior of adult rats prenatally exposed to MA depends on their gender. The aim of the present study was to compare the response to acute application of MA in adult male and female rats neonatally exposed to the same drug. Rat mothers during lactation period or their pups were treated subcutaneously by a daily injection of MA (5 mg/kg) or saline (SAL) throughout early neonatal period (from PD 1 to 11), which is a period of brain development that corresponds to human third trimester of gravidity. Spontaneous locomotor activity and exploratory behavior of adult animals were tested in Laboras apparatus (Metris B.V., Netherlands) for 1 hour. Rats were injected with MA (1mg/kg) or SAL prior to testing. Our results demonstrated that acute administration of MA in all groups increased locomotion, distance traveled, rearing as well as the velocity. In the groups, where mothers were treated and the drug crossed to the fetus via the breast milk, the MA-induced increase in exploratory behavior was higer in SAL-exposed males than MA-exposed males. In contrast, in the groups of injected pups, exploratory behavior after acute MA was higer in rats neonatally exposed with MA than with SAL. In all groups females displayed more locomotion and more rearing, faster velocity and longer distances traveled than males regardless of neonatal treatment. The sex-induced differences decreased as the time from the MA application progressed. Thus, our results indicate that adult MA injection affect behavior of adult males in sex- and prenatal drug exposure-specific manner.

Disclosures: I. Hrebícková: None. **M. Malinova:** None. **E. Macuchova:** None. **R. Slamberova:** None.

Poster

809. Amphetamine Reinforcement II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: CSM 7/CRP/2014

260045/SVV/2014

GAUK 545212

Title: What is the effect of acute methamphetamine exposure on anxiety-related behavior in female rats after prenatal treatment with the same drug?

Authors: *E. MACÚCHOVÁ, K. NOHEJLOVÁ, M. MALINOVÁ, I. HREBÍčKOVÁ, M. POMETLOVÁ, R. ŠLAMBEROVÁ;
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Abstract: Methamphetamine (MA) is among the most widely used addictive drugs, which leads to long-lasting changes of the developing brain of the fetus, when taken by pregnant women. Anxiety, panic attacks and mania have been reported among psychostimulant users, especially after the first use. Furthermore, females differ in the effect of psychostimulant drugs when compared to males. The aim of the present study was to examine the effect of acute MA exposure (1 mg/kg) on the female rats' behavior in the test of the elevated plus-maze (EPM) after prenatal treatment with the same drug (MA, 5 mg/kg) or saline (SA). EPM is commonly used for evaluation of anxiolytic and anxiogenic drug effects, based on the natural aversion of rodents to height and open spaces. Four groups of animals were tested (accordingly to prenatal/acute treatment): SA/SA, MA/SA, SA/MA, MA/MA. The parameters analyzed were divided into three groups: anxiogenic behavior, anxiolytic behavior and motor activity. As far as the anxiogenic behavior is concerned, the groups did not differ in a frequency of closed arms entries. However, females treated with MA in adulthood (regardless of prenatal treatment) showed increased frequencies of other parameters of anxiogenic behavior (protected head dipping and stretched-attend postures). Females from MA/MA group had increased frequency of open arms entries. This finding might not be taken as anxiolytic, as females from this group also had a higher frequency of all arms entries, and a higher frequency of rearing, when compared to other groups. So this effect was more likely caused by the locomotor activating effect of acute MA treatment. Additionally, the frequency of sniffing and the duration of sniffing episodes were decreased in the group of MA/MA females. Furthermore, we suggest that the effect of MA treatment on anxiety of female rats might be related to hormonal changes within the estrous cycle. Keywords: Methamphetamine, Elevated plus maze test, Anxiety, Motor activity

Disclosures: E. Macúchová: None. **K. Nohejlová:** None. **M. Malinová:** None. **I. Hrebíčková:** None. **M. Pometlová:** None. **R. Šlamberová:** None.

Poster

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Program#/Poster#: 809.17/Z30

Topic: C.17. Drugs of Abuse and Addiction

Support: Manitoba Health Research Council (JFW)

Canadian Institutes of Health Research (JFW)

Title: Chronic treatment with mood stabilizer lithium inhibits amphetamine-increased risk-taking behaviour in rats

Authors: Z. ZHOU[1], Y. WANG[1], H. TAN[1], Y. SUN[1], Y. CHE[2], *J.-F. WANG[1];
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Abstract: In humans frequent use of amphetamine causes symptoms that resemble psychotic mania such as hyperactivity, elevated energy, risk-taking, enhanced mood and racing thoughts. The drug lithium is commonly used to treat mania. In this study, we analyzed the effects of lithium on amphetamine-induced risk-taking behaviour in rats. Risk-taking behaviours were measured by elevated plus maze test. The number of stretch-attend postures, the open-arm duration and the open-arm entrance frequency were each recorded for 5 min. We found that treatment with amphetamine at 2 mg/kg for 10 and 14 days significantly increased the number of stretch-attend postures, the open-arm duration and the centre entrance frequency, but treatment with amphetamine for 1, 3 and 7 day has no effect on these behaviours. We also found that chronic treatment with lithium significantly inhibited amphetamine- increased open arm duration and entrance frequency. In addition, treatment with amphetamine for 14 days increased protein carbonylation in rat frontal cortex, and chronic treatment with lithium inhibited amphetamine treatment-increased protein carbonylation. These results suggest that repeated amphetamine treatment in rats displays risk-taking manic-like behaviors in rats, and this risk-taking behaviour can be inhibited by chronic lithium treatment. Our findings also indicate that the process of oxidative stress may be targeted by lithium for its anti-manic treatment.

Disclosures: Z. Zhou: None. **J. Wang:** None. **H. Tan:** None. **Y. Sun:** None. **Y. Wang:** None. **Y. Che:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: DGAPA Grant IN307414

Title: Acute food deprivation decreases the expression of methamphetamine-induced sensitization of motor activity in rats

Authors: *F. MIRANDA-HERRERA, J. C. JIMENEZ-MEJIA, M. BECERRA-DIAZ, I. BARRIENTOS-NORIEGA, J. A. MIRANDA-BARRIENTOS;
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Abstract: Addictive effects of psychostimulants such as cocaine, amphetamine (AMPH), and methamphetamine (METH) are associated with an increase in extracellular dopamine (DA) levels in the brain reward circuitry by facilitating the release of DA from presynaptic nerve terminals and/or inhibiting the reuptake. Cocaine inhibits the reuptake of DA by blocking the DA transporter (DAT). AMPH and METH facilitate the release of DA from presynaptic terminals by reversal the DAT function. DAT is, therefore, a critical regulator of DA levels. There is now evidence that insulin pathways in the brain reward circuitry play an important role in regulating the DAT activity. Some studies have reported that the hypoinsulinemia attenuates the DAT functions and, as a consequence, the psychostimulant-induced behaviors are also reduced. Galici et al. (2003) reported that the depletion of insulin by single injection of streptozotocin reduced AMPH self-administrations. In the present study we examined the effects of acute food deprivation, that it also reduces the insulin levels, on the METH-induced sensitization of motor activity in rats. In addition, in independent groups of rats, glucose and insulin levels were also examined. Male Wistar rats were divided in five groups (n=10) and treated with METH or saline during the days 3-7 (development phase). On day 10 (expression phase) rats were treated with METH or saline. On days 8-9, animals remained drug-free in their home cages. Groups M24M and M0M were treated with METH (1 mg/kg) during development and expression phase. Rats of group M24M were deprived of food 24 h before the expression phase. Groups S0M, S24S and S0S were treated with saline on the development phase. They were different because rats of group S0M were treated with METH (1 mg/kg) on the expression phase and rats of group S24S were deprived of food 24 h before the expression phase. The behavioral activity was recorded by 60 min on open-field cages and glucose levels were measured on days 7 and 10 before the experimental session. The glucose and insulin levels were also evaluated in independent groups of rats after 0 and 24 h of food deprivation. The results showed that repeated administration of METH induced a progressive increase in locomotor activity in rats on development phase. However, METH administration on the expression phase produced a decrease on locomotor activity after 24 h of food-deprivation on group M24M. The results also showed that as a result of food-deprivation it was observed a reduction in glucose and insulin levels. These results are in

line with previous studies and suggest that food-deprivation reduces some behavioral effects of the psychostimulants such as METH.

Disclosures: F. Miranda-Herrera: None. **J.C. Jimenez-Mejia:** None. **M. Becerra-Diaz:** None. **I. Barrientos-Noriega:** None. **J.A. Miranda-Barrientos:** None.

Poster

809. Amphetamine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: DoJ 2010-DD-BX-0517

DoJ 2010-DD-BX-0575

NIAAA 1R21AA020039-01

NIDA 2P50DA18165

Title: Methamphetamine, alcohol and nicotine dependent subjects have different neural signatures on a discounting task

Authors: *W. F. HOFFMAN[1,2], D. SCHWARTZ[2], V. WILSON[3], B. TREMBLAY[4], L. DENNIS[2], S. MITCHELL[5];
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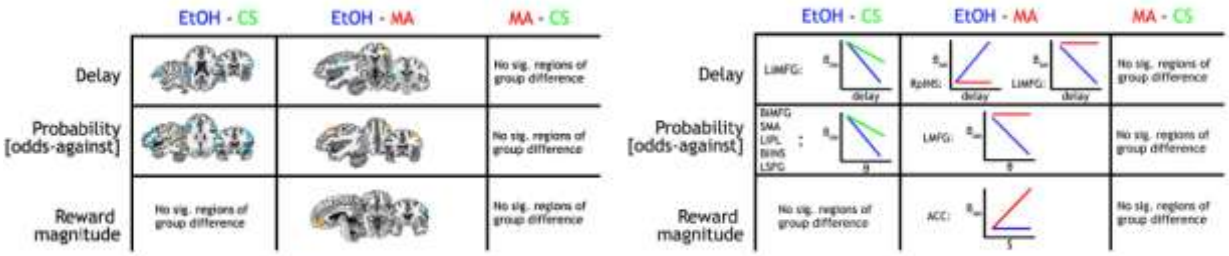
Abstract: We evaluated 38 alcohol dependent (EtOH), 25 methamphetamine dependent (MA) and 25 control subjects (CS) on an imaging task that differentiated neural networks representing reward magnitude, delay and probability. Subjects (52 male) were asked to choose between an immediate, certain monetary reward and an alternative reward that varied in magnitude, time to receipt and probability. Each aspect of the stimulus was presented separately and in pseudorandom order. When all aspects of the alternative were presented, the subject was signaled to choose and was informed of trial outcome. A standardized measure of impulsivity was obtained for each subject (IMP). We used amplitude modulated regression (AMR) to model the response to each stimulus aspect. The groups were well matched demographically; the EtOH group was more impulsive than the CS. We identified regions of group contrast in the AMR

coefficients (Figure 1) that suggest a qualitative difference in the neural signatures between the groups (Figure 2). EtOH subjects activate posterior insula for longer delays and LMFG for shorter delays and more certain outcomes. Conversely, MA users activate ACC with increasing reward, but EtOH do not. The difference between EtOH and CS does not show variability across regions, but rather quantitative differences within regions. EtOH show steeper activation decrease to increasing delay and decreasing probability than CS. Groups addicted to EtOH or MA respond differently to cardinal characteristics of monetary rewards.



Disclosures: **W.F. Hoffman:** None. **D. Schwartz:** None. **V. Wilson:** None. **B. Tremblay:** None. **L. Dennis:** None. **S. Mitchell:** None.

Poster

809. Amphetamine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NHRI Grant (02A1-NPPP01-021)

NSC Grant (NSC100-2320-B-400-002-MY3)

Title: Effect of memantine on treatment of methamphetamine-induced rewarding and drug-seeking in mice

Authors: P.-P. YANG[1,2], K.-Y. HSU[1], Z.-H. WU[1], *P.-L. TAO[1,2];
[1]Ctr. for Neuropsychiatric Res., Natl. Hlth. Res. Institutes/Center For Neuropsychiatric Res., Zhunan, Taiwan; [2]Dept. of Pharmacol., Natl. Def. Med. Ctr., Taipei, Taiwan

Abstract: Methamphetamine (MA) abuse and dependency are international problems. Studies have shown that neuronal inflammation and degeneration after long term use of MA.

Memantine, an Alzheimer's disease medication, has NMDA antagonist property and neuroprotective effects *in vitro* and *in vivo*. In this study, we evaluated whether co-administration or post-treatment of memantine (10-20 mg/kg, s.c.) would be effective to prevent or treat MA-induced rewarding and drug-seeking behavior in male ICR mice. A conditioned place preference test with 6 days of conditioning was used. We found that co-administration of memantine (10 or 20 mg/kg, s.c.) with MA (0.5 mg/kg, i.p.) significantly attenuated the MA-induced place-preference in mice. Post-treatment of memantine for 4 days after conditioning did not show statistical significant effect. The neurochemical and other addiction or inflammation related changes including dopamine turnover rate, striatal delta-Fos B level, plasma cytokines, etc. are under investigation.

Disclosures: P. Yang: None. **K. Hsu:** None. **Z. Wu:** None. **P. Tao:** 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.; Pao-Pao Yang.

Poster

809. Amphetamine Reinforcement II

Location: Halls A-C

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Program#/Poster#: 809.21/Z34

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA029815

Title: The effects of amphetamine exposure during adolescence on anxiety- and depression-like behavior in young adulthood

Authors: *S. KANG[1,2], A. P. SINGH[3,1], W. M. SOLER[3,1], M. WU[3,1], Z. P. DANIELS[3,1], R. GALVES[3,2,1], J. M. GULLEY[3,2,1];
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Abstract: We have previously demonstrated that repeated exposure to amphetamine (AMPH) during adolescence induces changes in dopamine D1 and D2 receptors in the medial prefrontal cortex (mPFC) that last into young adulthood. It is not clear whether this is the result of changes in the expression of these receptors or if AMPH also alters anxiety- and depression-like behaviors that have been previously shown to be sensitive to manipulations of dopamine receptor

function. To investigate this, we treated pair-housed male and female Sprague-Dawley rats with saline or 3 mg/kg AMPH (i.p.) every other day from postnatal day 27 to 45 (10 injections). One, 7 and 22 days after the last injection (i.e., on P46, P52 and P67), sucrose preference tests were performed to assess depression-like behavior. During each test, consumption of water and a 1% sucrose solution was measured for 48 hours. One day before the last sucrose preference test (P66), anxiety-like behavior was assessed by measuring activity in an open-field arena and in an elevated plus maze (EPM). On P68 or P69, rats were sacrificed and the mPFC and nucleus accumbens (NAC) were collected for subsequent western blot analysis of D1 and D2 protein levels. Our preliminary result suggested that AMPH exposure induced a decrease in sucrose preference for both sexes, especially during the first test session. However, there was no apparent effect of AMPH pre-exposure on anxiety-like behavior measured by EPM. The expression levels of dopamine receptors are currently being quantified, but findings from previous studies have led us to predict that AMPH exposure will likely induce a reduction of D1 and an increase of D2 receptor level in the mPFC and NAC. These preliminary results are consistent with our previous work suggesting AMPH exposure has a long lasting effect on the dopamine system in the mPFC and NAC. Future analysis will reveal whether the predicted changes in dopamine receptors levels are associated with disruptions in depression- and anxiety-like behavior.

Disclosures: S. Kang: None. **A.P. Singh:** None. **W.M. Soler:** None. **M. Wu:** None. **Z.P. Daniels:** None. **R. Galves:** None. **J.M. Gulley:** None.

Poster

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Program#/Poster#: 809.22/Z35

Topic: C.17. Drugs of Abuse and Addiction

Title: Acute methamphetamine induces reactive oxygen species in dopamine terminals of the nucleus accumbens

Authors: *S. C. STEFFENSEN[1], D. M. HEDGES[1], N. D. SCHILATY[1], E. Y. JANG[1], J. T. YORGASON[2], F. P. BELLINGER[3];
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Abstract: Methamphetamine (METH) is a powerful psychostimulant known to act on both the dopamine (DA) transporter (DAT) and the monoamine vesicular transporter (VMAT-2).

Ultimately, METH facilitates DA release by causing a reverse transport of the DAT while simultaneously blocking VMAT-2. Dopamine is subject to auto-oxidation and enzymatic degradation, resulting in formation of hydrogen peroxide (H2O2) and superoxide (O2-), reactive oxygen species (ROS). This induces significant oxidative stress for DA neurons. Using fast scan cyclic voltammetry (FSCV), we evaluated the role of ROS in acute METH enhancement of DA release. Using FSCV *in vivo*, IV administration of METH (1.0 mg/kg) markedly increased DA release in the nucleus accumbens (NAc) 340%. Similarly, superfusion of METH (0.1-100 μM) dramatically increased DA release in the NAc slice preparation (330% at 100 μM METH) *ex vivo*, but the effect was more transient than that found *in vivo*. Within 15 minutes of continuous superfusion, the evoked signal decreased back to baseline level, suggesting some 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%. Due to the inherent nature of METH to raise oxidative stress in DA neurons, we sought to determine if part of the DA enhancement by METH was due to ROS formation. We tested the effects of METH in the presence of glutathione (GSH). Glutathione (100 μM) decreased METH-induced DA release in the NAc approximately 50%. To determine if this effect of GSH was due to molecular antioxidant properties, we tested METH in the presence of 2 mM sodium ascorbate, which failed to alter METH enhancement of DA release. In addition, we have also tested TEMPOL, a SOD mimetic compound, both *in vivo* and *ex vivo*. In both experimental paradigms there was no significant decrease in METH's effect. These results indicate that GSH is able to decrease METH's effect likely through enzymatic processes. Taken together, these results indicate that acute METH elicits a significant cellular response in DA cells based on ROS formation. The enzymatic processes associated with ROS formation may prove to be a potential therapeutic target.

Disclosures: S.C. Steffensen: None. **D.M. Hedges:** None. **N.D. Schilaty:** None. **E.Y. Jang:** None. **J.T. Yorgason:** None. **F.P. Bellinger:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: Methamphetamine Abuse Research Center (P50 DA018165)

T32 NS007466

VA Merit Review and Research Career Scientist Programs

Title: The combined neurotoxic effects of 3,4-methylenedioxymethamphetmine (MDMA) and selected substituted methcathinones in a mouse model

Authors: *N. MINER, R. JOHNSON, A. ESHLEMAN, A. JANOWSKY;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The rise in popularity of substituted methcathinones (aka "Bath Salts") has increased the focus on the neurotoxic effects of these substances. Two of the more popular methcathinones, 3,4-methylenedioxymethcathinone (methylone or MDMC) and 3,4-methylenedioxypyrovalerone (MDPV) are similar in structure to MDMA, yet differ significantly pharmacologically. Many users, whether intentionally or unwittingly, are concomitantly ingesting these methcathinones along with the popular illicit drug MDMA. To examine potential neurotoxic effects of these drug combinations, we studied the effects on C57BL/6 mice of administering a single-day neurotoxic regimen (4 i.p. injections spaced 2 hours apart) of the drugs either by themselves: MDMA (15 mg/kg and 30 mg/kg), MDMC (20 mg/kg), MDPV (1 mg/kg), or in combination: MDMC with MDMA (20 and 15 mg/kg), MDPV with MDMA (1 and 15 mg/kg), in comparison to control animals receiving saline injections. Core temperature was continuously monitored during day of administration using telemetry and animals were euthanized either 2 or 7 days following the last injection. Dopamine (DA) levels were analyzed in the striatum *via* competitive ELISA and striatal glial fibrillary acidic protein (GFAP) expression was analyzed by sandwich ELISA. At 2 days following the last injection, striatal DA was significantly decreased only with the highest dose of MDMA, whereas GFAP levels were increased by both doses of MDMA. DA or GFAP levels were not significantly altered by either methcathinone administered alone or in combination with MDMA. There were also no significant changes from baseline in any groups for DA or GFAP, measured 7 days later, indicating these changes are transient. Mice in all treatment groups, with the exception of MDPV alone, developed varying degrees of acute hypothermia, followed by hyperthermia. These results indicate that, at the doses used here, the effects of MDMC and MDPV are not additive with the effects of MDMA on acute DA depletion, or on increased astrocyte activation in the striatum, but may potentially block astrogliosis caused by MDMA alone. Additionally, temperature responses to these drugs appear to be mediated by different mechanisms that are not correlated with DA and GFAP levels.

Disclosures: N. Miner: None. **A. Eshleman:** None. **A. Janowsky:** None. **R. Johnson:** None.

Poster

809. Amphetamine Reinforcement II

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Program#/Poster#: 809.24/AA1

Topic: C.17. Drugs of Abuse and Addiction

Title: Interactions between methamphetamine self-administration, early life stress, and MeCP2

Authors: *C. LEWIS, M. F. OLIVE;
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Abstract: Early life stress and epigenetic processes are known mediators of drug addiction, yet little is known how these two factors interact to contribute to the vulnerability to abuse various substances such as methamphetamine. The maternal separation (MS) paradigm is an animal model used to study the long-term effects of early life stress. Animals subjected to prolonged daily maternal separation during a critical period of neurological development display altered behavioral and neurological development as adults. We recently examined the effects of early life stress on methamphetamine self-administration in rats (Lewis et al., Frontiers in Psychiatry, 2013). Male rats subjected to MS conditions self-administered significantly more methamphetamine and acquired methamphetamine self-administration earlier than males without a history of MS. The effects of MS on multiple neural systems and behaviors are in part mediated by epigenetic regulation of target genes. Methyl-CpG-binding-protein 2 (MeCP2) is an epigenetic marker that binds to methylated DNA and attracts other regulatory complexes, and has previously been implicated in psychostimulant reward and reinforcement. In our study, methamphetamine self-administration was inversely related to levels of MeCP2 in the nucleus accumbens core (NAcc). In order to determine a causal role for MeCP2 levels in MS-induced increases in methamphetamine intake, we are currently conducting studies on the effects of virally mediated knockdown of MeCP2 expression in the NAcc using an AAV9-U6-shRNA virus. The effects of the active shRNA-expressing vs. control virus on methamphetamine self-administration, extinction, and drug primed reinstatement in male rats with and without a history of early life stress are being examined. These studies will hopefully provide a potential epigenetic basis for the ability of early life stress to increase vulnerability to drug intake, and thus offer insight into potential intervention strategies for individuals with a history of early life stress. This work was supported by DA025606.

Disclosures: C. Lewis: None. **M.F. Olive:** None.

Poster

810. Cannabis: Neural Mechanisms and Addiction

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Program#/Poster#: 810.01/AA2

Topic: C.17. Drugs of Abuse and Addiction

Support: McKnight Brain Institute, Department Development Award

Title: Passive exposure to cannabis smoke leads to signs of dependence in rats

Authors: *A. W. BRUIJNZEEL, X. QI, S. WALL, M. GOLD, M. FEBO, B. SETLOW; Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Cannabis is the most widely used illicit drug in the US and cannabis use among young adults continues to rise. Previous studies have shown that chronic systemic administration of delta 9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis smoke, or CB1 cannabinoid receptor agonists leads to the development of dependence. However, because smoking is the most popular route of THC self-administration it is critical to investigate the effects of cannabis smoke exposure. In previous work, we established a whole body tobacco smoke exposure protocol and showed that exposure leads to the development of nicotine dependence and an upregulation of nicotinic receptors. The goal of the current study was to investigate if exposure to cannabis smoke has behavioral effects and leads to the development of cannabis dependence, similar to what has been shown previously with THC administration. Cannabis cigarettes (5.8% THC) were obtained from NIDA and cannabis smoke was generated using an automated cigarette smoking machine. Adult rats (n=4) were exposed to cannabis smoke for 1 h per day, 5 days per week, for a total of three weeks in an exposure chamber. Control rats (n=4) were exposed to the smoking machine in the absence of smoke, for the duration of the exposure sessions. Blood samples were collected immediately after exposure and THC levels were determined using a THC ELISA kit. Exposure to cannabis during weeks 2 and 3 led to THC levels of 58.6 ± 1.8 and 59.4 ± 2.1 ng/ml, respectively. Similar THC levels have been detected in humans after smoking a cannabis cigarette. During week 2, the effect of cannabis smoke exposure on locomotor activity was investigated. Exposure to cannabis smoke led to a decrease in locomotor activity and rearing compared to control rats. Similar effects have been observed after THC administration in rats. When the rats were tested 3 days after cannabis smoke exposure there was no difference in locomotor activity or rearing between the two groups. At the end of weeks 2 and 3, the rats received the CB1 receptor antagonist SR 141716A (5 mg/kg, ip) and somatic withdrawal signs were recorded for 10 min. At both time points, the cannabis rats displayed about twice as many somatic withdrawal signs as the control rats. Finally, exposure to cannabis smoke led to a significant reduction in body weight gain during the 3-week exposure period. Taken together, the present data show that chronic exposure to cannabis smoke leads to an increase in serum THC levels, decreased activity levels, and early signs of cannabis dependence. This exposure model should be useful for separating the effects of THC from cannabis smoke and developing treatments for cannabis dependence.

Disclosures: **A.W. Bruijnzeel:** None. **X. Qi:** None. **S. Wall:** None. **M. Gold:** None. **M. Febo:** None. **B. Setlow:** None.

Poster

810. Cannabis: Neural Mechanisms and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 810.02/AA3

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH IRP (NIDA)

Title: Species differences of cannabinoid receptor 2 genes and their roles in cocaine self-administration

Authors: ***Q.-R. LIU**, H.-Y. ZHANG, E. L. GARDNER, Z.-X. XI;
Behavior Neurosci. Res. Br., Intramural Res. Program, NIDA/ NIH, BALTIMORE, MD

Abstract: The discovery of functional neuronal cannabinoid receptor 2 (CB2R) expressed in brain suggests a potential new therapeutic target for neurological and psychiatric disorders. However, species differences in CB2R gene structures, protein sequences and brain expression patterns confound the pharmacological and behavioral interpretations in different animal models. The pharmacological effects of the CB2R-selective agonist JWH133 on cocaine self-administration were different between mice and rats. Systemic administration of JWH133 (10, 20 mg/kg) significantly and dose-dependently inhibited intravenous cocaine self-administration under both fixed-ratio (FR) and progressive-ratio (PR) reinforcements in mice, but had little effect on intravenous cocaine self-administration under FR reinforcements, but increased PR break-point for cocaine self-administration in rats. We analyzed the molecular differences of CB2R in different species. In comparison with human and rat CB2R, mouse CB2R contains a premature stop codon that truncates the C-terminal 13 amino acids. We previously found that mouse and human brains express CB2A and CB2B isoforms, with mRNA levels of CB2A higher than that of CB2B in their respective brain regions. In this study, we discovered novel rat-specific alternatively spliced isoforms of rCB2C and rCB2D that are produced by inter- and intra-exonal splicing events, respectively. The ultrasensitive RNAscope *in situ* hybridization found that CB2R mRNA was expressed in ventral subiculum of hippocampus of both rat and mouse. These findings suggest that an accelerated evolution of CB2R gene in terms of alternative spliced isoforms, brain expression patterns, and variations of the protein functional

domains might in part contribute to the behavioral differences of CB2R-selective ligand effects in different animal models.

Disclosures: Q. Liu: None. **H. Zhang:** None. **E.L. Gardner:** None. **Z. Xi:** None.

Poster

810. Cannabis: Neural Mechanisms and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH P50 DA05312

NIH R01 DA12964

Title: Evoked dopamine dynamics in nucleus accumbens core following acute versus chronic administration of the CB1/CB2 agonist, CP55,940

Authors: *S. LEE[1], G. V. REBEC[1,2], K. D. BUNNER[1,2];
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Abstract: Marijuana, like many drugs of abuse, increases dopamine transmission in the nucleus accumbens (NAc). This effect is believed to enhance goal-directed behavioral responses, including the motivation to obtain natural and drug rewards. Cannabinoid receptors (CB) play a key role in the marijuana-induced dopamine increase, and acute exposure to cannabinoids promotes NAc dopamine by activating dopamine neurons in the ventral tegmental area. The effect of prolonged cannabinoid exposure on NAc dopamine, however, is unclear. Moreover, a recent *in vitro* study indicated a possible interaction between cannabinoids and the dopamine transporter, suggesting a role for cannabinoids on dopamine uptake (Pandolfo et al., Eur. J. Pharmacol., 2011). Here, we examined extracellular dopamine dynamics in the NAc core, which is involved in goal-directed behavior, in response to acute as well as chronic administration of CP55,940, a CB1/CB2 receptor agonist. Fast scan cyclic voltammetry was used to measure dopamine overflow in the NAc core evoked by electrical stimulation of the median forebrain bundle in anesthetized, male Sprague-Dawley rats. Dopamine release was assessed immediately following one or seven daily injections of CP55,940 (0.2mg/kg, ip) or corresponding vehicle. Whereas a single injection of CP55,940 increased stimulation-evoked dopamine release, repeated

exposure failed to increase dopamine above the vehicle response. In fact, compared to vehicle, chronic CP55,940 lowered basal dopamine release. Our results show that although CB1 receptor activation increases NAc dopamine release, tolerance develops to this effect and may even lead to a decrease in dopamine transmission. Thus, altered dopamine signaling in the reward pathway by acute and chronic cannabinoid exposure may contribute to differential changes in motivational state.

Disclosures: S. Lee: None. **G.V. Rebec:** None. **K.D. Bunner:** None.

Poster

810. Cannabis: Neural Mechanisms and Addiction

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Program#/Poster#: 810.04/AA5

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA035281

NIH Grant DA024105

Title: Development and validation of a device for the intrapulmonary delivery of cannabinoids and stimulants to rats

Authors: *M. A. TAFFE[1], S. M. AARDE[2], M. COLE[2];
[1]Cmte Neurobio. of Addictive Disorders, The Scripps Res. Inst., LA JOLLA, CA; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: The recent popularization of non-combustible methods for intrapulmonary delivery of psychoactive drugs to humans (Vape, Volcano, e-cigarette, etc) has stimulated interest in the intrapulmonary administration models for rodent studies. We have designed a sealed rodent chamber, with a well regulated air flow, that is suitable for the controlled exposure of rats to psychoactive substances. Use of e-cigarette type delivery systems was found to afford excellent dosing control for this purpose. Studies were conducted in male rats to verify the *in vivo* efficacy of drug delivery. Implantable radiotelemetry methods were used to demonstrate that a 20 min exposure to Δ9-tetrahydrocannabinol (THC), or the CB1 receptor full agonist JWH-018, produces a robust hypothermia. The temperature nadir was reached within 40 min of exposure, was of comparable magnitude to that found after 30 mg/kg THC or 1.1 mg/kg JWH-018, i.p. and had resolved within 3 hours compared with a 6 hour time course following injection. Studies also

demonstrated that 30 min of intrapulmonary exposure to methamphetamine (MA) significantly increased home cage locomotor behavior for up to 2 hrs. A final study showed that a 30 min intrapulmonary exposure to MA reduced drug intake during the loading phase of intravenous self-administration of MA. Finally, it is shown that rats will nosepoke for the delivery of MA vapor. These studies show that an electronic cigarette type delivery system can be successfully used to model intrapulmonary drug delivery in rats. These techniques will be of increasing utility as recreational users continue to adopt "vaping" for the administration of psychtropic drugs.

Disclosures: **M.A. Taffe:** None. **S.M. Aarde:** None. **M. Cole:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); La Jolla Alcohol Research, Inc..

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Chronic cocaine self-administration up-regulates cannabinoid CB_2 receptor expression in mice and rats

Authors: ***H. ZHANG**, Q.-R. LIU, G.-H. BI, E. L. GARDNER, Z.-X. XI;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Brain cannabinoid CB_2 receptors (CB_2Rs) have been recently reported to regulate nucleus accumbens dopamine (DA) release and intravenous cocaine self-administration (SA) in mice and rats, which appears to challenge a long-accepted view that CB_2Rs are expressed mainly in periphery, not in brain. It is well documented that CB_2Rs are up-regulated in microglia during neuroinflammation or several other CNS disorders, suggesting that brain CB_2Rs are inducible under certain pathological circumstances. However, little is known as to whether chronic drug use and abuse similarly alters brain CB_2 receptor expression. Here we report that chronic, but not acute, exposure to cocaine up-regulates brain CB_2 mRNA expression in both mice and rats. We first used quantitative real-time PCR (RT-PCR) assays to measure mouse brain CB_2 mRNA with two different Taqman probes that target the 5′ and 3′ regions of mouse CB_2 (mCB_2) gene, respectively. We found that chronic cocaine self-administration (0.5-1 mg/kg/infusion, 3

hrs/session/day with maximal 50 cocaine infusions/session, for 4~6 weeks) significantly up-regulated (4-5 fold) CB_2 mRNA expression in prefrontal cortex (PFC) and striatum of mice compared to oral sucrose self-administration (0.02 ml of 5% sucrose/infusion, 3 hrs/session/day with maximal 100 sucrose infusions/session, for 4~6 weeks) control mice or drug naïve mice. In contrast, a single injection (10, 20, 30 mg/kg, i.p.) or repeated injections of cocaine (15 mg/kg, i.p. for 7 daily injections) (i.e. locomotor sensitization dose regimen) failed to alter brain CB_2 mRNA expression. We then used *in situ* hybridization (ISH) assays with a mouse CB_2 RNAscope probe that targets the 3′ untranslated region (UTR) of the mCB_2 gene, and found similar mCB_2 mRNA up-regulation in cortical and striatal neurons. To further confirm this finding, we used the same RT-PCR and ISH assays to detect CB_2 mRNA expression in rat brains with one rat CB_2 (rCB_2)-specific Taqman probe and an rCB_2 RNAscope probe. We observed the same results - cocaine, but not sucrose, self-administration up-regulated CB_2 mRNA expression in both the PFC and striatum in rats. Taken together, these findings suggest that brain CB_2 receptors are inducible and responsive to chronic cocaine use and abuse. Thus, brain CB_2 receptors may constitute a new target in medication development for the treatment of drug addiction and possibly other CNS disorders.

Disclosures: H. Zhang: None. **Q. Liu:** None. **G. Bi:** None. **E.L. Gardner:** None. **Z. Xi:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: Barrow Neuroscience Foundation

BNI-BMS Seed Fund

Title: Selective activation of CB2Rs eliminates VTA dopamine neuronal bursting firing in rodents

Authors: *M. GAO[1], Z. XI[2], J. WU[1];
[1]Divisions of Neurol., Barrow Neurolog. Institute, St. Joseph's Hosp. and Med. Ctr., Phoenix, AZ; [2]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Recently, the "peripheral cannabinoid type 2 receptors (CB2Rs)" have been detected in various brain areas, suggesting that CB2Rs are also expressed in the central nervous system and they may participate in the modulation of neuronal functions under both physiological and pathological conditions. Our previous study demonstrated that the activation of central CB2Rs significantly reduced animal cocaine seeking behavior, which indicates an important role played by CB2Rs in mesolimbic circuit for drug addiction. It is well known that drug addictive behavioral is highly correlated with VTA dopamine (DA) neuronal firing activity, especially bursting firing. However, whether central CB2Rs modulate VTA DA neuronal bursting firing is unknown. We hypothesize that the selective activation of functional CB2Rs in the VTA eliminates VTA DA neuronal bursting firing through the enhanced small-conductance Ca2+-activated K+ channels (SK). In the present study, we test our hypothesis using *in vivo* and *in vitro* electrophysiological approaches. In anesthetized mice, we performed extracellular single unit recording and found that systemic injection of CB2Rs agonist (JWH 133, i.p., 10 mg/kg) moderately reduced VTA DA neuronal firing rate but dramatically reduced bursting firing fraction in WT, but not CB2R KO, mice. In WT mice, the JWH133-induced inhibition in DA neuronal firing can be prevented or reversed by injection of CB2Rs antagonist AM630 (i.p. 10 mg/kg), suggesting that systemic JWH133 alters VTA DA neuronal bursting firing through the activation of CB2Rs. In VTA DA neurons in slice, bath-applied JWH133 (1 μm) significantly enhanced the amplitude of apamin-sensitive after-hyperpolarization, suggesting that the altered SK conductance may underlie JWH133-induced reduction of neuronal bursting firing. To elucidate this possible mechanism, we induced DA neuronal bursting firing with NMDA (30 μM) in VTA slices both in rats and mice. We found that JWH133 significantly inhibited NMDA-induced bursting firing represented as a decrease in amplitude of the inter-burst hyperpolarization potential. Importantly, this inhibition can be blocked by either AM630, or a SK selective blocker NS8593. Furthermore, JWH133 also enhanced SK current amplitude in DA neurons of VTA slices. Taken together, our results suggest that the selective activation of VTA CB2Rs significantly eliminates the bursting firing of VTA DA neurons, which is mediated through enhanced amplitude of SK currents. Therefore, our findings provide directly experimental evidence to improve our understanding, for the first time, of how CB2Rs play a critical role in drug addiction and DA associated diseases.

Disclosures: **M. Gao:** None. **Z. Xi:** None. **J. Wu:** None.

Poster

810. Cannabis: Neural Mechanisms and Addiction

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Program#/Poster#: 810.07/AA8

Topic: C.17. Drugs of Abuse and Addiction

Title: Cortical glutamatergic type 1 cannabinoid receptor protects against diet-induced obesity: Potential role of odor function in the motivation for food intake

Authors: I. RUIZ DE AZUA, A. APARISI REY, S. RUHLE, F. REMMERS, M. HAMANN, *K. MONORY, B. LUTZ;
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Abstract: Palatable food can promote overfeeding, beyond energy homeostatic requirements, and is a major risk to develop obesity. In this context, the endocannabinoid system is a key regulator of appetite and energy balance through the modulation of type 1 cannabinoid (CB1) receptor activity in central and peripheral tissues. Previous studies have shown that mice with deficiency of the CB1 receptor in cortical glutamatergic neurons (Glu-CB1-KO mice) lacks the well-known endogenous orexigenic effect of cannabinoids in a hunger state, but Glu-CB1-KO mice did not show body weight differences on standard diet, when mice had "*ad libitum*" access to food. In the present study, we investigated Glu-CB1-KO mice in a diet-induced obesity model. Strikingly, when Glu-CB1-KO mice had free access to palatable food (34% high-fat enriched diet, HFD), the lack of CB1 receptor in these particular neurons was sufficient to protect against the deleterious effects of HFD. We also found a strong and significant reduction in food intake linked to lower body weight. Importantly, a fasting-re-feeding experiment was enough to reverse the body weight difference, suggesting that the reduction in food intake in Glu-CB1-KO mice was mainly responsible for the resistance to HFD treatment. It has recently been demonstrated that CB1 receptor promotes food intake in fasted mice by increasing odor detection (Soria-Gomez et al., 2014). The analysis of mRNA levels of CB1 in mutant mice under HFD pointed out that there were small reductions in most brain areas except for cortical olfactory areas (decrease around 75%), where the well-known centrifugal glutamatergic innervations of the olfactory bulb are located. Immunohistochemistry showed a strong reduction of CB1 receptor expression in internal layers of the olfactory bulb in Glu-CB1-KO mice. Therefore, we tested the olfactory function and found that Glu-CB1-WT mice had a significantly better exploratory performance as compared to Glu-CB1 KO mice after six weeks on HFD during an odor habituation test. Finally, "re-expression" of CB1 receptor locally in these olfactory cortical areas in Glu-CB1-KO mice, by AAV carrying a Cre-dependent CB1 receptor transgene (AAV-stop-CB1), was able to partially rescue the differences in terms of body weight, food intake, odor function and glucose metabolism between Glu-CB1-KO and -WT mice. In summary, these findings suggest that CB1 receptor in cortical glutamatergic neurons is particularly relevant to promote overconsumption, when mice have free access to palatable food, and uncover a new role of CB1 receptor in the olfactory system in the motivation for food intake and the emergence of diet-induced obesity.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: Intramural Research Program of NIDA, NIH, DHHS

Title: Involvement of cortico-striatal glutamatergic neurotransmission in the reinforcing effects of delta-9-tetrahydrocannabinol (THC)

Authors: ***M. SECCI**[1], Z. JUSTINOVA[1], P. MASCIA[1], H.-Q. WU[2], G. TANDA[1], R. SCHWARCZ[2], S. GOLDBERG[1];
[1]Preclinical Pharmacol., NIDA, IRP, NIH, DHHS, Baltimore, MD; [2]Maryland Psychiatric Res. Ctr., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Although it is not as addictive as cocaine or heroin, delta-9-tetrahydrocannabinol (THC, the active constituent of cannabis) produces DSM-defined dependence and it is used by increasingly large numbers of people. Gaining a better understanding of THC's effects could lead to improved treatments for cannabis dependence. Like all other addictive drugs, THC produces rewarding effects in the brain by increasing extracellular dopamine levels in the nucleus accumbens (NAc) shell and ventral tegmental area (VTA). However, the mechanism by which THC affects dopamine in these areas differs from other drugs and has yet to be fully elucidated. Our goal was to determine the effects of THC on the release of glutamate, which is the primary excitatory neurotransmitter in the brain and a key modulator of dopamine neurotransmission. Using in-vivo microdialysis in rats, we examined the effect of systemic injection of THC on glutamate levels in the NAc and VTA. We also studied the involvement of glutamatergic projections from the medial prefrontal cortex (mPFC) to the NAc shell on THC-induced dopamine release by using the GABA(B) receptor agonist baclofen. By giving local injections of baclofen (0.3 nmol/0.5 ul) into the mPFC, we also disrupted the release of glutamate by cells that project from the mPFC to the NAc shell, to determine whether this would affect THC-induced dopamine release. We found that systemic injection of THC (3 mg/kg i.p.) increased glutamate release in the NAc and VTA by 86.5% and 90%, respectively. This effect of THC was mediated by cannabinoid CB1 receptors, since it was blocked by pretreatment with the CB1 receptor

antagonist, rimonabant (1 mg/kg i.p.). Consistent with these glutamatergic effects of THC playing a role in THC reward, local injection of baclofen into the mPFC significantly blocked THC's ability to induce dopamine release in the NAc shell. As a result, this study shows for the first time that: 1) THC can increase glutamate levels in key brain reward areas; 2) that this increase is mediated by CB1 receptors; and 3) that THC-induced dopamine release in the NAc shell is modulated by glutamatergic projections from the mPFC. Thus, cortico-striatal neurotransmission seems to play an important role in the abuse-related effects of THC.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: Regione Autonoma Sardegna, L.R. N. 7/2007, Art. 13

Title: Cannabinoid withdrawal and hypodopaminergia: A role for rostromedial tegmental neurons?

Authors: *A. MUNTONI[1], S. ARONI[2], C. SAGHEDDU[2], M. PISTIS[2,1];
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Abstract: The mesolimbic dopamine (DA) system, which arises from the ventral tegmental area (VTA), shows a decline in its spontaneous activity after chronic cannabinoid exposure and withdrawal, the critical phases of addiction. These changes in neuronal plasticity are thought to play a role into withdrawal-induced negative affective states that eventually lead to relapse into drug taking. The rostromedial tegmental nucleus (RMTg), a GABA structure located just posterior to the VTA, is a key site implicated in aversion processes. The RMTg provides a major inhibitory projection to the VTA and is a substrate for cannabinoid actions on DA cells. Indeed, acute administration of cannabinoids suppresses RMTg inputs to the VTA, thus contributing to cannabinoid-induced DA neuronal excitation. Here we sought to verify whether RMTg GABA projections to VTA neurons are causally involved in the hypodopaminergic state that characterizes cannabinoid withdrawal. To this aim, we took advantage of single unit extracellular

recordings from RMTg and VTA neurons in anesthetized male Sprague-Dawley rats. To induce Δ^9-tetrahydrocannabinol (Δ^9-THC) dependence, rats were chronically treated with Δ^9-THC (15 mg/kg, i.p.) twice daily for 6.5 days. Administration of the cannabinoid antagonist SR141716A (5 mg/kg, i.p.) precipitated an intense behavioral withdrawal syndrome, whereas abrupt Δ^9-THC suspension produced only milder signs of abstinence. Electrophysiological experiments confirmed that Δ^9-THC withdrawal produced a marked decrease in the firing rate and burst firing of VTA DA neurons. As expected, RMTg stimulation elicited a complete suppression of DA neuron discharge activity. In Δ^9-THC withdrawn rats the duration of RMTg-evoked inhibition was increased when compared with controls, suggesting an augmented GABA inhibitory input onto DA cells. Whether or not spontaneous activity of RMTg GABA neurons is altered in cannabinoid-withdrawn rats is currently under investigation. While preliminary, our results support the hypothesis that enhanced GABA inputs from the RMTg might contribute to the hypodopaminergia induced by cannabinoid withdrawal, and confirm that the RMTg takes part in the neuronal circuits underlying drug dependence and addiction.

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Poster

810. Cannabis: Neural Mechanisms and Addiction

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Program#/Poster#: 810.10/AA11

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of restraint stress on zebra finch vocal production: Evidence for social context-dependence

Authors: *T. L. HOLLAND[1], K. SODERSTROM[2];
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Abstract: Chronic cannabinoid treatment during the sensorimotor stage of zebra finch vocal development, but not during adulthood, has persistent effects on song patterns, suggesting interaction with processes responsible for late-postnatal brain development. Given accumulating evidence of an endogenous role for cannabinoid signaling in mediating physiological responses to stress, we have begun to investigate the extent to which cannabinoid-altered learning may be attributable to interaction with stress responses. Male zebra finches learn a complex song during a sensitive period of development with similarities to human language acquisition. During an early auditory phase, birds listen to and memorize the song pattern of an adult. During a

following sensorimotor learning phase, birds gradually improve songs through auditory feedback during sensorimotor practice. By adulthood, formation of a stable song pattern is achieved. Mature zebra finch song is stereotyped, but performance of the song may vary. The bird may add, skip, or repeat notes that are typically present in a motif, deviating from the typical stereotyped song pattern. We predicted that this type of note variability would be increased in stressed animals. To test this, songs were recorded before and after 30 minutes of restraint stress (isolation in a paper bag). Female-directed and undirected social context groups were included. Post-stress songs of the undirected social context group were more variable in song note composition than pre-stress songs. This difference was not seen in the female-directed social context group. As undirected song is established to involve activation of both vocal motor and anterior forebrain pathways (e.g. pre-motor HVC and anterior Area X and lMAN) and female-directed song involves activation of a more limited motor pathway (e.g. HVC) these results implicate involvement of the anterior forebrain pathway in mediating stress effects on vocal performance. Given evidence of a synergistic effect of cannabinoid treatment and stress on corticosterone levels, in future experiments we will test the hypothesis that corticosterone and cannabinoid pre-treatments will increase the magnitude of stress effects on song performance. These effects are predicted to only occur in undirected song social context. Results will provide insight into the role of stress on acute cannabinoid responses. Since cannabinoids have developmentally significant effects, future studies will evaluate effects of concurrent acute stress and cannabinoid treatment in developing zebra finches.

Disclosures: T.L. Holland: None. **K. Soderstrom:** None.

Poster

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A.D. Williams Multischool Award

Title: CRIP1a knockout mice exhibit anxiolytic-like behaviors and enhanced CB1 receptor signaling in the amygdala and striatum

Authors: B. G. HOEGBERG[1], J. C. JACOB[1], H. SHIM[1], A. TOMARCHIO[1], A. C. HOWLETT[2], A. H. LICHTMAN[1], L. J. SIM-SELLEY[1], C.-K. J. CHEN[3], *D. E. SELLEY[1]; [1]Dept. Pharmacol & Toxicol, VA Commonwealth Univ., RICHMOND, VA; [2]Dept. of Physiol. and Pharmacol., Wake Forest Univ., Winston-Salem, NC; [3]Departments of Ophthalmology and Biochem. and Mol. Biol., Baylor Col. of Med., Houston, TX

Abstract: G-protein-coupled CB1 cannabinoid receptors (CB1Rs) are highly expressed in the central nervous system (CNS), and together with the endocannabinoids, anandamide and 2-arachidonoylglycerol, modulate appetite, reward, pain, memory and motor activity. Cannabinoid receptor-interacting protein 1a (CRIP1a) is a novel regulatory protein that binds to the C-terminus of CB1Rs but not CB2Rs. Studies in cell models suggest that CRIP1a negatively regulates CB1R signaling. To determine effects of loss of CRIP1a function, we generated Cnrip1 genetic null mice. Cnrip1 knockout (KO) mice are viable, fertile and do not express CRIP1a mRNA or protein, as determined using both qPCR and Western immunoblot analysis, indicating a true null phenotype. No differences in CB1R expression levels were observed between CRIP1a KO and wild-type (WT) mice, as indicated by [3H]CP55,940 Bmax values, in any CNS regions examined so far. These animals were then characterized for novel behavioral phenotypes as well as alterations in CB1R-mediated G-protein activation, determined by agonist-stimulated [35S]GTPgammaS binding, in multiple regions of the CNS. Several CB1R-expressing CNS regions, including cerebellum, hippocampus, prefrontal cortex and spinal cord, did not display enhanced CB1R-stimulated G-protein activity between CRIP1a KO and WT mice. However, the amygdala and striatum of CRIP1a KO mice both exhibited significant enhancement of CB1-mediated G-protein activation compared to WT mice, as determined by concentration-effect curves using both the potent synthetic cannabinoid agonist CP55,940 and the putative endocannabinoid noladin ether. Importantly, behavioral analysis revealed that CRIP1a KO mice displayed significant anxiolytic-like phenotypes relative to WT mice in both the light:dark box and marble burying tests. Treatment-naïve CRIP1a KO mice also displayed slightly elevated tail-flick latencies, indicating a modest antinociceptive phenoptype, but were not different from WT mice in body temperature, spontaneous motor activity or rotarod performance. Moreover, no significant genotype differences were observed in antinociception, catalepsy, hypothermia or impairment of rotarod performance after acute administration of CP55,940, suggesting that CRIP1a selectively modulates anxiety-like behavior without enhancing CNS-mediated cannabinoid side effects. Our studies are the first to investigate the role of CRIP1a function in live animals, and suggest that CRIP1a could serve as a promising pharmacological target for future study and potential treatment of anxiety disorders.

Disclosures: B.G. Hoegberg: None. **A.C. Howlett:** None. **C.J. Chen:** None. **J.C. Jacob:** None. **D.E. Selley:** None. **H. Shim:** None. **A. Tomarchio:** None. **A.H. Lichtman:** None. **L.J. Sim-Selley:** None.

Poster

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Title: Regulation of 2-AG-mediated cannabinoid CB1 receptor signaling and adaptation by beta-arrestin2

Authors: *L. J. SIM-SELLEY[1], B. M. IGNATOWSKA-JANKOWSKA[1], I. B. ADAMS[1], A. TOMARCHIO[1], B. F. CRAVATT[2], C. K. ARNATT[1], Y. ZHANG[1], A. H. LICHTMAN[1], D. E. SELLEY[1];
[1]Virginia Commonweatlh Univ., RICHMOND, VA; [2]Scripps Res. Inst., La Jolla, CA

Abstract: Cannabinoids such as delta ^{9}-tetrahydrocannabinol (THC) and the endogenous cannabinoids (eCBs) 2-arachidonoylglycerol (2-AG) and anandamide (AEA) modulate neurotransmission in the central nervous system (CNS), primarily by activating cannabinoid CB_1 receptors (CB_1R). CB_1Rs are G-protein coupled receptors (GPCR) that activate mainly $G_{i/o}$-family proteins. Like other GPCRs, CB_1Rs are regulated by and can signal through beta-arrestins, such as beta-arrestin2 (Barr2). Accordingly, the relative ability of ligands to activate G-proteins versus recruit beta-arrestins determines their short and long-term signaling. Because THC and other CB_1 agonists have psychoactive side effects, a promising approach to regulate CB_1R activity for therapeutic benefit is the use of catabolic enzyme inhibitors to enhance eCB levels. In the present study, we compared the potency and efficacy of eCB ligands and THC to activate G-proteins in CB_1R-expressing CHO cells and N18TG2 neuroblastoma cells using [^{35}S]GTPgammaS binding, and to recruit Barr2 in CB_1-CHO cells using an enzyme complementation assay. We then examined the effects of repeated administration of THC or the monoacylglycerol lipase inhibitor JZL-184, which elevates 2-AG to a greater extent than other

eCBs, on the pharmacological actions of THC *in vivo* and on desensitization of CB_1R-mediated G-protein activation *in vitro*, in Barr2 knockout (KO) and wild-type (WT) mice. Results showed that 2-AG and AEA were essentially full agonists for G-protein activation in both cell lines, whereas 2-AG was more efficacious but had similar potency compared to AEA to recruit Barr2. Both eCBs recruited Barr2 with lower potency but much higher efficacy than THC. Repeated THC or JZL-184 administration produced tolerance to the hypothermic and thermal antinociceptive actions of THC in WT and Barr2 KO mice, but significant cross-tolerance was not observed to THC-induced catalepsy in repeated JZL-184-treated WT mice. Repeated THC produced tolerance to catalepsy in WT mice, which was reduced in Barr2 KO mice. CB_1R-mediated G-protein activation was partially desensitized by repeated treatment with THC or JZL-184 in multiple CNS regions, including spinal cord, cerebellum, hippocampus, caudate-putamen and nucleus accumbens. However, less CB_1R desensitization was detected in the caudate-putamen and nucleus accumbens of Barr2 compared to WT mice, and this difference was more robust with JZL-184 than THC. These results indicate that 2-AG is a high efficacy CB_1R ligand to both activate G-proteins and recruit Barr2, and that CB_1R desensitization and tolerance to motor effects are regulated by Barr2 in the striatum.

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Poster

810. Cannabis: Neural Mechanisms and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA033877

Title: Effects of adolescent nicotine exposure on cannabinoid-induced place preference in young adult rats

Authors: C. P. PLANT, M. STONE, A. D. HARDIN, *C. A. CRAWFORD;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: Nicotine is one of the most commonly abused substances during adolescence and is associated with later illicit drug use. For example, cigarette smoking during adolescence is

highly associated with the increased use of marijuana in adulthood. The mechanisms underlying this relationship are unknown, but it may be the result of nicotine-induced changes to dopaminergic reward circuitry. Thus, the goal of the present study was to determine whether adolescent nicotine exposure alters the rewarding properties of the cannabinoid agonist, CP 55,940. To this end we exposed rats to nicotine during the adolescent period and later assessed CP 55,940-induced conditioned place preference (CPP) in adulthood. Male Sprague-Dawley rats were injected with nicotine (0.16, 0.32, or 0.64, sc) daily for 10 consecutive days beginning on postnatal day (PD) 31. On PD 60, nicotine-induced CPP was assessed using a 14-day biased CPP procedure, consisting of one preconditioning day, 10 conditioning days, one test day and two rest days. Rats were pre-exposed to CP 55,940 (0, 10, 20, or 30 µg/mg, ip) 30 minutes after the preconditioning session. The rats were pre-exposed to the same dose of CP 55,940 as they received in the conditioning phase. Repeated pairings of CP 55,940 with the non-preferred CPP compartment induced a preference for the drug paired room at the 20 µg/kg dose but not at 10 or 30 µg/kg. Adolescent exposure to nicotine altered CP 55,940-induced CPP in a dose dependent manner. Specifically, the high doses of nicotine (0.32 and 0.64 mg/kg) decreased preference for the drug paired room compared to saline treated control. These findings suggest that adolescent exposure to nicotine does not increase the rewarding nature of cannabinoid drugs.

Disclosures: **C.P. Plant:** None. **M. Stone:** None. **C.A. Crawford:** None. **A.D. Hardin:** None.

Poster

810. Cannabis: Neural Mechanisms and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 810.14/AA15

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant T32 DA007237

NIDA Grant R01 DA023204

NIDA Grant P30 DA013429

Title: Randomly-methylated β-cyclodextrin used to solubilize Δ(9)-THC has effects on long-term potentiation in wildtype, CB1 and CB1:CB2 knockout mouse models

Authors: *M. SPEROW, H. ZANIEWSKI, L. G. KIRBY, M. E. ABOOD;
Ctr. for Substance Abuse Res., Temple Univ., Philadelphia, PA

Abstract: Δ(9)-tetrahydrocannabinol (Δ(9)-THC) is the principal psychoactive compound in marijuana and its actions in the brain are primarily mediated through CB1 receptors. Effects of Δ(9)-THC have been robustly demonstrated to cause deficits in long-term potentiation (LTP) of the hippocampus[1,2]. However studying this very hydrophobic compound in physiological systems is an ongoing technical challenge. Hazekamp and Verpoorte [3] demonstrated that randomly methylated β-cyclodextrin (RAMEB) can help maintain the aqueous solubility of Δ(9)-THC up to 8 weeks at 14 mg/ml. Starting with wild type littermates we attempted to replicate previous work showing the negative effect of Δ(9)-THC on hippocampal LTP. We then progressed to CB1 and CB1:CB2 knockout (KO) mice to elucidate the role of the different cannabinoid receptor subtypes. The RAMEB buffer was incorporated to increase solubility of the Δ(9)-THC in artificial cerebral spinal fluid as previously reported[4]. The CB1 KO mice showed a decrease in potentiation compared to wild type mice, an effect not seen in the CB1:CB2 double KO mice. However, unexpectedly, the RAMEB buffer had a genotype-specific effect on LTP, which was independent of Δ(9)-THC. RAMEB potentiated the LTP response in wild type mice, an effect that was attenuated by the addition of 8μM Δ(9)-THC. In CB1:CB2 double KO mice, the RAMEB buffer alone depressed the LTP below baseline. This effect was again mitigated by the addition of 8μM Δ(9)-THC. This interesting phenomenon highlights the importance of carefully examining the effects of vehicle controls, and suggests that RAMEB alone can alter LTP. 1. Nowicky AV., Teyler TJ. and Vardaris RM. The modulation of long-term potentiation by delta-9-tetrahydrocannabinol in the rat hippocampus, *in vitro*. *Brain Research Bulletin*, *1986*. Vol. 19:663-672. 2. Hoffman AF., Oz M., Yang R., Lichtman AH. and Lupica CR. Opposing actions of chronic Δ^9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learning & Memory, 2007*. Vol 14:63-74. 3. Hazekamp A. and Verpoorte R. Structure elucidation of the tetrahydrocannabinol complex with randomly methylated β-cyclodextrin. *European Journal of Pharmaceutical Sciences, 2006*. Vol 29:340-347. 4. Laaris N., Good CH. and Lupica CR. Δ^9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. *Neuropharmacology, 2010.* Vol 59:121-127

Disclosures: M. Sperow: None. **H. Zaniewski:** None. **L.G. Kirby:** None. **M.E. Abood:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.01/AA16

Topic: C.17. Drugs of Abuse and Addiction

Support: DA031699

Title: Dissecting the contribution of cannabinoid type-1 receptor activation to reward circuitry function

Authors: *B. TURNER[1,3], B. A. GRUETER[2,3];
[2]Anesthesiol., [1]Vanderbilt Univ. Med. Ctr., Nashville, TN; [3]Vanderbilt Brain Inst., Nashville, TN

Abstract: Forebrain excitatory inputs to the nucleus accumbens shell (NAcSh) are essential for encoding the salience of reward-associated cues and initiating goal directed behavior. In addition to its established role in addiction, past work has shown that NAcSh pharmacological manipulations, including infusion of the endocannabinoid (eCB) 2-arachidonyl glycerol, can induce voracious feeding in satiated animals suggesting that the NAcSh may be a key node in appetitive nutrient-independent feeding circuits. Given the prevalence of obesity and its high correlation with overconsumption of calorically dense and highly palatable foods, understanding the circuitry of brain regions that regulate hedonic feeding may be beneficial to treating or preventing obesity. Interestingly, recent epidemiological studies have noted that, paradoxically, use of cannabis sativa correlates with a lower body mass index and a reduced prevalence of diabetes despite its hyperphagic effects. Activation of cannabinoid type-1 receptors (CB1Rs) by the exogenous ligand THC specifically at glutamatergic terminals in the forebrain is required to induce hyperphagia. The strength of forebrain-NAc connectivity is known to be influenced by exposure to rewarding stimuli, including drugs of abuse, in an eCB dependent manner, and the strength of these inputs can influence response strength following repeated exposures. Additionally, CB1R signaling is necessary for the generation of psychostimulant-induced behavioral plasticicty. Despite its involvement in multiple aspects of motivated behavior, it remains to be shown how eCB signaling impacts the strength of region-specific afferents and their integration at NAcSh medium spiny neurons in different neural states. Therefore, we have investigated excitatory transmission within the NAcSh using *ex vivo* electrophysiology techniques following metabolic challenge and an amphetamine induced locomotor sensitization task using region specific CB1R knock out animals. We have found that prolonged consumption of a high fat diet (HFD) or acute fasting is sufficient to cause enhanced sensitivity to synaptically-induced long term depression (LTD) of excitatory transmission within the NAcSh. Additionally, knocking out CB1R expression on PFC afferents is sufficient to blunt amphetamine induced behavioral plasticity. These data suggest that CB1R signaling at glutamatergic inputs to the NAcSh is influenced by metabolic state and that CB1Rs may play a role in processing rewarding stimuli by selectively altering the strength of specific afferent fibers and potentially gating hedonic food intake.

Disclosures: B. Turner: None. **B.A. Grueter:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

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Program#/Poster#: 811.02/AA17

Topic: C.17. Drugs of Abuse and Addiction

Support: DA031699

Title: Investigation of parvalbumin expressing interneurons in the nucleus accumbens

Authors: *D. GHOSE[1,2], E. B. BISEN-HERSH[2], B. A. GRUETER[2,3];
[1]Dept. of Psychology, Vanderbilt Univ., Nashville, TN; [2]Anesthesiol., Vanderbilt Univ. Sch. of Med., Nashville, TN; [3]Vanderbilt Brain Inst., Nashville, TN

Abstract: The nucleus accumbens (NAc) is a key brain structure involved in integrating signals from external and internal stimuli ultimately gating emotional and motivational states. The projection neurons of the NAc, GABA-ergic medium spiny neurons (MSNs), comprise 90%-95% of the neuronal population. The rest, 5-10%, are interneurons (INs). Based on morphological, physiological and histochemical properties, recent studies have identified four main classes of NAc INs. Of these inter-neuronal sub-types, parvalbumin containing interneurons (PV-INs) and calretinin containing interneurons are GABA-ergic in nature while choline acetyltransferase (Chat) and nitric oxide synthase expressing interneurons are the main sources of acetylcholine and nitric oxide in the NAc. It is well established that NAc MSNs receive the majority of their excitatory glutamatergic inputs from Prefrontal Cortex (PFC), basolateral amygdala (BLA) and ventral subiculum (vSub) while the inhibitory drive onto the MSNs arises mainly from two sources: GABA-ergic INs that synapse onto proximal dendrites and cell bodies of the MSNs, and neighboring MSN collaterals that preferentially synapse onto the distal dendrites of MSNs. Inhibitory synapses onto or near the MSN cell body allows the GABAergic INs to exert a strong influence on MSN activity by mediating feed-forward inhibition despite comprising a small proportion of the total NAc neuronal population. Hence we hypothesize that it is the balance between excitation and inhibition that controls MSN output and in turn influences reward-related behaviors under both physiological and pathological conditions. The function of excitatory synapses onto the MSNs and its influence on reward-related behavior has been widely studied. In contrast, less is known regarding excitatory transmission onto INs and the role of inhibitory control on NAc function. PV-INs were tagged with D1tdTomato by crossing PV-cre mice with Ai9 mice. We performed targeted PV-IN and MSN whole-cell patch-clamp recordings of electrically evoked EPSCs and IPSCs. This allowed us to measure excitatory drive onto the PV-INs and excitatory and inhibitory drive onto MSNs. In addition, the

differential role of specific excitatory inputs onto the PV-INs was investigated utilizing optogenetics. The results of these studies afford a more thorough understanding of the microcircuitry of the NAc.

Disclosures: D. Ghose: None. **E.B. Bisen-Hersh:** None. **B.A. Grueter:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.03/AA18

Topic: C.17. Drugs of Abuse and Addiction

Support: DA031699

Title: NPY and CCK interneurons lacking GAD1 have distinct behavioral responses to cocaine

Authors: *E. B. BISEN-HERSH[1], D. GHOSE[1], K. GARBETT[2], K. MIRNICS[2,3,4], B. A. GRUETER[1,3,4,2];
[1]Anesthesiol., [2]Psychiatry, Vanderbilt Univ. Med. Ctr., Nashville, TN; [3]Vanderbilt Brain Inst., [4]Vanderbilt Kennedy Ctr. for Res. on Human Develop., Vanderbilt Univ., Nashville, TN

Abstract: There are many diverse GABA-ergic interneuron cell-types with distinct function and expression patterns in different brain regions. Mature interneurons can be distinguished by protein expression, electrical properties and laminar distribution. However, cell type specific effects of distinct interneuron populations on behavior remain largely unknown. As behavioral adaptations depend on intricate communication within neural circuits, disruption of GABA-ergic transmission within specific cell types has been implicated in many neuropsychiatric disorders. To date, much emphasis has been put on the influence of inter-regional circuit connectivity on reward-related behavior and adaptations in these connections continue to be elucidated at a rapid pace. In contrast, there remains a relative paucity of information about the changes in behavioral outcomes as a result of modifications in interneuron microcircuits: information that is critical for a thorough understanding of the circuit adaptations underlying addiction. The present study used transgenic mouse lines that silence glutamic acid decarboxylase 1 (GAD1) transcript in either cholecystokinin (CCK) or neuropeptide Y (NPY) interneuron populations by expressing miRNA for GAD1 driven by the CCK and NPY promoters. GAD1 produces the majority of GABA in the brain. Using these mouse lines, the consequences of GAD1 deficiency in two interneuronal cell types on reward-related behavior was examined. Baseline assessment of locomotor activity and

thigmotaxis was measured during a 60 min open field assay. Cocaine-induced locomotor sensitization was compared between NPY-miGAD1, CCK-miGAD1, and wildtype mice by administration of cocaine (20 mg/kg, i.p.). Additionally, conditioned place preference was used to assess the rewarding effects of cocaine following GAD1 deficiency in these mouse lines. Finally, we analyzed synaptic properties from NPY- and CCK-miGAD1 mice treated with cocaine or saline. NPY- and CCK-miGAD1 mice displayed differential responses to cocaine in these assays. In summary, the present study characterizes the adaptations to reward-related behavior in mice lacking GABA in either CCK or NPY expressing cells and demonstrates differential cocaine-induced adaptations in these mouse lines. These results suggest that GABAergic signaling from NPY and CCK cells may serve as important, but distinct, neuronal targets for cocaine to induce behavioral alterations related to addiction.

Disclosures: E.B. Bisen-Hersh: None. **D. Ghose:** None. **K. Garbett:** None. **K. Mirnics:** None. **B.A. Grueter:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Program#/Poster#: 811.04/AA19

Topic: C.17. Drugs of Abuse and Addiction

Support: DA031699

Title: Deconstructing nucleus accumbens circuit functions with cell type-specific NMDA receptor deletions

Authors: *M. JOFFE[1], B. A. GRUETER[2];
[1]Pharmacol., [2]Anesthesiol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Dysregulation of the mesolimbic dopamine (DA) system is a hallmark of the pathophysiology of drug addiction and many other prevalent diseases. The nucleus accumbens (NAc), a region essential for the incentive and hedonic properties of drugs of abuse, is a primary site of these aberrant changes. 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: direct pathway MSNs project primarily to the midbrain DA areas and express D1 DA receptors, while indirect pathway MSNs project to the ventral pallidum and express A2A adenosine receptors. MSNs are generally quiescent, resting at

relatively hyperpolarized membrane potentials. Excitatory drive is therefore essential to governing the output of the NAc and subsequent behavioral outcomes. The predominant excitatory afferents to the NAc are the prefrontal cortex, ventral subiculum of the hippocampus, and basolateral amygdala. Respectively, these regions are thought to support behaviors related to motivational significance, contextual memories, and emotional reactivity. While much remains to be understood about reward-related changes in NAc circuit dynamics, drug-induced modifications of excitatory signaling (i.e. synaptic plasticity) in the NAc have been suggested to underlie the maladaptive behaviors observed in addiction. At glutamatergic synapses, plasticity is mediated in large part through AMPA receptor trafficking. In turn, AMPA receptor surface expression is modulated bidirectionally by activity of the NMDA receptor, a tetrameric glutamate receptor generally composed of two obligatory GluN1 subunits, and two GluN2 or GluN3 subunits. Recent data suggests that changes in NMDA receptor stoichiometry and function occur in the NAc following *in vivo* cocaine exposure, and that these events may necessarily precede the persistent synaptic changes observed alongside addiction-like behaviors. We hypothesize that *in vivo* cocaine experience alters AMPA receptor function at specific NAc synapses, and that NMDA receptor activity is essential for these phenomena and related behavioral abnormalities. Here, we used a bacterial artificial chromosome (BAC) transgenic Cre-Lox approach to genetically delete NMDA receptors via the D1 or A2A promotor. Our findings suggest that circuit-specific NMDA receptor function is important for the expression of reward-related behaviors that require learning and memory processes. Furthermore, we believe that excitatory synaptic strength and plasticity are compelling cellular mediators of these phenomena.

Disclosures: **M. Joffe:** None. **B.A. Grueter:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

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Program#/Poster#: 811.05/AA20

Topic: C.17. Drugs of Abuse and Addiction

Support: National Insitute on Drug Abuse - Intramural Research Program

Title: Incubation of methamphetamine craving after prolonged self-imposed abstinence in a contingency management alternative-reward procedure

Authors: ***D. CAPRIOLI**, M. VENNIRO, T. ZERIC, N. MARCHANT, Y. SHAHAM; Behavioral Neurosci., Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Background: We previously found that the rat's response to cues associated with methamphetamine progressively increases after forced abstinence from the drug (incubation of methamphetamine craving). Here, we determined whether incubation of methamphetamine craving would occur when abstinence is self-imposed (volitional), because of the availability alternative non-drug rewards, a condition that more closely mimics human abstinence. Methods: We trained non-food-deprived rats to lever-press for palatable food (6 days, 9 h/day; 5 pellets per reward delivery). We then trained them to lever-press for methamphetamine (12 days, 9 h/day 0.1 mg/kg/infusion). On abstinence day 1, we tested rats for cue-induced methamphetamine seeking in an extinction test and then assigned them to forced and volitional abstinence conditions. The forced-abstinence rats had no access to the drug- or food-paired levers for 20 days. The volitional-abstinence rats had access to the two levers during 20 daily mutually exclusive choices (10 min apart) between palatable food and methamphetamine. On day 21, we tested all rats for cue-induced methamphetamine seeking. Results: The rats reliably self-administered the palatable food and escalated methamphetamine self-administration over time. During the abstinence period, the volitional-abstinence group showed a strong preference (over 90%) for the palatable food over methamphetamine. The rats in both groups showed significantly higher cue-induced methamphetamine seeking on abstinence day 21 than on day 1. Conclusions: Availability of a non-drug reward during the abstinence period had no effect on incubation of methamphetamine craving. Our procedure may serve as an animal model of relapse after extended periods of contingency management in humans.

Disclosures: D. Caprioli: None. **M. Venniro:** None. **T. Zeric:** None. **N. Marchant:** None. **Y. Shaham:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.06/AA21

Topic: C.17. Drugs of Abuse and Addiction

Title: Persistent and inflexible palatable food preference in rats with a history of limited and extended access methamphetamine self-administration

Authors: *M. VENNIRO, T. ZERIC, Y. SHAHAM, D. CAPRIOLI;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Background: In recent studies of Ahmed, Lenoir, and colleagues reported that when rats are given a mutually exclusive choice between cocaine and palatable foods, most of them prefer the food over cocaine. Here, we used a discrete choice procedure to assess drug versus food preference in rats with a history of limited (3 h/d) and extended access (6 or 9 h/d) methamphetamine self-administration. Methods: On different daily sessions, we trained non-food-deprived rats to lever-press for either methamphetamine (0.1-0.2 mg/kg/infusion) or palatable food (5 pellets per reward delivery) for several weeks. We then assessed preference during the training phase, after priming injections of methamphetamine (0.5 and 1.0 mg/kg), after food-pellet exposure in the home cage (a satiety manipulation) prior to choice testing, and after extended abstinence (21 days). We also assessed progressive-ratio responding for food and methamphetamine. Results: We found that independent of the daily drug access conditions and the abstinence period, the rats strongly preferred the palatable food, even when the food pellets were freely available in their home cage. Progressive ratio for methamphetamine, which increased over time, did not predict preference. Conclusions: Under our experimental conditions, most rats strongly and inflexibly prefer palatable food pellets over methamphetamine, confirming previous studies using discrete choice procedures with cocaine. We currently assess food-methamphetamine preference during extended training periods (2-3 months).

Disclosures: M. Venniro: None. **T. Zeric:** None. **Y. Shaham:** None. **D. Caprioli:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

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Program#/Poster#: 811.07/AA22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: The cfos-tetO-Cre transgenic rat system: A new tool to examine neuronal ensembles in learned behavior

Authors: *F. C. CRUZ[1], R. M. LEAO[1], Y. ZHANG[2], K. R. BABIN[1], C. T. RICHIE[3], J. M. PICKEL[4], Y. SHAHAM[1], B. K. HARVEY[3], B. T. HOPE[1];
[1]Natl. Inst. on Drug Abuse - NIDA, [2]OTTC - Natl. Inst. on Drug Abuse - NIDA, [3]OTTC- Natl. Inst. on Drug Abuse - NIDA, [4]NIMH transgenic core facility, NIH, Baltimore, MD

Abstract: Learned associations are thought to be encoded within specific patterns of sparsely distributed neurons called neuronal ensembles. However it has been difficult to manipulate and examine their unique properties because their only distinction is increased activation state during behavior. To address this problem, we developed bitransgenic rats that contain two transgenes: (1) the cfos-tetO::Cre recombinase transgene has a c-fos promoter that drives expression of Cre recombinase in strongly activated neurons, and (2) the EF1a-TetR transgene has an EF1a promoter that drives constitutive expression of a Tet repressor protein that binds to the tetO site in the cfos-tetO::Cre transgene to repress Cre transcription. Systemic injections of tetracycline remove TetR repression and open a short time window for Cre induction than can then invert and activate DIO (double-floxed inverse open reading frame) versions of virally expressed transgenes only in those neurons recently activated during behavior. We determined the dose response and time course for tetracycline-induced opening of the c-fos activation window in ventral medial prefrontal cortex (vmPFC). We injected adeno-associated virus (AAV) with a Cre-dependent EF1a::DIO-eYFP transgene into vmPFC to label activated neurons with YFP. One week later, we injected tetracycline (0-10 mg/kg, i.p) 2 hr prior to injection of cocaine (30 mg/kg, i.p.). We found strong YFP expression following only 2.5, 5 and 10 mg/kg tetracycline. We then injected 5 mg/kg tetracycline 0-24 hr before injecting cocaine and found YFP transgene expression only when tetracycline was injected 0.5-6 hours, but not 12 or 24 hours, before the cocaine injections. We are currently using the cfos-tetO-Cre transgenic rat system to study the role of neuronal ensembles and their unique alterations in context-induced reinstatement of cocaine seeking.

Disclosures: F.C. Cruz: None. **R.M. Leao:** None. **Y. Zhang:** None. **K.R. Babin:** None. **C.T. Richie:** None. **J.M. Pickel:** None. **Y. Shaham:** None. **B.K. Harvey:** None. **B.T. Hope:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH NIDA IRP

Title: Role of prefrontal cortical neuronal ensembles in appetitive (palatable food) operant extinction learning in rats

Authors: *B. L. WARREN, F. C. CRUZ, R. M. LEAO, K. R. BABIN, K. MCPHERSON, Y. SHAHAM, B. T. HOPE;
NIDA IRP/NIH, Baltimore, MD

Abstract: In operant tasks, 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. Memories encoding reinforced and non-reinforced (extinction) responding are thought to be distinct and are likely encoded by different patterns of sparsely distributed neurons called 'neuronal ensembles.' Here, we assess activation of infralimbic and prelimbic mPFC neuronal ensembles during an operant appetitive task. We trained food-restricted rats to lever press for palatable food pellets for 7 days (60 min/day). Rats in the Extinction group underwent 7 daily extinction sessions while rats the No Extinction group were not. On test day, we exposed half of the rats from each group to a 15 min extinction session and perfused them with paraformaldehyde 75 min after testing. The other rats from each group were not tested. We then assessed Fos (a marker of neuronal activity) expression in infralimbic and prelimbic mPFC. Non-reinforced responding during testing was higher in the No Extinction than in the Extinction group. Fos immunoreactivity in infralimbic mPFC of the Extinction group was higher than in the No Extinction groups; an opposite pattern of results was observed in prelimbic mPFC. Our data suggest anatomical double dissociation between mPFC neuronal ensembles encoding rewarded operant responding and extinguished operant responding.

Disclosures: B.L. Warren: None. **F.C. Cruz:** None. **R.M. Leao:** None. **K.R. Babin:** None. **K. McPherson:** None. **Y. Shaham:** None. **B.T. Hope:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.09/AA24

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Role of histone deacetylase 5 in dorsal striatum in incubation of methamphetamine craving

Authors: *T. ZERIC[1], M. B. CARREIRA[2], H. CATES[3], P. KENNEDY[4], S. KAMBHAMPATI[1], J. M. BOSSERT[1], E. J. NESTLER[3], C. W. COWAN[2], Y. SHAHAM[1], X.

LI[1];
[1]NIDA, Baltimore, MD; [2]Dept. of Psychiatry, Harvard Med. Sch., Belmont, MA; [3]Fishberg Dept. of Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Dept. of Psychology, Univ. of California Los Angeles, Los Angeles, CA

Abstract: Background: We previously reported that cue-induced methamphetamine seeking progressively increases after withdrawal from extended access methamphetamine self-administration (incubation of methamphetamine craving). Here we studied the role of striatal histone deacetylase 5 (HDAC5), recently implicated in cocaine behavioral effects, in incubation of methamphetamine craving. Methods: In Exp. 1, we trained rats to self-administer intravenous methamphetamine or saline (control) for 10 days (9 h/d) and then measured HDAC5 mRNA and protein levels in ventral and dorsal striatum on withdrawal days 2 and 35. In Exp. 2, we injected adeno-associated virus bilaterally into dorsal striatum to express either GFP (control) or a mutant form of HDAC5 (MUTANT-HDAC5, which strongly localizes to the nucleus). After training rats to self-administer methamphetamine, we tested cue-induced drug seeking in extinction tests on withdrawal day 2 and 35. We then assessed re-escalation of methamphetamine intake and responding on a progressive ratio reinforcement schedule. Results: We found that methamphetamine self-administration increased HDAC5 mRNA and protein in dorsal but not ventral striatum on withdrawal day 35 but not day 2. MUTANT-HDAC5 viral expression in dorsal striatum increased cue-induced extinction responding on day 35 but not day 2; dorsal striatum MUTANT-HDAC5 viral expression also increased re-escalation of methamphetamine self-administration after prolonged withdrawal and progressive ratio responding. Conclusions: Our results suggest a role for nuclear HDAC5 in the dorsal striatum in both incubation of methamphetamine craving and methamphetamine reward. We are currently examining the effect of inhibiting endogenous HDAC5 in dorsal striatum on incubation of methamphetamine craving.

Disclosures: T. Zeric: None. **M.B. Carreira:** None. **H. Cates:** None. **P. Kennedy:** None. **S. Kambhampati:** None. **J.M. Bossert:** None. **E.J. Nestler:** None. **C.W. Cowan:** None. **Y. Shaham:** None. **X. Li:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Program#/Poster#: 811.10/BB1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Unique molecular alterations in dorsal striatal neuronal ensembles selectively activated during context-induced reinstatement of methamphetamine seeking in rats

Authors: *F. J. RUBIO;
Behavioral Neurosci. Res. Branch, NIDA IRP, NIH, Baltimore, MD

Abstract: Background: Learned associations between environmental cues and drug taking promote relapse in drug addicts. Here we assessed molecular alterations induced specifically in Fos-expressing dorsal striatal neurons that were activated by the drug-associated context. Methods: We trained rats to self-administer methamphetamine in context A, extinguished drug-reinforced responding in a distinct context B and assessed context-induced reinstatement under extinction condition in context A. In Exp. 1 we examined the effects of injecting the GABA agonists muscimol+baclofen into dorsomedial and dorsolateral striatum on context-induced reinstatement. In Exp. 2, we used immunohistochemical detection of the neural activity marker Fos and the neuronal marker NeuN to determine the degree of neural activation in dorsal striatum. In Exp. 3, we isolated Fos-positive and Fos-negative dorsal striatal neurons using fluorescence-activated cell sorting (FACS) and assessed gene expression from the sorted neurons using gene-targeted pre-amplification and quantitative PCR; we also used quantitative *in situ* hybridization with RNAscope® to assess expression of Grin2a gene encoding NMDA subunit NR2A in Fos-positive neurons from dorsal striatum brain sections. Results: Reversible inactivation of dorsolateral but not dorsomedial striatum decreased context-induced reinstatement. Context-induced reinstatement increased the number of Fos-positive neurons 2-3 fold in dorsal striatum. qPCR data from FACS-isolated neurons indicated selective upregulation of immediate early genes, as well as the NMDA receptor subunit gene Grin2a, in Fos-positive, but not Fos-negative, neurons. The RNAscope® assay confirmed that Grin2a mRNA was preferentially increased in Fos-positive neurons in dorsolateral, but not dorsomedial, striatum. Conclusions: Results demonstrate a role of dorsolateral striatum in context-induced reinstatement of methamphetamine seeking and that this reinstatement is associated with unique molecular alterations in activated neuronal ensembles in this brain region.

Disclosures: F.J. Rubio: None.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Program#/Poster#: 811.11/BB2

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Incubation of methamphetamine craving is associated with selective increases of the epigenetic HDAC5 gene in FACS-sorted Fos-positive activated neurons in dorsal striatum

Authors: *X. LI[1], F. J. RUBIO[1], T. ZERIC[1], R. CIMBRO[2], Q.-R. LIU[1], B. T. HOPE[1], Y. SHAHAM[1];
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Abstract: Background: We recently found that incubation of methamphetamine craving (time-dependent cue-induced methamphetamine seeking after withdrawal) is associated with increased (about 50%) histone deacetylase 5 (HDAC5) expressions in dorsal striatum homogenates. We also found that viral expression of nuclear HDAC5 in dorsal striatum enhanced this incubation (Zeric et al. SFN 2014). Here we used fluorescence-activated cell sorting (FACS) to examine whether incubation of methamphetamine craving is associated with increased HDAC5 gene expression in Fos-positive neurons selectively activated in dorsal striatum by methamphetamine-associated cues after prolonged withdrawal. Methods: We trained rats to self-administer intravenous methamphetamine for 10 days (9 h/d) and then either tested or not tested the rats between withdrawal days 30 to 50 for cue-induced methamphetamine seeking under extinction conditions. After extinction tests or no test, we used FACS to isolate dorsal striatum Fos-positive and Fos-negative neurons. We then measured mRNA expression of both immediate early genes (c-fos, Arc, Fosb, Egr1) and Hdac5 by gene-targeted pre-amplification and quantitative RT-PCR. Results: Cue-induced methamphetamine seeking during the late withdrawal extinction tests increased c-fos, Fosb (total), Arc and Egr1 mRNA expression in Fos-positive but not Fos-negative neurons. Moreover, Hdac5 mRNA expression was selectively increased (about 300%) in Fos-positive neurons of rats that underwent the extinction tests. Conclusions: Our results suggest that cue-selected activated neurons in rat dorsal striatum exhibit an HDAC5-regulated genetic profile that is distinct from that of non-activated neurons. Results also suggest that methamphetamine-induced neuroadaptations identified in brain homogenates likely underestimate the neuroadaptations induced in neurons activated by methamphetamine cues, which potentially play a role in drug relapse

Disclosures: X. Li: None. **F.J. Rubio:** None. **T. Zeric:** None. **R. Cimbro:** None. **Q. Liu:** None. **B.T. Hope:** None. **Y. Shaham:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.12/BB3

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Role of ventral medial prefrontal cortex neuronal ensembles in context-induced reinstatement of cocaine seeking

Authors: *R. M. LEÃO, F. C. CRUZ, K. R. BABIN, J. M. BOSSERT, Y. SHAHAM, B. T. HOPE;
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Abstract: In human addicts and rat models, cues associated with previous drug use can provoke relapse to drug seeking. Learned associations between cues, context and drug are thought to be encoded by specific patterns of sparsely distributed neurons called neuronal ensembles. We trained rats to lever-press for cocaine (0.5 and 1 mg/kg/infusion) for 10 days in Cocaine context A; lever-presses were paired with a tone-light compound cue . Next the rats underwent 12+ daily extinction sessions in Extinction context B. On test day, re-exposure to Cocaine context A, but not Extinction context B, reinstated cocaine seeking and increased expression of the neural activity marker Fos in 9.4% of ventral medial prefrontal cortex (vmPFC) neurons. To assess a causal role for these activated neurons in context-induced reinstatement, we trained cfos-lacZ transgenic rats that co-express β-galactosidase and Fos to self-administer cocaine in Cocaine context A and extinguish lever pressing in Extinction context B. On 'induction day', we exposed the rats to either Cocaine context A or a Novel context C (round bowl with bedding) for 30 min and injected the prodrug Daun02 or vehicle into vmPFC 60 min later. β-galactosidase catalyzes Daun02 into daunorubicin that lesions the activated neurons. On test day, three days later, reinstatement of cocaine seeking in context A and vmPFC neuronal activity were attenuated in rats previously injected with Daun02 after exposure to Cocaine context A, but not to Novel context C. We conclude that a small subset of vmPFC neurons form neuronal ensembles that encode learned associations between cocaine and the drug-associated context and play a role in context-induced reinstatement of cocaine seeking.

Disclosures: R.M. Leão: None. **F.C. Cruz:** None. **K.R. Babin:** None. **J.M. Bossert:** None. **Y. Shaham:** None. **B.T. Hope:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.13/BB4

Topic: C.17. Drugs of Abuse and Addiction

Title: Assessing dendritic spine plasticity in transgenic rat nucleus accumbens neuronal ensembles activated during amphetamine sensitization

Authors: *R. V. FALLON[1,2], F. C. CRUZ[2], B. T. HOPE[2];
[1]Cellular, Molecular, Developmental Biol. and Biophysics, Johns Hopkins Univ., Baltimore, MD; [2]Behavioral Neurosci. Br., NIDA IRP, Baltimore, MD

Abstract: We have previously shown that conditioned drug behaviors in rats are mediated by specific patterns of sparsely distributed neurons called neuronal ensembles. We are now developing a procedure for assessing morphological alterations of dendritic spines in Fos expressing neuronal ensembles after amphetamine sensitization. In our initial experiments, we injected rats with adeno-associated virus (AAV) that constitutively expresses a membrane-restricted version of GFP (memGFP) to label plasma membranes including dendritic spines. One week after low-titer AAV injections, rats were perfused and sections collected from the nucleus accumbens. We are currently using confocal microscopy to collect images for dendritic spine analysis. In future experiments, we will use our cfos-tetO-iCre transgenic rat system to induce memGFP exclusively in neuronal ensembles that were activated during context-specific amphetamine sensitization.

Disclosures: R.V. Fallon: None. **F.C. Cruz:** None. **B.T. Hope:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: INSERM ATIP-AVENIR

ERC StG SalienSy 335333

Title: Cellular mechanisms underlying cocaine-evoked plasticity in the lateral habenula

Authors: ***M. MAMELI**[1], S. LECCA[1], K. VALENTINOVA[1], L. MARION-POLL[1], J.-A. GIRAULT[1], S. MUSARDO[2], F. GARDONI[2], F. GEORGES[3], F. MEYE[1];
[1]Inserm UMR S839 Inst. Du Fer A Moulin, Paris, France; [2]Dept. of Pharmacol., University of Milano, Italy; [3]Bordeaux Neurosci. Inst., Inserm, France

Abstract: The lateral habenula (LHb) powerfully inhibits dopamine neurons through its projections to the rostromedial tegmental nucleus (RMTg), and has emerged as a key structure encoding aversive states. Addictive drugs, such as cocaine, functionally modify the habenulo-mesencephalic circuitry. However the underlying mechanisms remain so far elusive. We combined retrograde tracing, ex-vivo and *in vivo* electrophysiology to assess the influence of cocaine experience on excitatory transmission onto a subset of LHb neurons that specifically project their axons to the RMTg. We find that, in mice, cocaine exposure increases the amplitude of miniature excitatory postsynaptic currents (mEPSCs), but not their frequency, indicating an increased efficacy of glutamatergic synapses onto RMTg-projecting LHb neurons. The cocaine evoked plasticity requires dopamine 2 receptors activation as pretreatment *in vivo* with specific antagonists prevents the cocaine-induced synaptic adaptations. We next used a viral-based strategy that allows expressing a dominant-negative sequence that interferes with the activity dependent delivery of AMPA receptors at the membrane to test the postsynaptic requirements for the cocaine-evoked plasticity. Expression of the dominant negative in the LHb prevents the cocaine-evoked plasticity in RMTg-projecting neurons. Furthermore, we find that AMPA receptors exocytosis in the LHb depends on the phosphorylation of the serine 845 residue in the C terminal of GluA1 subunits, as in a mouse with a single mutation at the serine 845, cocaine does not produce synaptic plasticity. Using *in vivo* and *in vitro* electrophysiology we find that cocaine exposure increases the excitability of LHb neurons. Precluding AMPAR trafficking, not only blocked the synaptic adaptations but also the increased excitability. Altogether, drug-driven modifications in the LHb-to-RMTg pathway at the synaptic and cellular level may contribute to specific aspects of drug taking, highlighting the LHb as a potential hub controlling cocaine-evoked behavioral adaptations.

Disclosures: **M. Mameli:** None. **S. Lecca:** None. **K. Valentinova:** None. **L. Marion-Poll:** None. **J. Girault:** None. **S. Musardo:** None. **F. Gardoni:** None. **F. Georges:** None. **F. Meye:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA034943

DA015835

DA009621

DA029099

DA36963

Title: Differential calcium signaling mediated by NMDA and AMPA receptors in individual dendritic spines of nucleus accumbens medium spiny 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 seeking intensifies or incubates during withdrawal from extended-access cocaine self-administration and is mediated by GluA2-lacking, Ca2+-permeable AMPARs (CP-AMPARs) in the nucleus accumbens (NAc). A previous study from our lab (Ferrario et al. 2012) demonstrated that a smaller percentage of NAc medium spiny neuron (MSN) spines in "incubated rats" responded to glutamate uncaging, in the presence of CNQX, with NMDAR-mediated Ca2+ entry compared to saline controls. We speculated that unresponsive spines might be "new spines" that have accumulated CP-AMPARs but not NMDARs. The goal of this study is to assess Ca2+ entry into MSN dendritic spines via CP-AMPARs and/or NMDARs utilizing 2-photon microscopy, whole cell electrophysiology, and photo uncaging of NPEC-caged-(S)-AMPA and MNI-caged-NMDA compounds following withdrawal from saline or cocaine self-administration. In preliminary studies, the sequential photolysis of caged NMDA and caged AMPA allowed for the identification of different populations of dendritic spines based on their complement of NMDARs and CP-AMPARs (conclusions about the presence of GluA2-containing AMPARs cannot be drawn because they do not pass Ca2+). In both groups of rats, individual spines on MSNs in the NAc showed Ca2+ responses to: 1) caged AMPA but not caged NMDA, 2) caged NMDA but not AMPA, or 3) both caged AMPA and NMDA compounds. Our preliminary results suggest an increase in the percent

of spines responding to caged AMPA in the cocaine group. In addition, electrophysiological measures of somatic current following NPEC-AMPA but not MNI-NMDA application increased in cells from cocaine rats. These are some of the first experiments to utilize caged AMPA to characterize Ca2+ signaling in dendritic spines and the first to analyze cocaine-induced AMPAR plasticity in functional terms at the single spine level. **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

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA009621

DA015835

DA029099

DA036950

Title: Role of protein translation in regulating surface and synaptic AMPA receptor levels in cultured nucleus accumbens neurons

Authors: *M. E. WOLF, C. T. WERNER, K. K. Y. WOO, J. LOWETH;
Neurosci., Rosalind Franklin Univ. of Med. and Sci., NORTH CHICAGO, IL

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 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). Recently, it has been demonstrated that ongoing protein synthesis is required for the maintenance

of this adaptation. Thus, after NAc slices obtained from "incubated rats" were treated for 1 h with inhibitors of translation, NAc neurons in the slices no longer showed elevated CP-AMPAR levels (Scheyer et al., 2014). However, studying the role of protein synthesis in maintaining CP-AMPARs is difficult *in vivo*. Therefore, we are using a model system consisting of rat NAc neurons co-cultured with prefrontal cortex neurons from enhanced cyan fluorescent protein (CFP)-expressing mice. The cortical neurons restore excitatory input onto the NAc neurons but can be distinguished based on their fluorescence. NAc neurons 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). To determine the role of ongoing protein synthesis in regulating surface and synaptic levels of AMPARs in NAc neurons, we are examining surface expression, synaptic expression and turnover of the AMPAR subunits GluA1 and GluA2 after pharmacological inhibition of protein translation, as well as expression of proteins that regulate AMPAR levels, including Arc and Ube3A. 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: **M.E. Wolf:** None. **C.T. Werner:** None. **K.K.Y. Woo:** None. **J. Loweth:** None.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA009621

DA015835

DA029099

DA036950

Title: UPS activity in the nucleus accumbens: Parsing out effects of cocaine self-administration, withdrawal time, and memory retrieval

Authors: *C. T. WERNER, M. MILOVANOVIC, J. A. LOWETH, M. E. WOLF; Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: The ubiquitin-proteasome system (UPS), responsible for proteasomal-dependent protein degradation, has been implicated in synaptic reorganization associated with memory retrieval (Lee et al., 2008, Jarome et al., 2011), including drug reward memory retrieval after cocaine conditioned place preference (Ren et al., 2013). However, nothing is known about its role in memory retrieval after self-administration of cocaine or how the length of withdrawal from cocaine may influence the UPS. Here we examined UPS activity in the nucleus accumbens (NAc) of rats that self-administered cocaine for 6 h/day for 10 days, a regimen leading to withdrawal-dependent intensification ("incubation") of cue-induced seeking. Control rats self-administered saline. We used a ubiquitin, Lys-48 antibody to measure polyubiquitinated proteins bound for proteasomal degradation and a fluorescence assay to quantify catalytic (chymotrypsin- and trypsin-like) activities of the proteasome. First, we compared rats that received a cue-induced seeking test on withdrawal WD50-60 (memory retrieval groups) to rats that were killed after 55 days of withdrawal in their home cages (no retrieval). Responding in the active hole during the test delivers the drug-associated cue (light) but no cocaine. We expected the cocaine group to show elevated polyubiquitination after the test, since the NAc mediates the expression of incubated cue-induced seeking after prolonged withdrawal. To our surprise, we found significant increases in polyubiquitinated proteins in the NAc of the retrieval groups compared to the no retrieval groups, but no difference between cocaine and saline groups. This suggests that patterns of protein tagging for proteasomal degradation are affected by memory retrieval but are independent of prior drug treatment. We also quantified proteasome activity following retrieval. Consistent with our results on polyubiquitination, seeking tests conducted on WD50-60 were not associated with significant changes in proteasome activity in the NAc in rats that self-administered cocaine compared to saline. However, we did find changes in other brain regions examined, including the amygdala, dorsal striatum and medial prefrontal cortex. In contrast, we found that activity was significantly increased in the NAc, as well as the dorsal striatum and medial prefrontal cortex, in cocaine rats compared to saline rats that underwent a seeking test on WD1. We conclude that the relationship between UPS activity and memory retrieval is complex and dependent on training paradigm, brain region and the time elapsed since training.

Disclosures: C.T. Werner: None. **M. Milovanovic:** None. **J.A. Loweth:** None. **M.E. Wolf:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA009621

DA029099

DA036327

Title: Development of mGluR-LTD in the nucleus accumbens during withdrawal from extended-access cocaine self-administration

Authors: *A. F. SCHEYER[1], M. E. WOLF[2], K. Y. TSENG[3];
[1]Neurosci., [2]Dept. of Neurosci., [3]Dept. of Cell. and Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Prolonged withdrawal from extended-access cocaine self-administration (6 h/d, 10d) is associated with incubation of cocaine craving as well as several changes in medium spiny neurons (MSN) of the nucleus accumbens (NAc). These include accumulation of Ca2+-permeable AMPA receptors (CP-AMPAR) and a switch from group I metabotropic glutamate receptor (mGluR) 5- to mGluR1-mediated synaptic depression, typically after withdrawal day (WD) 40. More importantly, these adaptations are disrupted by protein-synthesis interference, indicating ongoing active processes required for their maintenance and therefore the maintenance of drug-seeking behavior. In order to better understand the mechanisms underlying the emergence of this novel form of plasticity, we conducted whole-cell patch-clamp recordings of NAc MSN at multiple time-points during withdrawal and examined the nature of mGluR-mediated synaptic depression through bath-application of the group I mGluR agonist, DHPG (50μM). In contrast to results obtained in saline controls or earlier in withdrawal from cocaine, DHPG application in slices obtained after WD15 induced a form of synaptic depression which persisted for >30min after washout of the drug (mGluR-LTD). This mGluR-LTD was maintained through WD45 and beyond. To determine if this mGluR-LTD was mediated by the removal of CP-AMPARs as indicated by previous work characterizing mGluR-mediated synaptic depression after prolonged withdrawal (>WD45), we used the rectification index in addition to the CP-AMPAR antagonist naspm (100μM) to quantify the contribution of CP-AMPAR transmission at numerous points during withdrawal. Surprisingly, we found that CP-AMPAR-mediated synaptic transmission in the NAc was significantly elevated only after WD25. Together, these results indicate that additional mechanisms underlie the development of mGluR-LTD at earlier withdrawal points. Ongoing studies will identify the mechanisms underlying the emergence of mGluR-LTD, thereby illuminating the processes that underpin the development of aberrant behaviors following prolonged withdrawal from extended-access cocaine self-administration.

Disclosures: **A.F. Scheyer:** None. **M.E. Wolf:** None. **K.Y. Tseng:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA009621

DA015835

DA029099

Title: Effects of extended access cocaine self-administration on inhibitory neurotransmission in the nucleus accumbens

Authors: *A. PURGIANTO, J. J. MIAO, M. MILOVANOVIC, M. E. WOLF;
Dept. of Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Excitatory synaptic transmission in the nucleus accumbens (NAc) undergoes time-dependent changes during withdrawal from extended access cocaine self-administration in concert with the incubation of cue-induced cocaine craving. The goal of this project is to determine if incubation of cocaine craving is also accompanied by plasticity of inhibitory transmission in the NAc. While prior studies have found changes in both presynaptic and postsynaptic aspects of inhibitory transmission after different cocaine regimens, very little is known about whether these measures are affected during incubation. Three types of studies are underway to compare rats after prolonged withdrawal (>45 days) from extended access cocaine or saline self-administration. The first is an immunocytochemical study of parvalbumin expression, which provides an index of the activity of parvalbumin positive (PV+) interneurons. We found no differences in cell count, indicating there was no loss or de novo generation of PV+ interneurons. However, we did find an increase in PV immunoreactivity in the NAc of the cocaine group compared to the saline group. When we separated our data into 3 rostral to caudal levels, we found that the increase in PV immunoreactivity was specific to middle- and caudal NAc. The second study aims to measure GABAA receptor subunit expression at several different withdrawal time points (2, 25, and 48 days) using biotinylation and immunoblotting. We found significant decrease in surface expression of the GABAA α2 subunit after 48 days of withdrawal from cocaine. No changes were observed for α1 on any withdrawal day or for α4 on withdrawal day 2. Our first two studies described above suggest potential pre- and post-synaptic alterations in inhibitory neurotransmission. Our third study uses electrophysiological local field potential (LFP) recordings to obtain a measure of overall inhibitory tone in the NAc. We are currently

analyzing LFP in the NAc after stimulation of medial prefrontal cortex (mPFC). In this mPFC-NAc circuit, there seems to be no alteration in basal synaptic strength, as indicated by lack of change in the input/output (I/O) curve. However, our results after train stimulations showed loss of potentiation after 5Hz and 10Hz stimulation in the cocaine group. To determine whether this is due to changes in GABA transmission, we are repeating these studies after intra-NAc injection of picrotoxin, a GABAA receptor antagonist. We are also investigating changes in the basolateral amygdala-NAc circuit. These studies are the first to explore changes in inhibitory neurotransmission in the NAc that may contribute to the withdrawal-dependent incubation of cocaine craving.

Disclosures: A. Purgianto: None. **J.J. Miao:** None. **M. Milovanovic:** None. **M.E. Wolf:** None.

Poster

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Title: Altering group I mGluR tone selectively affects surface expression of GluA2-lacking AMPA receptors in cultured nucleus accumbens neurons

Authors: *J. A. LOWETH[1], J. M. REIMERS[2], M. E. WOLF[1];
[1]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]UT Southwestern Med. Ctr., Dallas, TX

Abstract: After prolonged withdrawal from extended-access cocaine self-administration, Ca2+-permeable AMPA receptors (CP-AMPARs) accumulate in the nucleus accumbens (NAc) and mediate the withdrawal-dependent intensification ("incubation") of cue-induced cocaine craving. Using patch-clamp recordings in NAc slices from "incubated rats" (extended access-cocaine

self-administration and >40 days of withdrawal), we showed that acute bath application of the group I mGluR agonist DHPG rapidly removes CP-AMPARs from NAc synapses through an mGluR1-dependent mechanism and attenuates incubated craving. To study mechanisms underlying the interaction between mGluR1 and CP-AMPARs, we have taken advantage of the fact that Nac neurons co-cultured with prefrontal cortical (PFC) neurons contain a significant population of CP-AMPARs, making this a suitable *in vitro* model for NAc synapses in "incubated rats". To begin, we tested the effects of brief incubation with DHPG (50 μM, 10 min) on AMPAR surface expression in NAc neurons co-cultured with PFC neurons. Consistent with our findings in the incubation model, live cell labeling studies showed that DHPG significantly decreased GluA1 but not GluA2 surface expression, indicating that group I mGluR activation selectively internalizes GluA2-lacking, CP-AMPARs. Interestingly, in contrast to our findings in the incubation model, we found that incubation with either the mGluR1 antagonist LY 367385 (100 μM) or the mGluR5 antagonist MTEP (1 μM) prevented the DHPG-induced decrease in GluA1 surface expression, indicating that both receptor subtypes mediate DHPG-induced CP-AMPAR internalization in cultured NAc neurons. Additional studies focused on consequences of long-term changes in group I mGluR transmission. We found that 24 h of incubation with the mGluR1 antagonist LY 367385 (100 μM) leads to a significant increase in GluA1 surface area but no change in GluA2 levels. These studies are consistent with those in the incubation model showing that a decrease in mGluR1 tone during early withdrawal contributes to CP-AMPAR transmission in NAc synapses and incubation of cocaine craving. Studies are now underway to assess whether this effect is also produced in cultured NAc neurons following long-term inhibition of mGluR5-mediated transmission. By further characterizing group I mGluR-mediated regulation of AMPAR trafficking *in vitro*, these studies will provide insight into the mechanisms mediating enhanced AMPAR transmission and cue-induced cocaine seeking in the incubation model, as well as insight into mechanisms that mediate homeostatic adaptations in NAc synapses as a result of altered levels of glutamate transmission.

Disclosures: **J.A. Loweth:** None. **J.M. Reimers:** None. **M.E. Wolf:** None.

Poster

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Support: NIH K08 DA035972-01 (D. Bajic)

Trailblazer Award Department of Anesthesia at Boston Children's Hospital (D. Bajic)

NIH K24 (D. Borsook)

Title: Long-term behavioral effects after postnatal morphine exposure in a rat model

Authors: M. M. CRAIG, *D. BAJIC;
Dept Anesthesiol., Boston Children's Hosp., Boston, MA

Abstract: Prolonged morphine treatment in the developing brain is associated with a high incidence of opioid tolerance and dependence. This suggests that early postnatal opioid treatment runs the risk of significantly altering neural pathways. Our knowledge of the long-term consequences associated with prolonged post-natal opioid exposure is sparse. We hypothesized that prolonged morphine administration in a neonatal rat model of antinociceptive tolerance and dependence is associated with long-term behavioral changes in adulthood. Animals received either morphine (10 mg/kg; n=21) or equal volume of saline (n=17) subcutaneously twice daily for 13 ½ days (postnatal day (PD)1-14). Morphine treated animals underwent a weaning period of 10 days to prevent occurrence of any signs of withdrawal. Subsequently, all animals were tested in adulthood at PD55. The series of behavioral tests included: (1) Hot Plate test, (2) calibrated forceps test, (3) novel object test, (4) locomotor activity, and (5) forced swim test. These tests evaluated thermal and mechanical pain threshold, short-term memory, addiction potential, as well as anxiety/emotional processing, respectively. We combined data for male and female rats since no sex differences were found. Mean values were compared using t-test. Analysis revealed that thermal threshold (paw-withdrawal time (sec) ± SD) is increased in animals treated with morphine (12.4 s ± 3.0) in comparison to saline control (9.74 ± 2.77; p=0.015). No differences were found in adulthood between groups with respect to mechanical threshold, novel object recognition time, individual swim test activities (swimming, climbing, immobility), and total or individual locomotor activity (central, peripheral, rearing). Using a rat model of chronic postnatal morphine exposure, these novel results demonstrating selective long-term neuroplastic differences in sensory processing: thermal and not mechanical. Negative results are also important in implicating a lack of long-term alterations on memory, affective processing, and addictive behaviors.

Disclosures: M.M. Craig: None. **D. Bajic:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Title: Changes in expression of brain-derived neurotrophic factor and calbindin following behavioral sensitization to apomorphine in rats

Authors: D. J. LUSTBERG[1], M. P. HIND[1], *D. A. WELDON[2];
[1]Neurosci. Program, Hamilton Col., Clinton, NY; [2]Psychology, Hamilton Col., CLINTON, NY

Abstract: Behavioral sensitization has been demonstrated in response to chronic treatment with a variety of pharmacological agents and is thought to share common mechanisms with the development of drug dependence. Evidence has been provided for changes in a number of neurochemicals in the nucleus accumbens, prefrontal cortex, and hippocampus as a result of drug-induced sensitization. In the present experiment, we examined the expression of brain-derived neurotrophic factor (BDNF) and calbindin-D28k (CB) following sensitization to the dopamine receptor agonist apomorphine. Administration of 2 mg/kg (SC) of apomorphine for 14 days induced behavioral sensitization in adult male Sprague-Dawley rats. Control animals received an equivalent volume of isotonic saline. On Days 1, 7, and 14, the locomotor behavior of the animals was recorded for 20 minutes in an open field test conducted 15 minutes following injections. Sensitization was shown by a progressive increase in distance traveled along the peripheral zone of the open field across the three testing days in the APO group, with no changes in the distance traveled by the SAL group. Tracking records showed that the APO rats restricted their routes to the periphery of the open field. SAL rats entered the center zone more frequently than APO rats on each testing day. More BDNF and CB immunoreactivity was found in the ventral tegmental area, striatum, and hippocampus in APO animals compared to those of SAL animals. These increases in BDNF expression with APO-induced sensitization are similar to those previously demonstrated in rats sensitized to cocaine and amphetamine. In addition, the increase in the number of CB immunoreactive cells suggests that dynamic induction of this calcium-binding protein may be involved in the neuroadaptations associated with sensitization to dopaminergic drugs.

Disclosures: D.J. Lustberg: None. **M.P. Hind:** None. **D.A. Weldon:** None.

Poster

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Brain and Behavior Research Foundation Young Investigator Award

Title: A kappa opioid receptor antagonist reverses stress-induced neuroplasticity and cocaine-seeking even well after the stressor

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Abstract: Stress can be an important precipitating factor that drives escalation or relapse of drug-seeking behavior, and dopaminergic neurons in the ventral tegmental area (VTA) are an important site of convergence of drugs and stress. We previously identified a long-term potentiation of GABAergic synapses onto these neurons (LTP_{GABA}). Nitric oxide release by the dopaminergic neuron activates guanylate cyclase in the presynaptic GABAergic terminals, resulting in accumulation of cGMP, activation of PKG, and persistently increased release of GABA. Multiple drugs of abuse and stress block or inhibit LTP_{GABA} (Nugent et al, Nature, 2007; Niehaus et al, Eur J Neurosci 2010), suggesting that this common mechanism may play a role in addiction and stress-related diseases. Our recent work shows that the block of LTP_{GABA} by stress is dependent on both glucocorticoid (GR, Niehaus et al 2010) and kappa opioid receptors (KOR, Graziane et al, Neuron, 2013). We set out to understand how long the block of LTP_{GABA} lasts after stress and what is required to maintain the block. To address this, we performed whole-cell patch clamp recordings in slices prepared at various time points after acute cold-water swim stress. Here, we show that the block of LTP_{GABA} by stress is long lasting, persisting for five days after stress (control: IPSC amplitude 143±10% of baseline; 5 days post stress 101±13% of baseline). Administration of a glucocorticoid antagonist one hour after stress rescues LTP_{GABA} (IPSC amplitude 141±11%of baseline), however, by 24 hours after stress blocking glucocorticoid receptors is no longer effective in rescuing LTP_{GABA} (IPSC amplitude 113±8% of baseline). Surprisingly, antagonism of KORs with nor-binaltorphimine is effective at recovering LTP_{GABA} even when administered days after the stressor. When norBNI (10 mg/kg, i.p.) is given 2 hours, 24 hours or even 4 days after stress, LTP_{GABA} can be induced in slices (2 hrs: IPSC amplitude 131±7% of baseline; 24 hrs: 138±14% of baseline; 4 days (130±9% of baseline). In parallel, administration of nor-BNI 2 hours after stress also prevents reinstatement of cocaine

self-administration (Lever presses in reinstatement session: vehicle 36±7; norBNI 5±2). Therefore, a single exposure to acute stress can cause days-long changes in the reward circuitry that can be reversed with a kappa opioid receptor antagonist even well after the stressor has occurred.

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Poster

811. Neural Plasticity, Dependence, and Addiction

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 811.24/BB15

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA033684

Title: Identifying striatal gene networks and pathways associated with cocaine dependence

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Abstract: Multiple interacting genes and environmental factors underlie the risk of drug addiction. Drugs of abuse cause lasting change in ventral limbic brain areas that process reward, and addiction is a form of aberrant dopamine plasticity in the dorsal striatum that underlies drug craving and relapse. In this study, we used RNA-seq to quantify transcript changes and analyzed global gene expression in well-characterized cohorts to identify candidate gene markers that are regulated by cocaine abuse in striatal dopamine pathways. The dorsal caudate and the nucleus accumbens were sampled post-mortem from individuals who were chronic cocaine abusers (N=25) and from age-matched control subjects who died suddenly without a history of drug or alcohol abuse (N= 25). The RNA-seq read alignment and differential analysis were done using TopHat and Cufflinks packages. For the initial analysis, cutoffs were set as FDR < 5%, fold change >1.2 and RPKM > 1 for cases and control groups. The caudate nucleus had 383 up-regulated and 124 down-regulated genes that were differentially expressed ($p < 0.05$). Differentially expressed genes in the nucleus accumbens showed a strikingly different pattern

with only 34 genes up-regulated and 117 down-regulated in chronic cocaine abusers compared to drug-free control subjects. Gene network and pathway analysis by GeneGo demonstrated dysregulated neurophysiological processes in the dorsal caudate, including glutamate regulation of dopamine D1A receptor signaling, constitutive and regulated NMDA receptor trafficking, activity-dependent synaptic AMPA receptor delivery and PKA signal transduction. The most significant altered biological function genes in the nucleus accumbens were associated with growth factor function, developmental regulation of cytoskeleton proteins and oligodendrocyte precursor cell proliferation. Our study reveals human gene networks and canonical pathways associated with cocaine dependence. These data provide not only networks between genes, but also map significant pathways in the up- and down-regulated gene set in cocaine dependence for future development of novel therapeutic strategies.

Disclosures: **D.C. Mash:** Other; NIH Grant DA033684. **Z. Jiang:** None. **E. Capobianco:** None. **N.F. Tsinoremas:** None. **D.S. DeLuca:** None. **K. Ardlie:** None. **S.P. Garamszegi:** Other; NIH Grant DA033684. **G. Turecki:** Other; NIH Grant DA033684.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Support: Ministerio de Ciencia e Innovación [PSI2011- 29181]

FPU12/04059

FPI-PREDOC2009/05

PPF 2013 (13I087.01/1)

Title: Cocaine-induced metaplasticity in the cerebellum: Effects on the cerebellar Perineuronal nets

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Abstract: External factors might promote structural remodelling of brain circuitry by modulating the activity of regulatory molecules that restrict neuronal plasticity in order to stabilize circuits. These plasticity inhibitory mechanisms take place in a cartilage-like structure called Perineuronal net (PNN) consisting of several molecules of extracellular matrix. Deep nuclear cerebellar projection neurons, those receiving strong innervation from GABAergic Purkinje axons are enveloped by PNNs. Therefore, by reducing or over-expressing extracellular matrix components, drugs and environmental factors might be able to modify conditions for synaptic change in the outputs from the cerebellum. In the present research, we addressed three different studies exploring cocaine effects on PNNs of the large projection neurons in the medial cerebellar nucleus. First, we evaluated the effects of a repeated cocaine treatment followed by a one-week withdrawal period and then a new cocaine administration; second, we administered the same cocaine regimen but including a one-month drug free period. Third, chronic cocaine administration was evaluated in a conditioned-odour cue paradigm. To properly identify PNNs, we employed Wisteria floribunda agglutinin (WFA), and we immunolabelled medial nuclear neurons with antibodies recognizing SMI32 and. We sampled all the SMI32+ neurons per section and estimated the number of them being surrounded by WFA+. At the same time, we performed an analysis of WFA staining intensity. Each net was assigned to one of three categories of staining intensity, ranging from the lowest to the highest value of WFA intensity: faint= 0-33%, medium= 34-66%, strong= 67-100% of the maximum staining intensity We observed that short withdrawal after chronic cocaine administration results in an increase of the number of PNNs exhibiting strong WFA intensity in the deep medial nucleus. Conversely, when the withdrawal was extended up to a month, or cocaine was repeatedly associated with a cue there was an increase in the number of PNNs expressing faint WFA intensity. Nevertheless, cocaine-mediated regulation of the PNN expression in the medial nucleus appears to be an unconditioned effect of the drug, as it was unrelated to cocaine-induced conditioned preference. These results show for the first time that cocaine might alter PNNs expression, thereby, promoting or restricting structural remodelling of the synapsis.

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Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH K08 DA035972-01 (D. Bajic)

Trailblazer Award Department of Anesthesia at Boston Children's Hospital (D. Bajic)

NIDA K24 (D. Borsook)

Title: Long-term effects in rat resting state networks following postnatal morphine exposure

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Abstract: Exposure to prolonged morphine treatment during development is associated with increased incidence of opioid tolerance, dependence, as well as long-term neurodevelopmental delay, neurocognitive and motor impairments. Currently, the long-term consequences of neonatal morphine treatment on functional brain networks are not known. We hypothesized that functional activity in resting state networks following chronic morphine administration in adulthood will differ depending on previous exposure to opioids in postnatal period. Rats received twice-daily injections of either morphine (10 mg/kg, n=12, MMA Group) or equal volume of saline (n=7, SMA Group) subcutaneously for 13 ½ days (postnatal day (PD) 1-14). Morphine group underwent a weaning period of 10 days to prevent withdrawal. In adulthood, both groups received an additional two weeks of morphine treatment (PD55-70) following which they underwent high field functional magnetic resonance imaging (fMRI). Following anesthesia with isoflurane (0.5-2%), rats underwent an anatomical scan (RARE : 256x256 in-plane resolution FOV:3 cm, 0.6 mm 40 slices, TR/TE=5.3/33ms), and a functional scan (EPI: 64x64 in-plane resolution, FOV: 3cm, 1mm slices, TR/TE=1s/21.5ms, 600 volumes) using a 7T Bruker Biospec MRI. Analysis was performed with fsl. Preprocessing steps included brain extraction, motion correction, high pass filtering-100 s and spatial smoothing 0.7 mm. We used independent component analysis (ICA) to determine total group components and subsequently used dual regression to determine differences in connectivity between the groups. A rat brain template from our lab was used for registration and group comparison. Group ICA results were compared to published RSN rat templates (Becerra et al. NeuroImage 2011) for network identification. We identified 6 published rat brain networks (Becerra et al. PLoS One 2011): default mode (DMN), sensorimotor (SMN), interoception (ICN), cerebellar, basal ganglia and autonomic networks. We observed significant differences in connectivity in DMN, SMN, and ICN. The DMN displayed increased connectivity with orbito frontal (OFC), anterior cingulate, primary sensory, nucleus accumbens (NAc) and periaqueductal gray. The SMN displayed increased connectivity with OFC, NAc, Caudate Putamen, hippocampus and thalamus. The ICN only displayed decreased connectivity with primary motor, septal nucleus, retrosplenial, and hippocampus. These results suggest exposure to morphine in neonates produced brain alterations in adulthood when re-exposed to morphine.

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Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: UCLA Division of Life Sciences Recruitment and Retention Fund

Title: Voluntary aerobic exercise normalizes methamphetamine-induced changes in frontocortical BDNF and striatal D2 transcription

Authors: *A. B. THOMPSON[1], A. STOLYAROVA[1], Z. YING[2], F. GÓMEZ-PINILLA[2], A. IZQUIERDO[1];
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Abstract: Substance abuse is a complex, multifaceted disorder characterized by an inability to cease drug seeking/taking in spite of negative consequences and repeated attempts to discontinue use. Drug abusers show decreased cognitive flexibility and impulse control, two endophenotypes which are predictive of relapse after abstinence and continued drug seeking. These deficits in inhibitory control have been associated with decreased dopamine D2-like receptor availability in the striatum (Groman 2012). Aerobic exercise increases striatal dopamine D2 receptor availability in Parkinson's patients (Fisher 2013), suggesting that it may serve to normalize D2-like dysregulation in drug abusers. Exercise has already been successfully applied in a clinical setting as a therapy for methamphetamine abuse, with encouraging results (Mooney 2014). Aerobic exercise enhances cognitive flexibility in healthy human adults and promotes expression of neurotrophins such as BDNF (Masley 2009; Gómez-Pinilla 2013), which has been linked to improved learning rates in a strategy set-shifting task, a measure of cognitive flexibility (D'Amore 2013). In order to investigate the relationship between aerobic exercise and the addiction process, rats were allowed to voluntarily run on a wheel in their home cage following a 2-week escalating methamphetamine (mAMPH) treatment. Sedentary controls were housed in home cages without running wheels. Running behavior was recorded for 6 weeks, then rats were sacrificed to analyze changes in BDNF and D2 mRNA transcripts in the striatum and frontal cortex, regions that are associated with differential learning rates following mAMPH and exercise. Following an acute withdrawal period, mAMPH-treated rats ran significantly more than their saline-treated counterparts, and maintained this elevated running activity for the duration of the exercise period. This could indicate increased sensitivity to the rewarding effects of aerobic exercise. All BDNF and D2 transcription differences between saline-treated and mAMPH-treated rats were normalized by subsequent aerobic exercise. Interestingly, BDNF in the frontal

cortex and D2 in the striatum were altered in the same direction by mAMPH alone and by exercise alone, supporting the idea of a shared neurochemical mechanism. Future research will investigate the efficacy of exercise as a non-pharmacologic intervention for methamphetamine addiction, both by serving as a substitute for the addictive substance and by enhancing cognitive functioning, protecting against relapse.

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Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: University of Verona Cooperint 2013 grant

Title: Neuroadaptation induced by acute or chronic ketamine: Long-term effects of ketamine self-administration on zif-268 expression in rats

Authors: *C. V. CHIAMULERA[1], M. VENNIRO[1], D. W. S. CHEUNG[2], A. MUTTI[1], L. PADOVANI[1], M. DI CHIO[1], G. FUMAGALLI[1], D. T. YEW[2];
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Abstract: Ketamine was shown to own reinforcing properties, i.e. to initiate and maintain self-administration (S/A). Recently, we showed that single ketamine injection induced neuroadaptive changes in addiction-related brain areas (Tedesco et al., 2013). The objective of this study was to assess the expression of zif-268 as a marker of neuroadaptation in rats exposed to ketamine S/A trying to discriminate from the acute effects. Rats were daily trained to self-administer IV ketamine S/A (group 12x30). As a control, 2 separate groups of rats were exposed respectively to a single ketamine 0.5 mg/kg IV (group 1x1) or vehicle (group 0x1). Ketamine S/A was acquired and maintained for an average of 36 days. At endpoint, the total ketamine intake was 191.2 ± 82.1 mg/kg IV, with an average of 6.4 mg/kg ± 2.7 mg/kg IV of daily intake (mean ± SD). Brains were perfused and processed for immunohistochemistry in prelimbic (PRL) and infralimbic (IL) cortex, nucleus accumbens core (NACC) and shell (NACS), ventral tegmental area (VTA), hippocampus CA1, CA2 and CA3, and basolateral amygdala (BLA). Semi-

quantitative zif-268 cell counting showed a significant main effect of ketamine IV in all the brain areas except for hippocampal CA3. Post-hoc Tukey's multiple comparisons, showed a significant effect in the 1x1 vs. 0x1 group in PRL, NACC, NACS. Group 12x30 showed a significant increase of zif-268 vs. 0x1 group in all brain regions except CA3. In NACC, NACS, VTA, CA1 and BLA, this effect was also significantly greater than that observed in 1x1 group. Zif-268 cell counts from each brain area were analysed with Pearson's multiple comparison in order to identify correlated changes. In the 12x30 group zif-268 levels significantly correlated between hippocampal CA areas and dopaminergic nerve terminals areas such as PRL, IL and NAC areas. In group 1x1, significant correlations were found within the mesocorticolimbic pathway, and between CA3 and NAC areas. Our study is the first to report zif-268 expression in rats exposed to ketamine S/A. The comparison to single ketamine injection showed a significant difference in pattern and intensity of zif-268 signal, suggesting discrimination between the reinforcing (group 12x30) and potentially antidepressant (group 1x1) effects of ketamine.

Disclosures: **C.V. Chiamulera:** A. Employment/Salary (full or part-time):; Univ of Verona. **M. Venniro:** A. Employment/Salary (full or part-time):; Univ of Verona. **D.W.S. Cheung:** None. **A. Mutti:** 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.; Univ of Verona. **L. Padovani:** None. **M. Di chio:** A. Employment/Salary (full or part-time):; Univ of Verona. **G. Fumagalli:** A. Employment/Salary (full or part-time):; Univ of Verona. **D.T. Yew:** A. Employment/Salary (full or part-time):; The Chinese University of Hong Kong.

Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA019666

K02 DA035459

University of Minnesota MnDrive

Title: Optogenetic self-stimulation of the infralimbic-accumbens pathway blunts the development of cocaine sensitization

Authors: A. J. ASP, *E. B. LARSON, M. C. HEARING, M. J. THOMAS;
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Abstract: Discrete optogenetic manipulation of reward circuitry is reported to influence cocaine-mediated behavioral changes. For example, co-administration of cocaine and optogenetic stimulation of the afferents to the nucleus accumbens shell (NAcSh) enhances cocaine-induced psychomotor sensitization, while afferent inhibition during cocaine exposure attenuates sensitization (Britt et al. 2012). On the other hand, optogenetic activation of infralimbic cortex (IL)-NAcSh afferents during drug abstinence is reported to disrupt the expression of cocaine sensitization (Pascoli et al., 2012). One parameter that has not yet been well investigated as a potential source of variability in the influence of NAcSh optogenetic stimulation on cocaine-induced behavior is the temporal relationship between stimulation and drug exposure. We tested the idea that non-concurrent optogenetic activation of IL-NAcSh afferent and cocaine exposure would influence the development of cocaine sensitization. To do this, we used a spatial optogenetic self-stimulation task in which C57BL/6J mice expressing channelrhodopsin in IL cortex quickly learn to optically self-stimulate IL-NAcSh afferents (465 nm, 30 hz, 10 ms, 5 s max pulse train) upon entry into a visually unique "active quadrant." Spatial preference in this task was measured in daily sessions (30 min/day) for two weeks, during which preference for the treatment quadrant was robust and stable. Similarly, a separate group of mice expressing the inhibitory opsin, Archaerhodopsin, in the IL cortex with optical fibers in the NAcSh was tested in the same spatial optical self-stimulation task (525 nm, constant illumination in active quadrant). In this case, no preference for the treatment quadrant was observed. During the second week, each mouse completed a cocaine sensitization regimen (15 mg/kg i.p., 5 once-daily injections) administered 3-5 hours prior to the optogenetic stimulation. Interestingly, we find that non-concurrent optogenetic excitation of IL-NAcSh afferents attenuates the development of psychomotor sensitization while optogenetic inhibition of IL-NAcSh afferents increases cocaine-induced locomotion. We hypothesize that these two behavioral profiles result from differential NAcSh synaptic plasticity induced by repeated bouts of either optogenetic excitation or inhibition. These data fit an emerging model in which the timing of IL-NAcSh afferent stimulation relative to drug exposure is a fundamental factor in determining the direction of influence on cocaine-mediated behavior.

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Poster

811. Neural Plasticity, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA019112-06A1

5F30DA034428-03

Title: Alpha2a-adrenergic receptors filter parabrachial inputs to the bed nucleus of the stria terminalis

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Abstract: Addiction is a chronically relapsing disorder characterized by continued use of drugs despite negative consequences. Stress exposure increases an individual's risk of relapse. The bed nucleus of the stria terminalis (BNST) is a key player in rodent behavioral models of stress-induced reinstatement of drug-seeking behavior. The BNST receives a dense norepinephrine (NE) input and α_2-AR agonists locally administered into the BNST have been shown to attenuate stress-induced reinstatement behavior. α_{2A}-ARs have widespread expression in the BNST and heterosynaptically modulate glutamatergic signaling in this region. Yet, it is not clear what glutamate inputs are regulated. The parabrachial nucleus (PBN) relays important stress/arousal information from the brainstem to the BNST and thus may play a role in stress-induced reinstatement. The PBN is also interesting for its heavily somatic innervation of BNST neurons and little is known about the nature of PBN regulation of it's amygdalar targets. Thus, we used a combination of optogenetic and electrophysiological approaches to assess the PBN input to the BNST and to test the hypothesis that α_{2A}-ARs specifically filter excitatory transmission from this input to the BNST. To study excitatory inputs, an AAV-CaMKII-ChR2:YFP viral vector was microinjected into the PBN. 6-8 weeks following surgery, brain slices were prepared and the PBN afferents in the BNST were optogenetically activated to generate excitatory postsynaptic responses (oEPSCs/oEPSPs). Using current clamp analysis, we found that PBN stimulation leads to two major classes of postsynaptic actions in BNST neurons. In one class, a large EPSP followed by a burst of action potentials was observed, while in the other, substantial feed-forward inhibition was seen. We found these two types of responses to be differentially sensitive to the α_{2A}-AR selective agonist guanfacine. EPSPs recorded from the PBN-excited cells showing consistent decrease. IPSPs from PBN-inhibited cells showed more variable responses. Using voltage clamp analysis of the isolated PBN oEPSC, we found that guanfacine inhibited the PBN oEPSC in a manner that was subsequently reversed by the α_2-AR antagonist atipamezole. In

contrast, these drugs had no effect on oEPSCs from BLA. Moreover, we have found a line of Thy1-ChR2 mice in which ChR2 expression is not apparent in CGRP+ PBN inputs to the BNST. In these mice, we find that a low concentration of guanfacine transiently enhances optically evoked excitatory responses in BNST. In total, these data suggest that the PBN input plays an important role in regulating the responsiveness of BNST neurons, and that this role can be suppressed by α_{2A}-ARs.

Disclosures: S. Flavin: None. **R. Matthews:** None. **D.G. Winder:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: Intramural Research Program of NIDA, NIH, DHHS

NIH Grant R01DA012413

Title: The FAAH inhibitor PF-04457845 has THC-like rewarding and reinstatement effects in squirrel monkeys and increases dopamine levels in the nucleus accumbens shell in rats

Authors: *Z. JUSTINOVA[1], P. MASCIA[1], M. E. SECCI[1], G. H. REDHI[1], D. PIOMELLI[2], S. R. GOLDBERG[1];
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Abstract: FAAH inhibitors (like URB597), that increase brain levels of anandamide, OEA and PEA, show promise for treatment of several neuropsychiatric disorders, including drug dependence. The agonist substitution strategy has been effective for several substance use disorders, mainly nicotine and opioid dependence, and its therapeutic effectiveness is currently under investigation for cannabinoid dependence. We previously showed that the prototypical FAAH inhibitor URB597, unlike cannabinoid CB-1 receptor agonists like THC and anandamide, is not rewarding in squirrel monkeys (Justinova et al., 2008). Here we assessed a newer FAAH inhibitor PF-04457845, currently in clinical testing for treatment of cannabis withdrawal, for THC-like agonist effects. In squirrel monkeys trained to self-administer anandamide (FR10 schedule, 60-s timeout), PF-04457845 maintained higher numbers of self-administered injections

per session and higher rates of responding than vehicle at doses of 3 and 10 µg/kg/injection. The cannabinoid CB1 inverse agonist/antagonist rimonabant (0.3 mg/kg, i.m.) blocked self-administration of the peak dose of PF-04457845 (10 µg/kg/injection). In abstinent monkeys that had previously self-administered THC, PF-04457845 caused moderate reinstatement of drug-seeking behavior over a narrow dose range. In rats, PF-04457845, unlike URB597, significantly increased extracellular dopamine levels in the accumbens shell, but similarly to URB597 did not show THC-like subjective effects. It is clear now that FAAH inhibitors can produce a spectrum of outcomes ranging from no evidence of rewarding, reinstatement or dopaminergic effects (URB597) to significant self-administration, reinstatement and accumbal dopamine release (PF-04457845). FAAH inhibitors that produce THC-like actions may be useful for management of cannabis dependence via agonist substitution strategy.

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Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

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P20 GM104932

Title: Self-administration of the sigma-receptor agonist, DTG, in experimentally naïve rats

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Abstract: Previous studies demonstrated the reinforcing effects of the sigma-receptor (σR) agonists [1,3-di-o-tolylguanidine (DTG), PRE-084, (+)-pentazocine] in rats with a history of stimulant self-administration (Hiranita et al., 2010; 2013). However, none of the selective σ1R

agonists [PRE-084, (+)-pentazocine] were self-administered in naïve rats or those with a history of food reinforcement (Hiranita et al., 2013). The present study assessed the reinforcing effects of the non-selective σ1/2R agonist DTG in experimentally naïve, male Sprague-Dawley rats using a drug self-administration procedure. In marked contrast to the selective σ1R agonists, response-dependent injections of DTG (1.0 mg/kg/inj) increased and maintained responding over 28 daily sessions. The self-administration of DTG was long lasting; a response for which subjects had no history of reinforcement was newly conditioned with DTG, extinguished when injections were discontinued, and reconditioned when DTG injections again followed responses. The self administration of DTG was insensitive to omission of the visual stimulus (VS) that accompanied injections. Further, there was no appreciable difference in dose-effect curves for DTG (0.1-3.2 mg/kg/inj) self-administration with and without the VS changes, indicating a primary role of DTG pharmacological effects in its self administration. Moreover, the acquisition of DTG (1.0 mg/kg/inj) self-administration (with VS changes) over 15 daily sessions was blocked by presession treatment (i.p.) with the non-selective σ1/2R antagonist BD 1008 (10 mg/kg), the selective σ1R antagonist CM 304 (3.2 mg/kg) and the selective σ2R antagonist CM 398 (1.0 mg/kg). Subsequently, acquisition of DTG self-administration was obtained within 15 daily sessions in these same subjects when saline replaced pre-session antagonist treatments. Finally, acquisition of food-reinforced responding was obtained within 15 daily sessions in separate groups of subjects pre-treated with each σR antagonist. In marked contrast to the findings with selective σ1R agonists, the present results suggest reinforcing effects mediated by σ2Rs in naïve subjects. However, that the selective σ1R antagonist CM 304 blocked acquisition of DTG self-administration suggests a cooperative interaction among σ2 and σ1R-mediated reinforcing effects. That cooperation is unidirectional as evidenced by the lack of antagonism of self-administration of selective σ1R agonists by the selective σ2R antagonist CM 398 (previous results). Supported by NIDA IRP (JLK) and grants R01 DA023205 (CRM) and P20 GM104932 (CRM).

Disclosures: **T. Hiranita:** None. **T.A. Kopajtic:** None. **C. Mesangeau:** None. **C.R. McCurdy:** None. **J.L. Katz:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 812.03/BB24

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DK097351

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NIH Grant DA006214

Title: Role of orexin terminal regions during conditioned food seeking

Authors: *A. M. CASON[1], G. ASTON-JONES[2];
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Abstract: Previous studies in our laboratory demonstrated that signaling at the orexin 1 receptor (OxR1) is involved in operant responding for sucrose, and in cue-induced reinstatement of extinguished sucrose or saccharin seeking; however, the specific orexin circuitry involved in cue-induced food seeking remains unknown. The present study examined the involvement of the orexin system in cue-induced reinstatement of extinguished sucrose or saccharin seeking by examining Fos expression in orexin terminal regions implicated in hedonic feeding and activated by cues associated with food reward and after local microinfusions of the OxR1 antagonist SB-334867 (SB) or vehicle. Rats were trained to self-administer sucrose or saccharin (paired with a tone+light cue) and then extinguished as described previously (Cason and Aston-Jones, 2013). Reinstatement of extinguished food seeking was elicited by presentation of the previously paired tone+light cues. Prior to reinstatement testing, rats were given systemic injections of SB (30mg/kg) or vehicle (for Fos comparisons); or rats were given a bilateral microinfusion (0.3ul) of SB (1mM) or vehicle (artificial cerebrospinal fluid) into an orexin terminal region. Pretreatment with systemic SB decreased Fos expression in prelimbic (PL) and infralimbic (IL) cortices, medial amygdala (but not central or basolateral amygdala), and paraventricular nucleus of the thalamus (PVT) during cue-induced sucrose seeking, and in PL, but not IL, during cue-induced saccharin seeking. Furthermore, microinfusions of SB aimed at PL decreased cue-induced reinstatement to saccharin seeking. These results indicate that signaling at the OxR1 in PFC, PVT and MeA are involved in cue-driven seeking of palatable food rewards, and indicate that orexin increases motivation to seek palatable foods. Orexin may also act in other regions including the ventral tegmental area and nucleus accumbens to influence conditioned food seeking. Current experiments are underway to test this hypothesis.

Disclosures: A.M. Cason: None. **G. Aston-Jones:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 812.04/BB25

Topic: C.17. Drugs of Abuse and Addiction

Title: Behavioural response to a 5-HT_{1b} agonist following MDMA self-administration

Authors: D. ARONSEN, J. WEBSTER, N. BUKHOLT, N. ARNOTT-STEEL, *S. SCHENK; Victoria Univ. Wellington, Wellington, New Zealand

Abstract: A significant body of research has suggested a role of the serotonin 1B (5-HT_{1B}) receptor in drug reinforcement because of its ability to modulate dopamine (DA) neurotransmission. 5-HT_{1B} agonists increase DA release via activation of heteroreceptors localized on GABA and Glutamate afferents. Because MDMA preferentially stimulates the release of 5-HT, the possibility that repeated exposure might impact these receptors was investigated. The effect of MDMA self-administration on the behavioural responses to the 5-$HT_{1B/1A}$ agonist, RU 24969, was determined and compared to rats that had self-administered vehicle. Rats self-administered a total of 350 mg/kg of MDMA during 22-57 daily 2 hour sessions. The day following the last self-administration session, the effect of RU 24949 (0.3-3.0 mg/kg) on fluid consumption was measured. RU 24969 dose-dependently decreased fluid consumption in control rats. The dose-effect curve was shifted to the right in rats that had self-administered MDMA. These results suggest a desensitization of the 5-HT_{1B} receptor following MDMA self-administration.

Disclosures: D. Aronsen: None. **S. Schenk:** None. **N. Bukholt:** None. **J. Webster:** None. **N. Arnott-Steel:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 812.05/BB26

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA006214

R01 DA033049

T32 DA007288

Title: Motivation to self-administer methamphetamine in a behavioral-economics paradigm predicts the effect of oxytocin on relapse behavior

Authors: *B. M. COX[1], B. S. BENTZLEY[1], R. E. SEE[2], C. M. REICHEL[1], G. ASTON-JONES[1];
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Abstract: Human and animal studies indicate that females differ in their motivation to use methamphetamine (meth), and have increased propensity to relapse. However, addiction pharmacotherapies have primarily only been tested in males, which may not accurately predict treatment outcomes in females. Evidence indicates that oxytocin, an endogenous peptide well known for its role in social behaviors, childbirth and lactation, is a promising addiction pharmacotherapy. We have shown that oxytocin differentially affects meth seeking in males vs. females on a progressive ratio (PR) test, but attenuates reinstatement of meth seeking similarly in the two sexes. To further examine oxytocin's relationship to motivation and relapse we used a within-session behavioral economic (BE) paradigm, that allows measurement of drug demand at high effort (motivation; α) normalized for intake at low effort (baseline consumption; Q0). This approach also allows us to assess individual variability in meth demand in relation to relapse behaviors, and in response to oxytocin administration. Male and female Sprague Dawley rats were trained to self-administer meth, followed by daily BE sessions. Rats were tested with oxytocin (1 mg/kg, IP) or saline during BE, and then the effects of oxytocin were tested on cue-induced or meth-primed reinstatement responding. Compared to males, females showed greater motivation to seek meth (lower α) and higher meth intake (higher Q0). In both sexes, α correlated with both cue-induced and meth-primed reinstatement. Oxytocin decreased motivation to seek meth in both sexes during BE and reinstatement, and baseline α predicted the efficacy of oxytocin to attenuate cued reinstatement (e.g., low α predicted a greater effect of oxytocin). Motivation (α) assessed during BE accurately predicted relapse of meth seeking and the ability of oxytocin to attenuate relapse. Overall, this novel paradigm will help to delineate sex differences observed in the motivation to seek meth and may predict the efficacy of pharmacotherapies to treat meth addiction.

Disclosures: B.M. Cox: None. **B.S. Bentzley:** None. **R.E. See:** None. **C.M. Reichel:** None. **G. Aston-Jones:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 812.06/BB27

Topic: C.17. Drugs of Abuse and Addiction

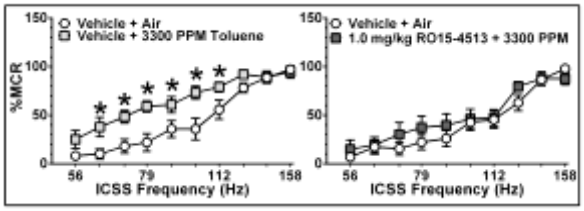
Support: F31DA034469

R01DA020553

Title: Pharmacological modulation of the reinforcement enhancing effects of toluene in intracranial self-stimulation

Authors: *M. E. TRACY, K. L. SHELTON;
Pharmacol. and Toxicology, Virginia Commonwealth Univ. Med. Ctr., Richmond, VA

Abstract: Inhalants are a diverse group of abused substances characterized by their route of administration. Despite widespread abuse, little is known about the mechanism underlying the reward-related effects of inhalants. Intracranial self-stimulation (ICSS) is one method by which these properties can be examined in rodents. In ICSS electrodes are surgically implanted into the medial forebrain bundle of C57BL6/J mice which are then trained to respond on a lever for direct electrical stimulation of the brain. Previously, we have demonstrated that brief exposure to toluene vapor significantly enhances responding for electrical brain stimulation. In the present study, we probed the contributions of the gamma amino butyric acid A receptor system (GABAA) to the reward related effects of toluene. In a group of 8 mice, the GABAA benzodiazepine site neutral antagonist flumazenil or the GABAA benzodiazepine-site negative modulator RO15-4513 were administered alone as well as prior to 3300 PPM toluene vapor exposure. When administered alone 1 or 3 mg/kg flumazenil did not affect ICSS compared to vehicle control. Similarly doses of 0.3 and 1 mg/kg RO15-4513 alone had no significant effect compared to vehicle control. A higher dose of 3 mg/kg RO15-4513 significantly decreased responding for ICSS relative to vehicle control. Preadministration of 1 or 3 mg/kg flumazenil had no effect on toluene vapor facilitated ICSS. However, both the 0.3 and 1 mg/kg RO15-4513 doses significantly decreased the facilitation of ICSS produced by 3300 ppm toluene vapor. These results suggest that the GABAA receptor system is involved in modulating the rewarding effects of toluene.



Disclosures: **M.E. Tracy:** None. **K.L. Shelton:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

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Program#/Poster#: 812.07/BB28

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant F30DA037649

NIH Grant R01DA033930

Title: Quantitative structure-activity relationships (QSAR) for abuse-related neurochemical and behavioral effects of para-substituted methcathinone derivatives in rats

Authors: ***J. S. BONANO**[1], M. L. BANKS[1], R. A. GLENNON[1], J. S. PARTILLA[2], M. H. BAUMANN[2], S. S. NEGUS[1];
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Abstract: Methcathinone (MCAT), the β-keto analog of methamphetamine, is a potent central nervous system stimulant and the parent compound to an emerging class of abused drugs that includes mephedrone and methylone (bath salts components). This study examined quantitative structure-activity relationships (QSARs) for MCAT and six para-substituted MCAT derivatives on two experimental endpoints: (1) *in vitro* potency to function as substrates for dopamine and serotonin transporters (DAT and SERT, respectively), and (2) *in vivo* modulation of intracranial self-stimulation (ICSS) in male Sprague Dawley rats. Correlation analysis was used to compare neurochemical and behavioral effects to each other, as well as to steric (Es), electronic (σ) and lipophilic (π) constants for the seven para substituents: -H (methcathinone), -F (flephedrone), -OCH3 (methedrone), -Cl, -Br, -CH3 (mephedrone), and -CF3. For *in vitro* neurochemical studies, drugs were tested in a rat-brain synaptosome preparation to assess potency of compounds to promote monoamine release through DAT or SERT. All seven compounds

functioned as substrates at DAT and SERT, and DAT vs. SERT selectivity varied across compounds (H>F>Cl>CH3>Br>OCH3>CF3). For *in vivo* behavioral studies of ICSS, rats were implanted with electrodes targeting the medial forebrain bundle and trained to respond under a fixed-ratio 1 schedule for varying frequencies of electrical brain stimulation (56-158 Hz). Brain stimulation maintained a frequency-dependent increase in response rates, and drug were evaluated for their effects on ICSS frequency-rate curves and on the total number of stimulations self-administered across all frequencies. All drugs dose-dependently altered ICSS, and selectivity to facilitate low ICSS rates vs. depress high ICSS rates varied across compounds (H>F>Br>Cl>OCH3> CH3>CF3). *In vitro* DAT selectivity correlated with *in vivo* potency to facilitate ICSS (R=0.92, p=0.003). In addition, the steric parameter (Es) of the para substituents correlated with both *in vitro* DAT selectivity (R=0.78, p=0.04) and *in vivo* facilitation of ICSS (R= 0.81, p=0.03). Together, these results suggest that DAT vs. SERT selectivity of MCAT derivatives is a key determinant of abuse-related facilitation of ICSS by these compounds, and that steric aspects of the para substituent of the MCAT scaffold are key determinants of DAT vs. SERT selectivity. Thus, MCAT derivatives containing para substituents with low steric bulk display DAT selectivity and have high abuse liability, while MCAT derivatives containing para substituents with greater steric bulk display SERT selectivity and have lower abuse liability.

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Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 812.08/BB29

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Drug Supply Program

Title: Constituents of "bath salts", mephedrone and methylenedioxypyrovalerone (MDPV), enhance behavioral sensitization to psychostimulants in rodents

Authors: *M. BERQUIST, JR, M. M. PEET, H. K. TRAXLER, A. M. MAHLER, L. E. BAKER;
Psychology, Western Michigan Univ., Kalamazoo, MI

Abstract: Recreational use of illicit methcathinones, more commonly known as “bath salts”, is a growing public health problem. Recent investigations of methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone or mephedrone (MEPH) indicate their behavioral and neurochemical effects are comparable to the amphetamines and cocaine. Moreover, frequent use of these drugs in combination may pose increased risks for abuse, cardiovascular side effects, and neurotoxicity. However, a paucity of experimental research exists on the combined neurobehavioral effects of these substances. It is well documented that following repeated daily exposure to psychostimulants, ambulatory responses in rodents are sensitized to subsequent drug treatment after a washout period. Concomitant with the development of behavioral sensitization, repeated exposure to psychostimulants produces neuroadaptive changes in mesolimbic dopaminergic pathways. The present study investigated mixtures of MEPH or MDPV with d-amphetamine (AMPH) for behavioral sensitization in rodents. In Experiment 1, 30 female mice were randomly assigned (n=6-8 per group) to receive subcutaneous injections of saline, AMPH (1.0 mg/kg), MEPH (3.0 mg/kg), or AMPH (1.0 mg/kg) + MEPH (3.0 mg/kg) on seven days over an eight day period. Activity was assessed on day 1 and day 8. After a 10 day washout, 1.0 mg/kg AMPH was administered to all animals. Compared to mice treated with AMPH or MEPH, those treated with AMPH + MEPH displayed stronger indices of behavioral sensitization on day 8 and in response to AMPH after the washout period. Experiment 2 utilized similar procedures in 32 male Sprague-Dawley rats (n=8 per group) injected with saline, MEPH (0.5 mg/kg), MDPV (0.5 mg/kg), or MEPH (0.5 mg/kg) + MDPV (0.5 mg/kg) once per day for seven consecutive days. After a 10 day washout, all rats were given 5 mg/kg cocaine. MDPV induced sensitization by day 7 and MEPH appeared to attenuate these effects. However, both MDPV and MEPH + MDPV treatment produced cross-sensitization to cocaine. The results of these experiments suggest methcathinone derivatives can enhance sensitivity to the behavioral effects of d-amphetamine and cocaine. Considered together with recent findings that MEPH and MDPV have different sites of action, but may act synergistically on dopaminergic synapses, further research on the abuse liability of these drug mixtures is warranted.

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Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 812.09/BB30

Topic: B.02. Ligand-Gated Ion Channels

Support: BNI-BMS Seed Found

ANN Medical Student Research Scholarship

Barrow Neurological Foundation

Title: Activation of cannabinoid type 2 receptors in the ventral tegmental area modulate dopamine excitability via m-type potassium channels

Authors: *J. WU[1], B. LARSEN[2], Z. XI[3], M. GAO[4];
[1]Barrow Neurolog Inst., PHOENIX, AZ; [2]Univ. of Arizona Med. College, Phoenix, PHOENIX, AZ; [3]Natl. Inst. on Drug Abuse, Phoenix, AZ; [4]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Objective: An electrophysiological study to determine the mechanism of dopamine (DA) activity depression after cannabinoid type 2 receptor (CB2R) activation in the ventral tegmental area (VTA). Background: CB2Rs are known to be expressed in the peripheral tissues of the immune system and microglia in the brain. Recently, CB2Rs have also been demonstrated on VTA DA neurons using immunoflourescent imaging, and CB2R activation has been involved in the modulation of self-administration of cocaine in mice. Design/Methods: Dissociated VTA DA neurons were prepared using typical brain slice preparation in mice. After a pronase treatment, the VTA was identified and single cells were mechanically dissociated out of the tissue. Healthy DA neurons were selected and neuronal activity recorded using conventional patch clamp techniques. Hyperpolarizing voltage steps were used to illicit M current (I_m) and evoked action potentials (AP), while a selective CB2R agonist was bath-applied to activate CB2Rs. Results: The CB2R agonist caused a 135.2±5.9% increase in I_m amplitude (n=19), demonstrating I_m activation. Significant changes were also noted in four AP parameters including: initiation time (296.9±74%, $p<0.05$, n=11), amplitude (90.4%±4%,$p<0.05$, n=11), half width (87.7%±3%,$p<0.01$, n=11), after hyperpolarization (117.5%±5%,$p<0.01$, n=11), and frequency (73.2%±7%,$p<0.01$). The DA neurons were also hyperpolarized when given the CB2R agonist, demonstrating a 137.9%±10% increase in resting membrane potential ($p<0.01$, n=11). A classic I_m activator elicited similar AP changes, further suggesting that CB2R activation is acting via I_m. Conclusions: These results suggests that activation of the CB2 receptor on VTA DA neurons causes decrease neuronal firing due to subsequent enhancement of I_m currents on the postsynaptic cell body. We recognize that other channels may also play a role in this mechanism and are currently under investigation.

Disclosures: J. Wu: None. **B. Larsen:** None. **Z. Xi:** None. **M. Gao:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 812.10/BB31

Topic: B.03. G-Protein Linked Receptors

Support: Peter F. McManus Charitable Grant (DLG)

NIH R01DK085712 (AG)

NIH R01DA013975 (AG)

NIH R21DA035588 (GDS, AG)

Title: Mapping glucagon-like peptide-1 receptor expression in the brain

Authors: *D. L. GRAHAM[1,2], H. H. DURAI[1], A. GALLI[3,4], G. D. STANWOOD[1,2,3,5];
[1]Pharmacol., [2]Vanderbilt Brain Inst., [3]Neurosci. Program in Substance Abuse, [4]Mol. Physiol. & Biophysics, [5]Kennedy Ctr. for Res. on Human Develop., Vanderbilt Univ., Nashville, TN

Abstract: Glucagon-like peptide-1 is an incretin hormone with a myriad of functions in the periphery, including inducing insulin secretion and delaying gastric emptying. It is not surprising then that its receptor, GLP-1R, is an efficacious target for treating diabetes and obesity. Moreover, recent data from our lab and others have demonstrated that central GLP-1R stimulation attenuates the hedonic properties of food and drugs of abuse. Thus, understanding GLP-1Rs within the brain are important in identifying how they produce these effects. Despite emerging data on brain GLP-1R function, there is limited information regarding where and to what extent it is expressed. GLP-1R is a G-protein coupled receptor (GPCR), a class of proteins that is notoriously difficult to label with antibodies. As such, current evidence of GLP-1R localization in the brain is based primarily on gene transcript expression and binding assays. We have therefore created a bacterial artificial chromosome (BAC) transgenic mouse, whereby the expression of a red fluorescent protein (mApple) is driven by the GLP-1R promoter, in order to characterize the expression patterns of mApple/GLP-1R. We used fluorescent immunohistochemistry (IHC) with an anti-dsRed antibody to amplify mApple signal. Overall, our results are consistent with previous mRNA and receptor binding studies but with much higher sensitivity. mApple reporting of GLP-1R demonstrated high expression within specific hypothalamic nuclei, corroborating previous gene expression mapping and reports that hypothalamic GLP-1Rs are critical for feeding behavior. We also found substantial expression in the bed nucleus of stria terminalis, amygdala, and lateral septum--all components of stress and emotional processing circuits. Extensive mApple labeling was noted within the dentate gyrus and CA3 region of the hippocampus, consistent with published reports indicating that GLP-1R activation facilitates learning and memory consolidation. We also identified sparse-to-moderate

GLP-1R-expressing cells in many other brain regions, including dorsal striatum, cerebral cortex, brainstem, and cerebellum. Additionally, studies are currently underway to determine the cell-type specificity of GLP-1R expression within these regions by double-label IHC using antibodies against neuronal markers. Our results thus far indicate that GLP-1R within the lateral septum colocalizes significantly with GAD67, a marker of GABAergic neurons. Identifying the regional and cellular expression of GLP-1R in the brain is critical in understanding its role in a number of functions, such as addiction, cognition, feeding, and neuroprotection.

Disclosures: D.L. Graham: None. **H.H. Durai:** None. **A. Galli:** None. **G.D. Stanwood:** None.

Poster

812. Drug Self-Administration: Behavioral Pharmacology and Molecular Mechanisms

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 812.11/BB32

Topic: C.17. Drugs of Abuse and Addiction

Support: Biology Department, Indian University of Pennsylvania.

Title: Evaluation of serotonergic, dopaminergic, and cholinergic drug effects and interactions in conditioned place preference in planarians

Authors: C. TROSTLE[1], H. PROUGH[1], A. HAWKINS[1], H. HOLMES[1], *D. V. WIDZOWSKI[2];
[1]Biol., [2]Indiana Univ. of Pennsylvania, Indiana, PA

Abstract: Drugs of abuse such as nicotine and cocaine can induce drug-seeking behavior in a variety of organisms including humans, mice and planarians. It has been shown that 5-HT2C receptor agonists can reduce nicotine seeking behavior in mice and rats. However, this hypothesis has not been tested in planarians. Given that cocaine interacts with serotonin, norepinephrine, and dopamine transporters, we wanted to test the hypothesis that a selective dopamine transporter inhibitor, GBR 12909, would induce drug-seeking behavior in planarians. To test these hypotheses, we used conditioned place preference (CPP) procedures for planarians which consisted of either a single, one-day, conditioning session or repeated sessions (nicotine/GBR 12909 or vehicle) over 4 day period followed by a preference test. Planarians were exposed to nicotine or GBR 12909 in the non-preferred light environment and vehicle in the dark. Preliminary tolerability experiments showed that 150 μM nicotine and 100 nM of GBR 12909 were tolerated (i.e., no stereotypic or seizure-like movements). Similar to previously

published results, nicotine-induced a preference for the paired condition (light). Application of the 5-HT2C receptor agonist Ro 60-0175 at 200 nM after nicotine conditioning but before preference testing did not reduce nicotine-seeking behavior. These results do not support the hypothesis that a 5-HT2C receptor agonist can reduce nicotine seeking in planarians under the conditions used in this study. At a test concentration of 100 nM of GBR 12909, did not induce place preference with single or repeated conditioning sessions. Given that inverted U-shaped concentration response functions have been observed for cocaine and methamphetamine in place preference in planarians, further evaluation of the effects of other concentrations of nicotine, Ro 60-0175 and GBR 12909 may help to elucidate the potential interactions of these drugs.

Disclosures: C. Trostle: None. **H. Prough:** None. **A. Hawkins:** None. **H. Holmes:** None. **D.V. Widzowski:** None.

Poster

813. Neuropeptides and Behavior

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 813.01/BB33

Topic: C.18. Behavioral Pharmacology

Support: CNPq

FAPDF

DPP/UnB

CAPES

Title: Antiepileptic activity of the Neuropolybin peptide: Electroencephalographic study and potential pharmacologic target

Authors: *J. SILVA[1], L. L. COUTO[1], M. R. MORTARI[1], F. M. M. GOMES[1], R. O. BELEBONI[2];
[1]Univ. of Brasilia, Brasilia, Brazil; [2]Univ. of Ribeirão Preto, Ribeirão Preto, Brazil

Abstract: Epilepsy is a disorder associated with a dysfunction of the Central nervous System that, according to the WHO, affects 50 million people worldwide. Despite the widespread availability of AEDs, the treatment is still ineffective for the several clinical phenomena of epilepsy, being of great importance the search for new alternatives that can generate drugs with

fewer adverse effects. In this study, a promising peptide was isolated from the venom of the social wasp Polybia paulista, named Neuropolybin. After high performance liquid chromatography, the neuropeptide was subjected to MALDI TOF/TOF mass spectrometry to obtain the molecular mass, the amino acids sequence and the purity degree. To evaluate the antiepileptic activity, Wistar rats presented epileptic seizures acutely induced by subcutaneous Pentylenetetrazole (105 mg/kg). Occurrence and latency of the maximum epileptic seizure were recorded for both behavioral and electoencephalographic (EEG) tests. Protection curves were obtained by using three doses of Neuropolybin in the behavioral test (4.5, 3 and 1.5 ug/animal) and ED50 for the EEG recordings. It was noted that the latency for the onset of behavioral and electrographic maximum seizures were the same, moreover the Neuropolybin treatment increased this latency time significantly in a dose-dependent manner ($p<0.05$) and reduced the severity of the epileptic seizures. Aditionally, in an attempt to elucidate the mechanism of action of these compounds radioactive binding assays of glutamate and GABA receptors and glutamate uptake in rat cortical synaptosomes were done but no significant results were obtained. Furthermore, to evaluate possible changes in spontaneous general activity of rats, the open field bioassay was performed, and also to evaluate the motor impairment, the rotarod test was used. The results showed that Neuropolybin did not cause changes in the normal activity of rats and also did not cause motor impairment for the same doses tested on PTZ model. Thus, Neuropolybin showed high efficacy in the epilepsy model used with no adverse effects, and may represent a promising tool for the development of new neuroactive drugs. This study also demonstrated how wasp's venoms may be important resources in the search for new molecules with selectivity for the neurotransmission.

Disclosures: J. Silva: None. **L.L. Couto:** None. **M.R. Mortari:** None. **F.M.M. Gomes:** None. **R.O. Beleboni:** None.

Poster

813. Neuropeptides and Behavior

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Program#/Poster#: 813.02/CC1

Topic: C.18. Behavioral Pharmacology

Support: RO1DA024746

Title: The role of melanin-concentrating hormone in repetitive behavior

Authors: *N. M. SANATHARA, C. GARAU, O. CIVELLI;
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Abstract: Melanin-Concentrating Hormone (MCH) is a neuropeptide expressed in the lateral hypothalamus and zona incerta that is involved in the regulation of reward and anxiety. MCH acts through its G protein-coupled receptor, MCHR1, which is highly expressed in the nucleus accumbens and prefrontal cortex, areas containing neural networks important in obsessive-compulsive behavior. MCH has been shown to decrease compulsive grooming in rats. In the present study, we examined the effects of acute MCH administration in marble-burying behavior, a paradigm to screen repetitive/perseverative types of behavior. MCH significantly decreased marble-burying behavior in comparison to vehicle treatment without affecting locomotor or exploratory activity in mice. This effect was blocked by pharmacological administration of a MCHR1 antagonist. These results suggest a potential role for drugs acting on the MCH system in modulating compulsive behavior.

Disclosures: N.M. Sanathara: None. **C. Garau:** None. **O. Civelli:** None.

Poster

813. Neuropeptides and Behavior

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Title: A novel regulator of motivated behavior: The role of Neuromedin U Receptor 2

Authors: *D. L. MCCUE[1], C. R. BENZON[2], J. M. KASPER[2], J. D. HOMMEL[2];
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Abstract: Motivation for food is thought to contribute to excess caloric intake in obese individuals. A novel regulator of motivation for food may be Neuromedin U receptor 2 (NMUR2), a highly-conserved neuropeptide receptor which influences food intake. Administration of the endogenous NMUR2 agonist, Neuromedin U (NMU), into the

paraventricular nucleus of the hypothalamus (PVN) significantly decreases food consumption, while NMUR2 knockout phenotypes display hyperphagia. Furthermore, our previous studies indicate that RNAi knockdown of NMUR2 in the PVN increases preference for higher-fat foods and potentiates binge eating. We hypothesize that NMUR2 signaling regulates motivation for highly reinforcing foods (e.g. high-fat diet). Progressive-ratio (PR) operant responding measures reinforcement efficacy, which is sensitive to changes in motivation. Successively greater numbers of lever-press responses are required in order to gain consecutive reinforcers, providing insight into the degree of motivation to gain a reinforcer. New studies indicate that systemic treatment with NMU causes a decrease in PR responding in rats. Furthermore, we found that knockdown of NMUR2 in the PVN potentiates PR responding for a high-fat diet, following abstinence. These results indicate that NMU-NMUR2 signaling can regulate the motivation for highly reinforcing foods.

Disclosures: D.L. McCue: None. **C.R. Benzon:** None. **J.M. Kasper:** None. **J.D. Hommel:** None.

Poster

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Topic: C.18. Behavioral Pharmacology

Support: CNPq

FAPDF

DPP/UnB

Capes

Title: Anxiolytic activity of fractions isolated from the venom of the social wasp Synoeca surinama

Authors: *F. M. GOMES, C. K. PANIAGO, D. O. FREIRE, L. C. DOS ANJOS, M. R. LIMA, M. R. MORTARI;
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Abstract: Epidemiologic data show that at least one-third of the population in several countries experienced one episode of pathologic anxiety. Benzodiazepines and selective serotonin reuptake inhibitors are the most commonly used drugs in its treatment; however, a major concern is their high potential to lead to abuse or dependence. Furthermore, side effects, such as tolerance and sedation, are limitations and have restricted their use. Due to the high affinity and specificity, neuroactive compounds isolated from the venom of wasps may represent alternatives for the treatment, as well as for the study of neural changes that trigger these disorders. Thus, the purpose of this study was analyze the anxiolytic activity of components isolated from the social wasp Synoeca surinama using the elevated plus maze and evaluate possible side effects of these components. The wasps were collected in Brazil and the venom reservoirs were extracted, homogenized and centrifuged. Venom compounds were then separated by high performance liquid chromatography (HPLC). Molecular mass spectral analyses of the fractions were performed on a MALDI-TOF mass spectrometer. For the bioassays, female Wistar rats received an intracerebralventricular (icv) injections of the fractions of the venom: Sysu13 and Sysu16 (0.5, 3, 10 and 20 μg) 10 minutes later were placed on the elevated plus maze and filmed for 5 min. The control groups received vehicle 10 min before entering the maze, the anxiolytic Diazepam (DZP; 2 mg/kg, ip) or the anxiogenic Pentylenetetrazole (PTZ; 30 mg/kg, ip) 30 minutes before of the test. The results were expressed as percentage of entries and time spent in the open and closed arms, time spent in exploratory activity and immobility, the number of rearing and grooming. In this study, the time spent in open arms data demonstrated that lower dosages of the fractions (0,5; 3 e 10 μg) exerted an anxiolytic effect when compared to negative control ($p<0.05$). The higher dose (20 μg) not presented significantly differences compared with the controls, demonstrating a decrease of the anxiolytic effect. Hyperexploration behavior was observed after administration of the higher dosage of the Sysu16. Therefore, a potent anxiolytic effect of the fractions Sysu 13 and Sysu16 was observed, presenting an inverted U curve, similar to the observed by other neurotoxins isolated from arthropods venoms. Furthermore, fractions administrated in higher doses promoted alterations at the animal motor activity. Neuroactive compounds isolated from social wasps' venom have great therapeutic potential, especially for the investigation of pathological processes and for the synthesis of new drugs to control anxiety.

Disclosures: **F.M. Gomes:** None. **C.K. Paniago:** None. **D.O. Freire:** None. **L.C. dos Anjos:** None. **M.R. Lima:** None. **M.R. Mortari:** None.

Poster

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Title: Social behavioral assessment of mice lacking oxytocin receptor expression in serotonergic cells

Authors: *B. L. TENG, D. C. STOPPEL, G. J. SALIMANDO, M. P. ANDERSON;
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Abstract: Oxytocin and serotonin are important modulators of social behavior. Prior studies found oxytocin receptor (Oxtr) expression in serotonergic cells of the raphe nucleus. The potential functional significance of these co-localized neuromodulatory systems was recently assessed using conditional inactivation of Oxtr *via* AAV-Cre stereotaxic injection in the dorsal raphe region of homozygous floxed-Oxtr mice. This intervention blocked social conditioned place preference (Dolen et al., Nature 2013). Other studies showed that germline deletion of Oxtr eliminates social preference in the three-chamber social choice task and impairs social recognition (Takayanagi et al. 2005; Sala et al. 2011, 2013; Pobbe et al. 2012). Based on these observations, we hypothesized that conditional deletion of Oxtr in serotonergic neurons might impair some of these social behaviors. In mice carrying homozygous floxed-Oxtr and ePet-Cre lacking Oxtr in serotonergic cells, no deficit in social preference in the three-chamber social choice task was observed. Additional measures of social learning and recognition are being assessed.

Disclosures: B.L. Teng: None. **D.C. Stoppel:** None. **G.J. Salimando:** None. **M.P. Anderson:** None.

Poster

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Title: Oxytocin attenuates changes in bdnf iv expression following chronic predator odor stress

Authors: C. E. KING[1], C. L. FERLAND[2], W. C. GRIFFIN, III[1], J. F. MCGINTY[2], *H. C. BECKER[1];
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Abstract: The brain-deprived neurotropic factor (Bdnf) gene is thought to be a key player in mediating the effects of chronic stress and related psychopathologies. Recent literature suggests that long-term changes in BDNF mRNA expression in response to stress correspond to remodeling of chromatin at specific Bdnf gene promoters. Furthermore, lasting and specific changes in histone modifications were observed after chronic antidepressant treatment that prevented stress-induced alterations in Bdnf expression. The neuropeptide oxytocin (OXT) has been shown to exert anxiolytic and stress-protective effects in rodents, suggesting a potential interaction with Bdnf related adaptations to chronic stress exposure. This study aimed to investigate the role of oxytocin in reducing chronic stress-induced alterations in Bdnf expression. C57BL/6J male mice were exposed to predator odor stress (TMT) or control (saline) for 15-min daily sessions for 5 consecutive days, a procedure shown to significantly elevate blood corticosterone (CORT) levels as compared to controls. Mice were treated with 1 mg/kg OXT or saline (ip) 30 min prior to each predator odor exposure. Immediately following the last stress session, brain tissue samples were collected for qPCR analysis of mRNA expression of OXT and Oxytocin Receptor (OxtR), Bdnf, and histone modifying enzymes. Results indicated that TMT treatment significantly decreased OXT mRNA expression in hypothalamus compared to saline-controls. In contrast, OXT alone and when given prior to TMT treatment, significantly increased hypothalamic OXT mRNA. Neither OXT, TMT nor OXT+TMT treatments altered OxtR mRNA expression in the hypothalamus. Additionally, there was a significant increase in Bdnf IV mRNA in prefrontal cortex of saline +TMT exposed animals as compared to controls, along with a corresponding decrease in the epigenetic marker Hdac5. Pre-treatment with OXT attenuated the decrease in Hdac5 mRNA and normalized Bdnf IV mRNA in the TMT-exposed animals. Similar analyses are being conducted with hippocampal tissue. Taken together this data suggest a protective role for oxytocin in preventing long-term adaptive changes associated with chromatin architecture at the Bdnf gene in response to chronic stress. Supported by NIAAA grants P50 AA10761and U01 AA014095, DoD/US Army Institute for Molecular Neuroscience 803-94, and VA Medical Research.

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Poster

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Support: CNPq

FAPDF

DPP-UNB

Title: Neuroprotective activity of a new peptide isolated from the venom of a social wasp against degeneration induced by pilocarpine model of epilepsy

Authors: *M. R. MORTARI, B. R. ARAÚJO;
Physiological Sci., Univ. of Brasilia, Brasilia, Brazil

Abstract: Modern pharmacology has developed two mainly lines of action against neurodegenerative diseases: etiopathogenic, which aims to prevent cell death and promote the recovery of already affected areas, for example the search of drugs that modulate the biochemical pathways involved in neuroinflammatory processes. And pathophysiological, that attempt to prevent, delay or reduce the appearance of typical symptoms of each disease. However, pharmacological treatment currently available for neurodegenerative disorders is only symptomatic and does not alter the course of the underlying pathologic condition. According to these considerations, it is necessary to develop new, more effective and less toxic neuroprotective drugs. In this context, the venom of social wasps represent a potential source since these venoms have compounds that act with affinity and specificity on elements of synaptic transmission in the mammalian brain. The aim of the present work was identify new peptides that are able to reduce the progression of neurodegeneration induced by intrahippocampal injection of pilocarpine. After wasp collection, the venom was manually extracted and separated by high performance liquid chromatography on a semi-preparative C18 column. The venom was solubilized in 5% ACN + 0.1% TFA and eluted under a linear gradient of acetonitrile (5-60%) in 60 min. Molecular mass spectral analyses of the peptide were performed on a MALDI-TOF mass spectrometer. To evaluate the neuroprotective effect, the peptide was administered during silent phase (for 10 days immediately after pilocarpine injection). After this period, the animals were monitored for 24h within 10 days to verify the incidence of recurrent seizures. After the experimental period, the animals were euthanized and the morphology of hippocampal formation was evaluated. The results showed that the peptide was able to decrease the degeneration

induced by pilocarpine. Moreover, the number of recurrent seizures was lower in the groups treated with the peptide compared to control rats with SE ($p<0.05$). Wasp venoms are noteworthy due to the presence of neuroactive compounds, which are highly selective to neuronal structures of mammals, including humans. These compounds could be useful in studies for new drugs or in the development of tools for the research of normal and dysfunctional CNS. Our results demonstrate the neuroprotective effect of the peptide isolated from social wasp, and reinforce the potential use of toxins isolated from animals for the identification and characterization of new neuroactive compounds.

Disclosures: **M.R. Mortari:** None. **B.R. Araújo:** None.

Poster

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MH18399-25

Title: Effect of predator stress on expression of CRF family transcript in mouse brain

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Abstract: Corticotropin-releasing factor (CRF) and its cognate receptors, CRF1 and CRF2, mediate the mammalian endocrine, autonomic, and behavioral response to stress. Dysregulation of CRFergic signaling has been implicated in the pathophysiology of mood and anxiety disorders, most notably post-traumatic stress disorder (PTSD) and major depressive disorder (MDD). However, the mechanism through which stress leads to pathological changes in CRF signaling is poorly understood as is the relationship between the CRF1 and CRF2 receptors. Previous studies have shown that exposure to 30 minutes of restraint stress decreases expression of CRF1 while increasing expression of CRF2. In the present study we sought to determine whether a physiologically relevant predator stress leads to similar changes in expression patterns. Male C57Bl6/J mice were placed in a room with a cat for ten minutes. The cat is allowed to paw and chase the mouse but does not physically injure the mouse. Mice were sacrificed two hours,

24hrs, 7 days, or 14 days after predator stress. Control mice were handled but did not experience cat exposure. Surprisingly, CRF peptide transcript was unaltered by predator stress. CRF1 transcript was also unaltered. There was a time-dependent increase in CRF2 transcript; in the anterior septum CRF2 transcript increased at the 24hr time point. The increase in CRF2 transcript at 24hrs supports the hypothesis that CRF2 contributes to recovery from stress exposure. The lack of effect on CRF1 and CRF peptide transcripts could mean that the involvement of the CRF/CRF1 system in predator stress-induced anxiety does not rely on changes in transcription.

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Poster

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Topic: C.18. Behavioral Pharmacology

Title: New microdialysis probe design - Improved probe recovery of energy metabolites, neurotransmitter and neuropeptides

Authors: *J. LIETSCHE[1], J. GORKA[2], S. HARDT[1], M. KARAS[2], J. KLEIN[1];
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Abstract: Microdialysis is an established technique for measuring small molecular weight substances (e.g. neurotransmitter and energy metabolites) in the extracellular space. Substances with a higher molecular weight than 1000 Da are difficult to dialyse with standard microdialysis probes, which have a molecular weight cut-off (MWCO) of 10,000 Da. In this work, a probe assembly has been developed and validated to build custom-made probes for large molecules. Probe recoveries of substances with different molecular weights (range of 100-1600 Da) and different physicochemical properties were compared for three different probes (custom-made 10kDa and 30kDa probe and the CMA-12 Elite (CMA Microdialysis, Sweden) probe with a MWCO of 20kDa). Glucose, lactate, acetylcholine, choline, ATP and the neuropeptides angiotensin II, substance P and somatostatin were selected as test compounds. Our experiments revealed that the 10kDa probe is only applicable for compounds with a molecular weight below

1000 Da. With this probe, low molecular weight substances exhibited recoveries around 15% (glucose 12.6 ± 0.6 %, lactate 9.5 ± 0.4 %, ATP 13.1 ± 0.7 %, acetylcholine 19.4 ± 1.0 %). Compounds with a higher molecular weight could not be dialysed efficiently by the 10kDa probe. The CMA-12 Elite probe and the custom-made 30kDa probe are capable to dialyse high molecular weight substances. They exhibited comparable recovery values of 2-3 % for all tested neuropeptides. However, the recovery of ATP was much lower for the Elite-12 probe (2.9 ± 1.3 %) than with the 30kDa probe (22.4 ± 0.7 %), an effect that may be due to the strongly negative charge of ATP. Furthermore, neuropeptides have the characteristic to adhere to polymeric materials (e.g. membrane, probe and outlet tubing). For further improvement of the microdialysis of neuropeptides, we have developed a new technique using a ZipTip® µC-18 (Merck Millipore, Germany) to replace the outlet tubing and to purify the dialysate for MALDI-MS measurement. MALDI spectra of neuropeptides concentrated with ZipTip technology revealed less oxidation and minor mass shifts which significantly improved the signal for MALDI measurement.

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NSF GRFP

Title: Insulin acts as a reward signal in the nucleus accumbens

Authors: ***C. WOODS**, M. STOUFFER, Z. GUTTMAN, S. CABEZA DE VACA, K. CARR; New York Univ., New York, NY

Abstract: Insulin is a metabolic hormone synthesized in beta cells of the pancreas and released upon detection of blood glucose via consumption of food. 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 wide 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 electrically evoked extracellular dopamine in the Nucleus Accumbens (NAc). Further, food restricted rats showed a significant decrease in electrically evoked extracellular dopamine at baseline compared to ad libitum fed rats, but were more sensitive to the enhancing effect of insulin. Our current research works to understand the behavioral significance of insulin in the striatum. Therefore, we use flavor preference - a behavioral learning paradigm with acquisition and expression mediated by NAc dopamine. During 1-bottle flavor preference conditioning sessions, the experimental group received microinjections in NAc shell of Insulin antibodies (InsAb) with one of two flavors, and on alternating sessions, mock microinjections paired with the other flavor. The control group received mock microinjections alternated with microinjections of either phosphate buffered saline (PBS) or immunoglobulin G (IGG). Both flavored solutions contained 0.8% glucose. During the 1-bottle conditioning sessions, InsAb microinjection significantly decreased consumption compared to vehicle during the 3rd and 4th infusions . By contrast, both groups consumed the same volume of solution during all four mock injection sessions. After a total of 8 conditioning sessions, flavor preference was assessed in a 2-bottle test in which rats had access to both flavored solutions simultaneously. The InsAb group consumed significantly less of the InsAb-paired flavor compared to the mock-paired flavor, whereas the vehicle group showed no flavor preference, implying that intact insulin signaling contributed to selection of a sweet caloric solution. Control microinjection had no effect on flavor preference, arguing against the possibility of a non-specific effect of InsAb microinjection on consumption or flavor preference. Overall, these data suggest that insulin in the NAc plays a role in reinforcement of preference for a flavor that signals a glycemic load. This work was supported by NIH grants DA033811 (KC & M. Rice & M. Reith) and the NSF GRFP.

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Title: Anxiogenic and pro-depressant effects of PACAP

Authors: *M. SEIGLIE, K. L. SMITH, A. IEMOLO, R. DORE, P. COTTONE, V. SABINO; Dept. of Pharmacol. and Psychiatry, Boston Univ. Sch. of Med., Boston, MA

Abstract: Major Depressive Disorder (MDD) is characterized by depressed mood, irritability, and a diminished ability to experience pleasure in acts that were once enjoyable. MDD is very often comorbid with symptoms of anxiety, which have a major impact on the course of the depressive illness. Stress is one of the leading predisposing factors for the onset of both depression and anxiety-related disorders. The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor PAC1 have been shown to play a key role in mediating the endocrine and behavioral responses to stress. The aims of the present study were to further characterize the role of PACAP in depressive- and anxiety-like behavior in rats and to determine whether the endogenous PACAP-PAC1 system is recruited by stressors. PACAP-38 was microinfused into the lateral ventricle and its effects on behavioral paradigms assessing anxiety and anhedonia were examined. I.c.v. PACAP-38 (0, 1, 3 μg/rat) decreased the time spent in the bright compartment in the light-dark box test, indicating an increase in anxiety-like behavior. We found that i.c.v. PACAP dose-dependently increased the intracranial self-stimulation (ICSS) thresholds, and reduced the intake and the preference for a saccharin solution. Both increases in ICSS threshold and reduction in saccharin preference are interpreted as measures of anhedonia, a core symptom of depression. Finally we observed that an acute, one hour exposure to immobilization stress (restraint), which was able to reduce interaction in a social interaction test, produced an elevation in PAC1 receptor mRNA and protein levels in the Central nucleus of the Amygdala. Altogether, these findings suggest that PACAP induces both anxiety-like behavior and anhedonia, and that the PACAP-PAC1 system is recruited following acute exposure to stress.

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Poster

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Title: Genetic disruption of the vasopressin 1b receptor reveals its complex role in nociception

Authors: *J. C. MORALES-MEDINA, H. CALDWELL;
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Abstract: The neuropeptides arginine vasopressin (Avp) and oxytocin (Oxt) are known to act as strong antinociceptive agents. However, the receptors that mediate the antinociceptive effects of Avp and Oxt remain unknown. While the physiological effects of Avp are mediated by three receptors Avpr1a, Avpr1b, V2; the effects of Oxt are modulated a single receptor subtype, the Oxtr. However, all four receptors are sensitive to both nonapeptides. In the present study, we aimed to investigate whether genetic deletion of the Avpr1b (Avpr1b KO), in mice, had any effect on their response to nociception in the absence of injury and after peripheral inflammation. The von Frey test (VFT) of mechanical sensitivity, the pin prick test of mechanical hyperalgesia, the acetone test of cold allodynia and the hot plate assay (HPA) of thermal sensitivity were assessed in the absence of injury. We also evaluated the analgesic-related properties of Avp, Oxt and osmotic stress (OS) challenge. Finally, we measured the pronociceptive effects of intraplantar injection of complete Freud's adjuvant (CFA). In the absence of injury, Avpr1b KO mice showed increased latencies in the HPA, with normal thresholds in all other assays tested. Avpr1b KO mice displayed stress-induced analgesia as observed after repeated measures in the VFT. Avpr1b KO mice had increased mechanical thresholds after OS, Avp and Oxt administration. Most notably, Avpr1b KO mice had reduced mechanical hypersensitivity after CFA administration, with no changes in paw edema. In summary, the lack of the Avpr1b inhibits the behavioral signs of thermal transient nociception, augments the antinociceptive effects of endogenous Avp, exogenous Avp and Oxt and reduces the mechanical hyperalgesia after CFA. Thus, these results suggest this receptor mediates, at least partially, the antinociceptive effects of Avp and Oxt and may represent an important novel target for the management of nociceptive-related disorders.

Disclosures: J.C. Morales-Medina: None. **H. Caldwell:** None.

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FAPDF

FINATEC

CAPES

DPP/UnB

Title: Neuroprotective activity in a murine model of Parkinson's disease of the peptide fraction isolated from the venom of the wasp Parachartergus fraternus

Authors: *A. B. MAYER, K. G. MOREIRA, R. B. D. GODINHO, L. A. CAMPOS, M. R. LIMA, M. R. MORTARI;
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Abstract: Parkinson's disease (PD) is the most common neurodegenerative disease related to movement, and currently affects about 1% of the population over 60 years old, and its prevalence increases over the years. The chronic use of dopamine precursors causes strong side-effects (dyskinesias), moreover the drugs used in the treatment does not modify disease progression. Therefore, it is necessary the development of new and more effective antiparkisonian drugs with lower side-effects. Wasp venoms are composed by a cocktail of bioactive molecules, with a high molecular variability and several compounds shows selectivity to Central Nervous System of mammals. So, the objective of this study was to identify and isolate components in the venom of the social wasp Parachartergus fraternus with neuroprotective activity in a murine model of Parkinson's disease. Fractionation of the venom components was performed by high performance liquid chromatography on a semi-preparative C18 column. The venom was solubilized in 5% ACN + 0.1% TFA, eluted under a linear gradient of acetonitrile (5-60%) in 60 min. Three peptides fractions were selected and tested, however only one showed an interesting activity. The composition of the peptide fraction was evaluated by mass spectrometry. For the experimental model, mices were divided in 3 groups (n=8): saline, damaged group by 6 -hidroxidopamine (6-OHDA) and the group treated with wasp peptide fraction after injury by 6-OHDA. The peptide fraction was injected one hour after lesion and during the next two days, always at the same time. One week after surgery, the animals were tested and the rotatory behavior was observed after subcutaneous administration of apomorphine (5 ug/animal, s.c.), and the number of the viable neurons counted after immunostaining (Tyrosine-hydroxylase reaction). The damaged group showed a higher number of contralateral rotations induced by dopamine agonist when compared to saline group ($p < 0.001$). Administration of the peptide fraction produced a decrease significantly in the number of rotations in relation to the damaged group ($p < 0.001$). Moreover, the peptide fraction decreased the degeneration in the substantia nigra induced by 6-OHDA. This study revealed a promising peptide fraction of the social wasp venom that was able to prevent the progression of the neuronal loss in a murine model of Parkinson's Disease.

Disclosures: **A.B. Mayer:** None. **K.G. Moreira:** None. **R.B.D. Godinho:** None. **L.A. Campos:** None. **M.R. Lima:** None. **M.R. Mortari:** None.

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CAPES

CNPq

Title: Antidepressant-like effect induced by Orexin A injection into the ventral medial prefrontal cortex (vMPFC): participation of TrkB receptors

Authors: *L. A. STANQUINI[1,2], A. SCOPINHO[2], S. JOCA[3];
[2]Pharmacol., [3]Physics and Chem., [1]Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: Introduction: Orexin-A (OXA) is a neuropeptide produced by a small group of neurons in the lateral hypothalamic and perifornical areas, regions classically implicated in the control of mammalian feeding behavior. Orexin neurons project throughout the central nervous system (CNS) where it may modulate neurochemical and behavioral responses to stress. For instance, OXA modulates the release of BDNF, a highly expressed brain neurotrophin, which trough the interaction with TrkB receptors, has been implicated in mediating behavioral adaptation to stress and antidepressant effects. Therefore, the aim of this study was to investigate the effect OXA microinjection into the in vMPFC of rats submited to the forced swimming test (FST), an animal model predictive of antidepressant-like effect. In addition, we investigated the participation of TrkB receptors in such effects. Methods: In the first experiment, male Wistar rats with guide cannulas aimed at the vMPFC were submitted to a 15 min swimming session and, 24h later they received a bilateral microinjection of vehicle or OXA (10, 50 and 100 pmol/200nL). 10 min after the injection the animals were submitted to the FST (5 min), when the immobility time was assessed by an investigator that was blind to the treatments. An independent group received a microinjection on the vMPFC of vehicle or OXA (100pmol/200nL) and were submitted to open field test (OFT) to assess the effect of this drug on locomotor activity. In a

third experimental group, the animals received a microinjection of K252 (an antagonist of trk receptors; 10pmol/200nL), OXA (100pmol/200nL) or Vehicle. Results: Microinjection of OXA induced an antidepressant-like effect at the dose of 100pmol/200nL ($F_{3,16}$= 4,643, $p<0,005$) and this dose was not able to induce locomotor effects ($p<0,005$). Besides that, the antidepressant-like effect of OXA was reversed by prior administration of K252 ($F_{3,30}$ =6,650, $p<0,005$). Experiments with OXA antagonist is under development. Conclusions: Our results suggest that the antidepressant like effect of microinjection of OXA in the vMCPF depends on the activation of trkB receptors and may possibily involve modulation of BDNF release. Financial **Support:** FAPESP, CNPq, FAEPA and CAPES.

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Poster

813. Neuropeptides and Behavior

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Title: Nanotechnology to deliver neuropeptides to the brain: Distribution and effects on anxiety-like behaviors

Authors: N. VINZANT, C.-M. WU, T. KINDLE, J. SCHOLL, V. KUMARASWAMY, R. SOLANKI, R. KOODALI, *G. L. FORSTER;
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Abstract: The corticotropin-releasing factor type 2 receptor (CRF_2) represents a novel target for treating anxiety states. Direct infusion of CRF_2 receptor antagonist antisauvagine (ASV) into the brain immediately reduces anxiety states in rodent models of early life stress and drug withdrawal. However, like many neuropeptides, ASV cannot cross the blood brain barrier (BBB) if administered systemically. Nanoparticles such as iron oxide can cross the BBB and can be designed to carry drug cargo. This study examined whether iron oxide nanoparticles with ASV cargo cross the BBB by determining the distribution within the rat brain following systemic

administration. Iron oxide nanoparticles (Fe_2O_3) were synthetized, size confirmed by transmission electron microscopy (5.0 +/- 1.1 nm), and were functionalized with 3-aminopropyltriethoxysilane (1:4 Fe_2O_3:APTES). Next, ASV (10:1 Fe_2O_3:ASV) was attached to the functionalized iron oxide nanoparticles and monitored hourly for five hours with fourier transform infrared (FTIR) spectroscopy to ensure stability of the nanoparticle+ASV complex over this time frame. Nanoparticle solution (87.7 μg /kg Fe_2O_3) with FITC tag, with or without ASV (200 μg/kg) was injected (ip.) 30 minutes prior to transcardial perfusion and brain fixation. Sections throughout the brain were processed using immunohistochemistry and imaged with confocal microscopy. Sections were analyzed for nanoparticle association with neurons (NeuN), with CRF_2 receptors, and with iron-related proteins such as ferritin and transferrin. Results suggest systemically-administered nanoparticle with ASV associates with neurons, including those that express CRF_2 receptors. To determine the anxiolytic effect of ASV delivered as nanoparticle cargo, rats were pretreated with either amphetamine (2.5 mg/kg) or saline and underwent two weeks withdrawal, which has been shown to increase anxiety-like behaviors. Rats were either treated with nanoparticle+ASV (87.7 μg /kg Fe_2O_3; 200 μg/kg ASV, ip.) or nanoparticle+vehicle 30 mins prior to testing on the elevated plus maze (EPM). Results thus far suggest that ASV delivered by nanoparticles reduces anxiety-like behaviors. Overall, the findings demonstrate a novel approach to drug delivery across the BBB and provide insight as to the neural distribution and efficacy of drug treatments delivered via nanotechnology.

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Title: The anxiolytic effect of oxytocin in the medial prefrontal cortex is sub-region specific

Authors: ***S. SABIHI**[1], S. DONG[1], J. KREIN[1], B. LEUNER[2];
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Abstract: The neuropeptide oxytocin (OT) has anxiolytic effects in both rodents and humans. However, the specific brain regions where OT acts to regulate anxiety requires further investigation. Recent studies from our lab have shown that OT delivered into the prelimbic (PL) region of the medial prefrontal cortex (mPFC) attenuates anxiety-related behavior in both male and female rats. In addition to the PL region, the mPFC of the rodent brain also includes the infralimbic (IL) and anterior cingulate (Cg1) cortices. These various sub-regions of the mPFC show different patterns of connectivity with subcortical and cortical structures and thereby differentially contribute to a variety of physiological and behavioral processes, including anxiety regulation. Thus, here we examined whether the anxiolytic actions of OT in the mPFC are sub-region specific. To do so, separate groups of male Sprague-Dawley rats were administered OT (0.1µg/0.5µl or 1.0µg/0.5µl) or an equal volume of saline vehicle bilaterally into one of the three mPFC sub-regions and anxiety-like behavior assessed in the elevated plus maze (EPM) and social interaction (SI) tests. As previously reported, rats receiving OT in the PL mPFC spent a greater percentage of time in the open arms of the EPM and made more open arms entries than saline-treated rats. Similarly, infusion of OT into the PL mPFC decreased anxiety-like behavior in the SI test as demonstrated by an increase in the amount of time spent interacting with an unknown conspecific. In contrast, OT did not affect anxiety-like behavior when infused into either the Cg1 or IL sub-regions of the mPFC. Indeed, rats receiving OT infusion in Cg1 or IL did not differ from saline treated rats in the amount of time spent in the open arms of the EPM, number of open arm entries, or time spent interacting with an unknown rat. Locomotor activity, as measured by the number of closed arm entries in the EPM, was not altered by OT treatment or infusion site. Taken together, these results suggest that the anxiolytic actions of OT are sub-region specific and localized specifically to the PL region of the mPFC.

Disclosures: S. Sabihi: None. **S. Dong:** None. **J. Krein:** None. **B. Leuner:** None.

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Support: Région Rhône-Alpes

Title: Paradoxical sleep (REM sleep) regulation in a mouse model of narcolepsy with cataplexy

Authors: C. PEYRON[1], A. ROMAN[1], S. ARTHAUD[1], *N. L. URBAIN[2], P.-H. LUPPI[1];
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Abstract: Objective: The symptomatology of narcolepsy suggests an impaired paradoxical sleep (PS, also called REM Sleep) regulation, as seen by the presence of cataplexy, sleep paralysis and sleep-onset into REM Sleep periods (SOREMp). Only one study conducted with only 6 narcoleptic patients has looked at PS homeostatic regulation (Vu et al., 2009) and none were done on narcoleptic mice. Here, we studied hypocretin/orexin Knock-out narcoleptic mice (KO), by depriving them for 48hrs of PS and analyzing the following recovery period. Methods - Results: We first used the platforms-over-water technique for PS deprivation. During the recovery period, KO mice (n=9) showed a PS rebound similar to wild type mice (WT; n=13) indicating that PS homeostatic regulation is maintained in KO mice. However, PS latency during recovery was much shorter in KO (20±4.2min) than WT (113±5.6min). As it could be due to a higher PS pressure, we then used our newly developed automatic PS deprivation method to objectively evaluate it (Libourel et al, 2014). When PS is detected, a TTL-signal is sent by the computer to the cage floor to move it up and wake-up the mouse. Of interest, KO were stimulated more often (782.3±60.7) than WT (367.6±42.0) mice revealing a stronger need to enter PS, thereby a stronger PS pressure. WT mice had a higher PS pressure during the light phase than dark phase in accordance to the well-established PS circadian distribution. KO mice however accumulated PS pressure similarly during light and dark phases and similarly to WT during the light phase. Conclusion: These findings may reflect a lack of inhibition of PS during the dark phase in KO narcoleptic mice. Acknowledgements: AR receives fellowship from Région Rhône-Alpes. The study is supported by CNRS, Université-lyon1, SFRMS. References: . Vu et al (2011) Selective REM sleep deprivation in narcolepsy. J Sleep Res. 20(1 Pt 1):50-56. . Libourel et al (2014) Self-learning adaptative algorithm for automated sleep scoring and sleep deprivation. Submitted.

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Poster

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Support: CNPq

FAPDF

FINATEC

CAPES

DPP/UnB

Title: Antinociceptive effect and identification of a peptide isolated from social wasp venom Parachartergus fraternus

Authors: *P. GALANTE, J. C. GONCALVES, G. A. A. CAMPOS, R. B. M. GODINHO, E. F. SCHWARTZ, M. R. MORTARI;
Univ. of Brasilia, Brasília, Brazil

Abstract: Wasp venoms are formed by several molecules, such as biogenic amines, acylpolyamines and peptides, these latter being the most abundant components. The neuroactive compounds have a great interest by presenting a relevant potential to design new drugs, in particular they are important to prevent and/or treat diseases as chronic pain, epilepsy and Parkinson`s disease. The social wasp Parachartergus fraternus has a remarkable peculiarity: although these wasps live in community, their venom acts paralyzing reversibly and non-lethally the prey, just like solitary wasp, indicating the presence of a large number of the neuroactive peptides. For this reason, the aim of this study was the identification and isolation of antinociceptive peptides in the venom of the P. fraternus. The nest was collected in the University of Brasilia after required permission from IBAMA. To peptide's identification, two strategies were adopted concomitantly: first, the venoms were extracted, centrifuged and ultrafiltered (<3000 Da). Low molecular compounds were separated by HPLC and a specific fraction was identified by mass spectrometry. The other strategy used was the construction of the cDNA library with 20 gland sacs and the venom extracted after anesthetizing of the wasps at -20oC during 1 hour. To the antinociceptive test, Swiss mice (n=5/group) were tested in the model of thermal stimulus (Hotplate). The peptides were infused via i.c.v, at doses of 8 and 16 nmol/animal (in vehicle dilution), four days after the implantation of the guide cannula. The positive control was produced by morphine (26 nmol/animal), and vehicle was used as negative control (2µL/animal). This isolated peptide from wasp venom showed an analgesic activity, and the major effect was induced at 90 and 120 min in the dose of the 8 nmol ($p<0.05$). The highest dose produced a decreased effect in relation to 8 nmol, probably due to neurotoxic effect. The primary sequence, ILGTILGFLKGL-NH2, was identified by mass spectrometry and confirmed by cDNA Library Construction. This peptide was firstly identified in Protonectarina sylveirae and known as protonectin, which induce chemotaxis in polymorphonuclear leukocytes and presents antimicrobial activity, but not hemolytic. These results corroborate the hypothesis that wasp's venoms have a group of bioactive molecules, which act in the CNS of mammals, capable

to contribute with neuroscience development in the elucidation of synaptic transmission as to design new drugs.

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Poster

813. Neuropeptides and Behavior

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Topic: C.18. Behavioral Pharmacology

Title: Vasopressin V1A receptor blockade within the latero-anterior hypothalamus reduces anxiety-like behaviors in Syrian hamsters treated throughout development with anabolic/androgenic steroids

Authors: *T. R. MORRISON, L. RICCI, R. MELLONI, Jr.;
Psychology, Northeastern Univerisity, Boston, MA

Abstract: Anabolic androgenic steroid (AAS) exposure has been shown across numerous animal models to affect both aggressive and anxious behaviors. For example, we have recently shown that Syrian hamsters exposed to moderate dose AAS throughout adolescence display enhanced aggressive behavior in the presence of reduced anxiety-like behaviors. During AAS-withdrawal, however, aggression returns to control levels, while anxiety-like behaviors increase. AAS-induced neuroanatomical alterations correlate with the emergence of enhanced aggressive behavior and reduced anxious responding during the exposure period. Namely, arginine vasopressin (AVP) afferent fibers within the latero-anterior hypothalamus (LAH) are increased during AAS exposure and microdialysate data show that AVP release within the LAH is increased in AAS-treated animals over vehicle-treated controls during an aggressive encounter with a conspecific. Indeed, AAS-induced aggression is closely associated with the AVP system as blockade of AVP V1A receptors within the LAH inhibits aggressive behavior in these animals. In various models, AVP receptor stimulation has also been shown to affect anxious responding. For example, AVP V1A antagonists delivered into the lateral septum and paraventricular nucleus (PVN) reduce anxiety-like behaviors in normal rats and rats bred to display high levels of anxiety-like behavior (HABs), respectively. HABs also show higher levels of V1A mRNA expression in the PVN than those bred for low anxiety, and accordingly, V1A receptor knockout mice exhibit low levels of anxious responding. In the present study, we investigated the effects of the V1A receptor antagonist, Manning compound, on anxious behaviors in hamsters treated throughout adolescence with AAS. Manning compound increased the time spent in the open arms of the elevated plus maze while reducing the amount of time spent in the closed arms without affecting measures of locomotion. These results are counterintuitive at a superficial level to previous data from our lab that show that AAS exposure

enhances the AVP system within the LAH and reduces anxiety. The behavioral data from the present study likely reflect the complex neuroanatomical changes that occur in the LAH as a result of developmental exposure to AAS ~alongside~ AAS-induced alterations to AVP structures. Nevertheless, these data lie in support of the hypothesis that AVP V1A receptors play a role in anxious responding in adolescent AAS-treated animals.

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Poster

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Support: CNPq

FAPDF

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CAPES

Title: A new antiepileptic peptide: Efficacy and adverse effects

Authors: *G. A. CAMPOS, J. SILVA, R. ARAUJO, M. MORTARI;
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Abstract: Invertebrate's venom has attracted considerable interest and has been helpful in the search for novel drugs for the treatment of neurological disorders, as epilepsy. This disorder comprises a variety of symptoms caused by distinct pathological processes in the brain. Although a great diversity of antiepileptic drugs (AED) is available, the treatment of epilepsy is ineffective in some cases and can produce several adverse effects. Therefore, it is highly necessary to search for new alternative AED that are more effective and less toxic. In this context, a promising antiepileptic peptide has been isolated from social wasp Polybia paulista's venom, named Neuropolybin. In this work, the antiepileptic activity of the natural peptide and a modified was evaluated in a model of epileptic seizures induced by pentylenetetrazole (PTZ). In order to assess behavior alterations caused by the administration of the peptide, open field and Rotarod tests were also performed. To evaluate the antiepileptic activity, the peptide injections (2 µL; solution

with deionized water) were administered via icv at the following doses: 2.5, 1.25 e 0.4 nmol/animal for the natural and 3.0, 1.5 e 0.5 nmol/animal for the modified (n=5/group). After 30 minutes of the peptide injection, the mice received pentilenetetrazole (PTZ; 95 mg/kg) subcutaneously and their behavior for 30 minutes was analyzed. The open field test consisted in placing the animal in a circular arena 30 min after injection of the peptides via icv; animals were recorded for 20 min and the time spent in four behaviors (immobility, exploration, elevation and grooming) was analyzed using X-Plo-Rat software. In the Rotarod test, the mice received the compounds and were placed on the cylinder at times 10, 20, 40, 60 and 120 min after injection. The latency to fall from the cylinder was registered and analyzed. For both open field and Rotarod, six groups (n=4-5 per group) were used: two control groups received vehicle (icv, 2 μL) or Diazepam (ip, 4mg/kg); the other groups were administered with peptides via icv at 2,5 or 25 nmol/animal for Neuropolybin and 3 or 30 nmol/animal for the modified. Both the analogue and the modified versions of this peptide were tested and proved to be able of increase latency to onset of seizures induced by PTZ in a dose-dependent manner ($p<0.01$). Moreover, only the ten times higher dose of the peptides showed behavioral alterations, reinforcing that this peptide presents potential for epilepsy treatment.

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Poster

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Title: Local oxytocin tempers anxiety via GABAA receptor in the female prairie vole hypothalamic paraventricular nucleus

Authors: *A. S. SMITH, M. TABBAA, K. LEI, P. EASTHAM, M. J. BUTLER, R. ALTSHULER, L. LINTON, Z. WANG;
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Abstract: Brain oxytocin (Oxt) regulates aspects of emotionality and stress coping, releasing in response to anxiogenic stimuli in various hypothalamic and extrahypothalamic brain areas. Pharmacological studies have documented that inhibition of Oxt action in brain regions that release Oxt in response to anxiogenic stimuli (e.g., paraventricular nucleus of the hypothalamus (PVN) and central amygdala) can potentiate the behavioral stress response and activation of one of the major biological stress pathways, the hypothalamic-pituitary-adrenal (HPA) axis. Yet, the mechanism for the anxiolytic effects of Oxt is not well known. In a series of experiments, we investigated in female prairie voles (Microtus ochrogaster) the anxiolytic action of Oxt in the PVN as well as if Oxt recruits GABA neurons to inhibit stress-induced HPA axis activity and behavior. In Experiment 1, females received intra-PVN injections of Oxt either 15 min before or immediately following exposure to a 10-min elevated platform stressor (EPS). EPS significantly increased plasma corticosterone levels, which was inhibited by ante-stress injections of Oxt in the PVN but was unaffected within that same time frame by post-stress Oxt injections. In Experiment 2, the impact of EPS and ante-stress, intra-PVN injections of Oxt on neuronal activity in the PVN was determined by immunohistochemical labeling of c-Fos, an immediate-early gene product, and various neurochemical markers. Intra-PVN injections of Oxt limited the stress-induced increase of c-Fos expression in corticotrophin-releasing hormone (CRH) immunoreactive (-ir) neurons and anxiety-like behaviors on the elevated plus maze (EPM) while promoting c-Fos expression in GABA-ir neurons. In Experiment 3, the modulator effect of GABAA receptors on the anxiolytic effects of Oxt was assessed via site-specific administration of Oxt and a GABAA receptor antagonist in the PVN. The anxiolytic effects of intra-PVN injections of Oxt were blocked with concurrent injects of a GABAA receptor antagonists. Together, our data demonstrate ante-stress treatments of Oxt in the PVN inhibit stress activation of the HPA axis through the recruitment of GABAergic neurons, providing local circuitry and potential therapeutically relevant mechanisms. (Supported by NSF Graduate Research Fellowship and NIMHF31-095464 to AS and NIMHR01-058616 to ZW).

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Poster

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Topic: C.18. Behavioral Pharmacology

Title: Motivation factors alter the response-suppressant effects of the neurotensin NTS1 receptor agonist PD149163 in rats: Implications for the assessment of neurotensin compounds in animal models

Authors: C. R. BROWN, R. M. KIRKENDALL, E. L. RIDGE, *A. PRUS;
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Abstract: Neurotensin receptor agonists have been studied as novel treatments for cognitive disorders, drug addiction, and schizophrenia. However, behaviorally effective doses of these compounds tend to suppress locomotor activity, precluding a more thorough evaluation of these compounds in animal models. The behavioral suppression observed with these compounds may be directly related to the motivational conditions employed. To evaluate this, we conducted a study to compare rates of lever pressing in food-deprived rats trained to earn food reinforcers and in rats trained to avoid an intermittent footshock after administration of the neurotensin NTS1 receptor agonist PD149163. In addition, this study also assessed the effects of the dopamine D2/3 receptor antagonist raclopride and the dopamine receptor agonist apomorphine. PD149163 significantly reduced responding at doses ranging from 0.1 to 10.0 mg/kg in the food-reinforced rats and significantly reduced responding at a 1.0 mg/kg dose, but not a 10.0 mg/kg dose, in the shock-avoidance group. Raclopride also significantly reduced responding in the food-reinforced group at a 0.1 mg/kg dose, but differences in responding were not found in the shock-avoidance group. Similarly, a significant reduction in responding was shown for apomorphine, which occurred at a 1.0 mg/kg dose, but a reduction was not shown in the shock-avoidance group. For each compound, responding persisted at higher doses in the shock-avoidance condition compared to the food reinforcement condition. For PD149163, in particular, response reductions were not shown following administration of a 10.0 mg/kg dose, whereas responding was suppressed at doses higher than 0.1 mg/kg in the food reinforcement group. These findings suggest that aversive stimulation may be necessary in order to study higher doses of neurotensin receptor agonists in animal behavioral models.

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Title: Trk B receptor agonist, 7,8-Dihydroxyflavone, suppress sleep and orexin levels

Authors: *P. FENG[1], A. AKLADIOUS[2], Y. HU[1], P. J. SMITH[2];
[1]Res., Case Western Reserve Univ/Cleveland VA, CLEVELAND, OH; [2]Louis Stokes Cleveland DVA medical Ctr., Cleveland, OH

Abstract: Introduction: Brain-derived neurotrophic factor (BDNF) has been broadly studied for effects on nerve growth, neural protection and mood regulation. BDNF primarily binds on Tropomyosin-receptor-kinase (Trk) B receptors that regulate synaptic strength and plasticity in the mammalian nervous system. 7,8-Dihydroxyflavone (7,8-DHF) is a recently identified small molecular Trk B agonist and has been demonstrated for antidepressive effect, memory consolidation and neuroprotective effect. 7,8-DHF also affects fear response but there is lack of information on how 7,8-DHF affects sleep and sleep regulation. Methods: Experiment was conducted in mice (n=7 for each group) implanted with electrodes for EEG and EMG recording after 10 days of post surgical recovery and sufficient adaptation to recording cages. After 24 hours baseline recording, 20 mg/kg of 7,8-DHF or vehicle (DMSO) was injected (i.p.) at the beginning of dark phase. Animals were sacrificed on the next day one hour after another dose of treatment, and orexin A was quantified using ELISA kit. Sleep and wake data were evaluated using two way ANOVA on 4 hour data segments. Orexin A data was evaluated by t-test. Results: Compared with the vehicle (51.38%), total sleep was significantly ($q=2.98$; $p=0.035$) decreased in the 7,8-DHF group (41.25%) in the third 4-hour segment, i.e., 8 hours post drug treatment (still in the dark phase). Further analysis indicated that this difference was due to significant ($q=3.077$; $p=0.03$) decrease of NREM sleep (36.54% in 7,8-DHF vs. 45.75% in vehicle) but not REM sleep (4.71% in 7,8-DHF vs. 5.62% in vehicle). Interestingly, hypothalamic level of orexin A was also significantly ($t=2.616$, $p=0.017$) decreased in the 7,8-DHF group (97 pg/mg in 7,8-DHF vs. 132 pg/mg in vehicle). However, differences of orexin B between groups was not significant. Conclusion: 7,8-DHF suppressed NREM sleep in a later dark phase but suppressed orexin A early phase. More work needs to be done to determine the relationship between 7,8-DHF induced changes in orexin and sleep. **Support:** This study is supported by VA Merit Award BX001814 and VA Rehabilitation Research & Development award RX000150 and Cleveland VA Medical Research Service.

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Poster

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Title: Modification of thalamocortical transmission by the thalamic reticular nucleus: Computation and optical studies

Authors: *B. J. SLATER[1], A. M. WILLIS[3], D. A. LLANO[2];
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Abstract: The role of the thalamic reticular nucleus (TRN) in thalamocortical (TC) transmission is not known. Many investigators have examined the potential for reciprocal TRN-TC interactions to contribute to oscillatory phenomena, such as spindle oscillations and absence seizures. However, there is emerging evidence that open-loop circuits are found between TRN and TC cells. The implications of open-loop configurations are not understood, particularly when they include time-dependent nonlinearities in TC cells such as low-threshold bursting. We hypothesized that low-threshold bursting in an open-loop circuit could be a mechanism by which the TRN could paradoxically enhance TC activation, and that this enhancement would depend on the relative timing of TRN vs. TC cell stimulation. To test this, we performed both computational studies as well as optical studies in a brain slice preparation. For the computational studies, we modeled small circuits containing a TC neuron, TRN neuron and a layer 4 thalamorecipient cell. We found that open-loop TRN stimulation, rather than universally depressing TC activation, increased cortical output across a broad parameter space and primarily functioned to enhance the likelihood that high-frequency inputs could activate the cortex. This TRN-based enhancement disappeared when T-type calcium channel currents were removed. We tested the predictions made by the model using a recently-developed colliculo-thalamocortical slice preparation in mice. We stimulated the inferior colliculus (IC) and measured the resulting thalamic and cortical activation using flavoprotein autofluorescence imaging. We found that UV laser-based glutamate uncaging in the TRN just prior to inferior colliculus stimulation can modify colliculo-thalamocortical transmission, enhancing cortical activation when the TRN stimulus preceded the IC stimulus by 50-100 ms. Thus, the dynamics of the TRN-based GABAergic inputs to TC cells play a major role in processing and gating of information between the thalamus and the cortex by controlling the firing mode of thalamocortical cells.

Disclosures: B.J. Slater: None. **A.M. Willis:** None. **D.A. Llano:** None.

Poster

814. Auditory Cortex

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Title: A feedforward inhibitory circuit mediates lateral refinement of auditory cortical processing in upper layer 2/3

Authors: *L.-Y. LI[1], X. JI[4], F. LIANG[4], H. W. TAO[2], L. I. ZHANG[3];
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Abstract: Sensory information undergoes ordered and coordinated processing across cortical layers. While cortical layer 4 (L4) faithfully acquires thalamic information, the superficial layers appear well staged for a deep processing of L4-relayed signals to generate corticocortical outputs. However, the specific role of superficial layer processing and how it is specified by local synaptic circuits remain not well understood. Here we show that upper L2/3 circuits play a crucial role in refining L4-relayed neuronal selectivity by sharpening auditory receptive fields and enhancing the contrast of frequency representation. The refinement can be attributed to a lateral suppression mediated by more broadly recruited inhibition than excitation, with the inhibition predominantly originating from inhibitory neurons in the same layer. By comparing the onsets of synaptic inputs as well as of spiking responses of different types of neuron, we find that the broadly tuned, fast responding inhibition observed in excitatory cells can be primarily attributed to feedforward inhibition from parvalbumin (PV)-positive neurons, while somatostatin (SOM)-positive inhibitory neurons respond much later compared to the onset of inhibitory inputs to excitatory neurons. We propose that the feedforward circuit mediated lateral inhibition enables the L2/3 excitatory neurons to rapidly refine sensory representation.

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Poster

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Title: Detecting causal connectivity from correlated spiking activity

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Abstract: The link between structure and function at the level of local neocortical circuits is largely unknown. While patterns in the cross-correlation of neuronal spiking can reflect underlying connections (Gerstein and Perkel 1969), it is difficult to use spiking correlations to identify causal synaptic connectivity. This is because individual connections are weak compared to the amount of synaptic input required to drive an action potential. Moreover, the strength of a synaptic connection depends on recent and long-term history. In order to achieve a mechanistic understanding of cortical computation, it is necessary to delineate the role of synaptic connectivity in the production of dynamic patterns of multineuronal activity. Using 2-photon Ca^{2+} imaging, we imaged spiking activity from up to 1000 neurons in auditory cortex. We then computed functional circuit diagrams quantifying reliable lagged firing using iterative Bayesian inference. These diagrams form graphs—a succinct representation of activity in which each neuron is a node and each reliable spike progression is an edge. To guide experimental design and benchmark inferred relationships, we have implemented a network model of LIF neurons with conductance-based synapses. A null model, consisting of unconnected Poisson neurons, does not replicate the high degree nodes, long continuous paths, or strong edges seen in reconstructions of connected populations. Thus, these maps delineate emergent patterns in the flow of activity, beyond what is expected by chance coincidence in spike times. Next, we tested whether topological features of the activity maps were informative of underlying synaptic connectivity. At fast scan rates, strong edges coincided with monosynaptic connections significantly more often than expected by chance, particularly for the strongest synaptic connections. Auditory cortical circuitry produces highly reliable and temporally precise firing activity (Sadovsky and MacLean 2013), and our modeling shows that these features are the most useful for indicating a causal connection. This fact makes auditory cortex the ideal model for

verifying our predictions of underlying connectivity. Modeling shows that a diversity of activity greatly enhances our ability to detect the underlying synaptic topology. Therefore spontaneous activity, which exhibits diverse pairwise spiking correlations, may be particularly efficacious to detect underlying causal connections. By exploring the relationship between network connectivity and activity, we demonstrate a set of 'top-down' heuristics to map function onto structure.

Disclosures: B. Chambers: None. **J.B. Dechery:** None. **J.N. MacLean:** None.

Poster

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Title: Activation of GABAergic interneurons by thalamocortical afferents and during cortical network activity

Authors: *B. M. KRAUSE[1], S. M. GRADY[2], A. RAZ[2], D. J. UHLRICH[3], M. I. BANKS[2]; [1]Neurosci. Training Program, [2]Anesthesiol., [3]Neurosci., Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Spiking responses to thalamocortical input in auditory cortex *in vitro* and *in vivo* occur primarily during brief evoked UP states. Somatostatin (Sst+) interneurons receive strong intracortical excitation and likely contribute to regulating the balance between excitation and inhibition during UP states. Sst+ interneurons can also be driven directly by thalamocortical input. Feed-forward activation of dendrite-targeting Sst+ interneurons can alter dendritic integration and may decrease the influence of top-down inputs during thalamocortical activity. Here, we show that Sst+ neurons are preferentially activated both by direct thalamocortical input and also during evoked cortical network activity, and that activation of Sst+ interneurons can regulate the occurrence of cortical UP states. **Methods:** Auditory thalamocortical (TC) slices were prepared from the offspring of Sst-Cre driver mice crossed with tdTomato Cre-reporter mice (Ai14) to label Sst+ neurons. TC fibers were activated with brief (100 ms) electrical stimulus trains (40 Hz) via bipolar electrodes. Ca imaging (OGB-1 AM) was

used to identify spiking cells as a function of laminar depth and combined with td-tomato fluorescence to identify spiking Sst+ neurons. Cells that spiked at low stimulus intensities or with UP states blocked using high divalent cations were targeted for whole-cell patch clamp and filled with biocytin. In other experiments, Sst-Cre mice were injected in auditory cortex with AAV carrying Cre-dependent channelrhodopsin [hChR2(H134R)] to selectively activate Sst+ neurons. **Results:** Although only about 6% of cortical neurons are Sst+, 18.4% of cells spiking in response to minimal intensity TC stimuli were Sst+. At higher stimulus intensities that evoked UP states, 10.3% of responding cells were Sst+. At high stimulus intensities, but with UP states blocked using high divalent cations, 36.7% of responding cells were Sst+, all located in layer 5. Targeted patch clamp of spiking cells in high divalent cation experiments showed that the responding Sst+ cells were Martinotti cells that had axons ascending to layer 1, had a burst-firing/low-threshold spiking pattern, and received facilitating TC EPSPs. Light-activation of Sst+ neurons expressing channelrhodopsin suppressed TC-evoked UP states. **Conclusions:** These data show that Sst+ interneurons are preferentially activated both by direct TC input and in the context of cortical UP states. Sst+ interneurons may regulate the threshold or intensity of cortical UP states. Additionally, early Martinotti cell activation may suppress inputs to the distal dendrites of pyramidal cells during TC input.

Disclosures: B.M. Krause: None. **A. Raz:** None. **S.M. Grady:** None. **M.I. Banks:** None. **D.J. Uhlrich:** None.

Poster

814. Auditory Cortex

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Topic: D.02. Auditory

Title: Learning-related neuronal circuit dynamics in mouse auditory cortex investigated by chronic two-photon calcium imaging

Authors: *B. LAURENCZY, F. HELMCHEN, H. LÜTCKE;
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Abstract: The rodent auditory system is an established model system for studying adult cortical plasticity during learning. Training on a pure tone frequency discrimination task, for example, leads to extensive changes which lead to the rewarded frequency becoming overrepresented in the tonotopic map. However, the corresponding changes in specific populations of neurons in

auditory cortex remain poorly defined because it has been difficult to track the activity of defined neurons and populations throughout the learning process. Moreover, it remains unclear how persistent learning-related changes are and whether the map is renormalized after the end of the learning period. To address these questions, we used chronic two-photon calcium imaging in layer 2/3 of mouse primary auditory cortex while the animals were learning a water-rewarded Go/No-Go 'cloud-of-tones' discrimination task. Mice were trained to discriminate between low- and high-frequency centered 'cloud-of-tones', which consist of sequences of overlapping short pure tones ranging from 4 to 20 kHz. Animals readily learned this task and reached expert levels (criterion: d' > 2) within 2 to 6 days (1000 to 2000 trials). To measure activity of the same neuronal populations throughout learning, we expressed the genetically-encoded calcium indicator yellow-cameleon Nano140 (YC-Nano140) in the auditory cortex and implanted chronic cranial windows. Stable expression of YC-Nano140 without apparent nuclear filling was observed over weeks, allowing us to identify populations of 50 - 100 neurons and measure their trial-related activity at three different stages: before learning (naive), during learning and when animals had reached stable task performance (expert). Preliminary analysis revealed a recruitment of auditory-evoked neurons during learning, which was stronger for the target stimulus than for the distractor. In addition, we found no clear evidence for re-normalization of activity once animals performed at expert levels.

Disclosures: B. Laurenczy: None. **F. Helmchen:** None. **H. Lütcke:** None.

Poster

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Helen Hay Whitney Foundation Postdoctoral Fellowship

Title: Dissecting local and long-range circuits involved in the movement-related modulation of auditory cortex

Authors: *D. M. SCHNEIDER, A. NELSON, R. MOONEY;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: The auditory system processes sounds that arise from the environment as well as sounds that are generated by an animal's own movements, and differentiating these two sound classes is important for normal hearing and behavior. To make this distinction, the auditory system likely integrates sensory information with copies of internally generated motor commands, including at the level of the auditory cortex (ACtx). Although movement-related modulation of spontaneous and sound evoked activity has been observed in the ACtx of mice, monkeys and humans, the local and long-range circuits that convey movement-related signals to the ACtx are not known. Here, we made sharp intracellular recordings in the ACtx of mice on a treadmill to monitor spontaneous and sound-evoked synaptic activity during periods of movement and rest. Movement was preceded and accompanied by stereotyped changes in membrane potential dynamics of ACtx excitatory neurons, including decreased variability, a slight depolarization and decreased tone-evoked responses. More than half of the movement-related suppression of tone-evoked responses was accounted for by local cortical suppression, and this suppression was likely mediated by increased local inhibition, since movement was also accompanied by decreased excitability and input resistance in ACtx excitatory neurons. In agreement with this observation, extracellular array recordings revealed that inhibitory neurons - including identified PV+ cells - increased their spiking prior to and during movement, whereas putative excitatory cells decreased their spiking. A population of neurons in secondary motor cortex (M2) synapses in the ipsilateral ACtx, including onto PV+ interneurons, forming a potential substrate for conveying movement-related signals to the ACtx. We found that M2 neurons, including those that project to ACtx, increased their activity prior to and during movement. Optogenetically activating M2 axon terminals in the ACtx was sufficient to reproduce movement-like membrane potential dynamics and to suppress tone-evoked responses. Moreover, optogenetically silencing ipsilateral M2 - but not contralateral M2 - quickly recovered rest-like membrane potential dynamics and tone-evoked responses in the ACtx, even as movements continued. These experiments identify a long-range circuit through which movement-related information infiltrates the ACtx and a local mechanism of increased PV+ interneuron activity that suppresses ongoing activity and sensory response in ACtx excitatory neurons.

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Poster

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Support: NIMH Silvio Conte Center 1P50MH094271

Nancy Lurie Marks Family Foundation

Canadian Institute for Advanced Research

Title: A disinhibitory circuit for auditory thalamocortical plasticity

Authors: *A. E. TAKESIAN[1,2], L. J. BOGART[2], A. L. COVELLO[2], T. K. HENSCH[2,1]; [1]Neurol., Boston Children's Hosp., Boston, MA; [2]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Inhibitory cells within cortical layer (L) 1 have been recently identified as important regulators of state-dependent sensory processing and associative learning. However, little is known about how these cells function within the circuits to influence cortical activity and plasticity. Here we examined the role of a subclass of L1 cells that express ionotropic 5-HT_{3A} receptors. In mouse auditory cortical slices, these cells were activated by local application of either 5-HT_3 or nicotinic acetylcholine receptor (nAChR) agonists. Optogenetic activation of these cells produced inhibitory responses in parvalbumin (PV)-expressing inhibitory cells that suppressed spiking responses to thalamic stimulation. Consistent with this functional inhibition, we observed that a subset of brainbow-expressing 5-HT_{3A} cells form putative contacts with PV cells within both superficial and deep cortical layers. In pyramidal cells, 5-HT_{3A} cell activation reduced thalamic-driven disynaptic inhibition, leading to greater and prolonged excitation. We further asked whether this disinhibitory circuit is involved in the robust sensory plasticity that occurs during developmental critical periods. During a 3-day critical period in the mouse, auditory thalamocortical connectivity is restructured by passive sound exposure. We examined this plasticity in Lynx1 knockout (KO) mice, which show heightened nAChR function and enhanced L1 cell activity. These mice continued to show robust sound-induced modification of thalamocortical connectivity beyond the critical period. Together, this study suggests that the recruitment of 5-HT_{3A} receptor-bearing L1 cells by neuromodulators may engage a disinhibitory circuit that promotes thalamocortical plasticity. The results highlight these cells as potential therapeutic targets to reinstate plasticity in auditory cortex beyond early critical periods.

Disclosures: A.E. Takesian: None. **L.J. Bogart:** None. **A.L. Covello:** None. **T.K. Hensch:** None.

Poster

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T32GM007507

Title: Modulation of spontaneous and evoked activity in auditory cortex by just-hypnotic doses of three anesthetic agents

Authors: *M. I. BANKS[1], N. S. MORAN[1], T. J. STILP[1], B. M. KRAUSE[2], S. M. GRADY[2]; [1]Dept. of Anesthesiol., [2]Neurosci. Training Program, Univ. of Wisconsin, Madison, WI

Abstract: Introduction: The mechanism whereby anesthetics cause loss of consciousness (LOC) is poorly understood. Current theories suggest that alterations of information and connectivity within cortico-thalamic networks underlie LOC. There have been few studies focused on the mechanisms of LOC that are common across diverse agents. We investigated changes in stimulus representation and connectivity in cortical networks upon LOC by recording spontaneous and stimulus-evoked spiking activity in auditory cortex using chronically-implanted electrode arrays. We tested the effects of three anesthetic agents with diverse molecular targets: isoflurane (iso), which acts at multiple pre- and postsynaptic loci, propofol (pro), which acts primarily on GABAA receptors, and dexmedetomidine (dex), an α2 adrenergic agonist. Methods: Multiunit activity was recorded from auditory cortex in rats chronically implanted with 16 channel microwire arrays (TDT 2x8). Stimuli consisted of pure tones, click trains and animal vocalizations. After recording under waking conditions, animals were exposed to sub-hypnotic and just-hypnotic doses of iso, pro and dex. Spike trains were characterized by measuring pre-stimulus (baseline) firing rate, driven rate (response rate - baseline) and correlation index (shuffled auto-correlogram at lag 0). In addition, pairwise noise correlations were measured across channels either via trial-by-trial spike counts or by the integral of the cross-correlogram. Results: Under control conditions, median noise correlation coefficients decreased with distance between electrodes, from ~0.8 at <0.5 mm to ~0.6 at >1 mm. All three agents decreased noise correlations in a dose-dependent manner (-13%, -58% & -22% for iso, pro & dex, respectively, at just-hypnotic doses). There was a modest interaction between drug and distance, with correlation values decreasing to a greater extent with distance under just-hypnotic doses. Consistent effects across agents were observed on baseline firing rate (-64%, -69% & -39% for iso, pro & dex, respectively, at just-hypnotic doses), driven rate (-33%, -60% & -63% for iso, pro & dex, respectively, at just-hypnotic doses), and on correlation index (+53%, +8.7% & +39% for iso, pro & dex, respectively, at just-hypnotic doses). Conclusions: These data indicate that agents with diverse molecular targets have common effects on population and network activity in cortex, though quantitative differences are observed across agents. However, all observed effects

were dose-dependent, and no qualitative difference was observed at just-hypnotic versus sub-hypnotic doses for any one measured parameter.

Disclosures: M.I. Banks: None. **N.S. Moran:** None. **T.J. Stilp:** None. **B.M. Krause:** None. **S.M. Grady:** None.

Poster

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Title: Effects of local inhibition on stimulus-specific adaptation across laminae of primary auditory cortex

Authors: *J. BRIGUGLIO[1], R. G. NATAN[2], L. MWILAMBWE-TSHILOBO[2], M. N. GEFFEN[2];
[1]Physics and Astronomy, [2]Dept. of Otorhinolaryngology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The majority of neurons in the primary auditory cortex (A1) exhibit stimulus-specific adaptation (SSA), a property in which responses to frequently occurring stimuli are suppressed, while those to rare stimuli are relatively enhanced. SSA is thought to underlie a range of auditory processing mechanisms such as detecting deviant sounds in complex acoustic environments. It has been hypothesized that SSA is generated by intra-cortical inhibitory circuits. Here, we tested whether intra-cortical inhibitory circuits control SSA propagation across cortical laminae. We recorded local field potentials across all cortical layers in A1 of lightly anesthetized head-fixed

mice, using linearly arranged multi-site electrodes, and computed current-source density profiles across laminae. Mice were exposed to classical oddball stimuli designed to reveal SSA in neuronal activity. Using an optogenetic technique, we selectively suppressed parvalbumin (PV+) or somatostatin (SOM+) positive interneuron activity during interleaved trials. Inactivation of either PV+ or SOM+ interneurons reduced SSA in local field potentials and current-source density profiles. To investigate how interneurons influence the propagation of SSA within A1, we compared the effects of SSA suppression in granular, supra- and infra-granular layers. SSA was stronger in infra-granular layers as compared to the thalamo-recipients layers III-IV, suggesting that SSA is passed on and amplified through local circuits between granular and infra-granular layers. Consistent with this interpretation, inactivation of either PVs or SOMs resulted in greater reduction in SSA in the infra-granular than in granular or supra-granular layers. Our results suggest that local inhibitory interneurons may differentially control the adaptive feedback from A1 to cortical and subcortical targets.

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Title: Characterization of cell-type specific inputs to auditory cortex with intersectional presynaptic tracing

Authors: *A. NELSON, R. MOONEY;
Duke Univ., Durham, NC

Abstract: The auditory cortex is a highly complex and interconnected structure critical to auditory perception and high-order processing of sounds. Extensive efforts using classical tracing methods have revealed massive interconnectivity between auditory cortex and other brain regions at a population scale. Despite these advances, recent experiments have only begun to detail the function and organization of neuronal circuits that provide input to the distinct classes of excitatory and inhibitory neurons that comprise local auditory microcircuits and complicate

the interpretation of nonspecific tracing experiments. To address this issue, we took advantage of intersectional cell type-specific rabies presynaptic tracing methods to identify brainwide neuronal inputs to distinct classes of excitatory and inhibitory neurons of the mouse auditory cortex. Using combined anterograde and presynaptic retrograde tracing, immunostaining, and stereological reconstruction, we identified and characterized the distinct cortical and subcortical populations of neurons providing input to genetically-identified neurons, as well as recurrent organization embedded within these circuits. To begin to characterize how these inputs function and influence their neuronal targets, we used 2-photon calcium imaging to monitor the activity of a subset of inputs to auditory cortical neuron subtypes in behaving mice. These experiments provide insight into the brainwide organization and function of inputs to cell type-specific auditory cortical circuits.

Disclosures: A. Nelson: None. **R. Mooney:** None.

Poster

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Title: Connections of auditory cortex with premotor areas in the marmoset monkey

Authors: *L. A. DE LA MOTHE[1], M. MITCHELL[1], C. T. MILLER[2];
[1]Psychology, Tennessee State Univ., NASHVILLE, TN; [2]Psychology, Univ. of California San Diego, San Diego, CA

Abstract: Part of understanding speech and communication is not just gaining insight into auditory processing, but also understanding the role of vocal-motor interactions. In companion studies, neurons in premotor areas 6d and 6v were found to be responsive to auditory stimuli and vocalizations. In light of the auditory responsiveness of these premotor areas the aim of the current study is to examine potential sources of auditory input. The current working model of primate auditory cortex identifies three levels of processing corresponding to regions: primary core, secondary belt, and a third level of processing the parabelt. Romanski et al. (1999) have

reported previously that auditory projections to frontal cortical areas arose predominantly from the parabelt region. Based on this work we hypothesize that auditory input to premotor areas 6v and 6d would originate mainly from the parabelt region. To investigate the connection patterns, injections of fluororuby (FR) combined with Cholera Toxin Subunit-B (594) were made into area 6v and biotinylated dextran amine (BDA) into area 6d of the marmoset monkey. The tissue was processed for tracers and other histology and then imaged microscopically. Tissue sections were outlined and the x-y position of retrograde labeled cells was plotted to identify inputs to areas 6v and 6d. Areal borders were determined from reconstruction of the tissue. Retrograde labeled cells identified from both injections in 6d and 6v were found predominantly in the parabelt as well as the belt region of auditory cortex; consistent with what Romanski et al. (1999) reported for frontal connections. Future studies will focus on other potential sources of auditory information into premotor cortex. By identifying the connections involved in vocal-motor interactions we edge closer to understanding the underlying structure and organization of communication.

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Poster

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RO1EY022720

Title: Crossmodal plasticity alters correlated neuronal activity in auditory cortex

Authors: *K. ORZECHOWSKI[1], P. KANOLD[2];
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Abstract: Although within-modality sensory plasticity is limited to early developmental critical periods, crossmodal plasticity has been shown to occur even in adult sensory systems. Previous studies have revealed significant changes that occur in the auditory cortex (ACX) of visually-deprived adult mice, including improved frequency selectivity, increased frequency discrimination, and strengthened thalamocortical synapses in ACX, whereas this enhancement

was not mirrored in the visual cortex (Petrus et al. 2014). However, the methodology employed in these studies revealed only the changes occurring on a single-cell and synaptic level, while failing to capture the changes in local network connectivity and population dynamics as a result of crossmodal sensory deprivation. Changes in population dynamics could underlie altered sound encoding performance of cortical circuits. Here we investigated the changes that occur in ACX in mice visually deprived for a short period of time after the critical period using *in vivo* two-photon calcium imaging, which allows the simultaneous measurement of dozens of neurons' responses with single-cell resolution. To quantitatively measure the tone-evoked population responses of ACX both in dark-exposed and normally-reared mice, we compute signal correlations (a measure of stimulus-dependent correlated activity) and noise correlations (trial-to-trial stimulus-independent covariance) between simultaneously imaged cells. We find that both correlation measures change in dark-exposed animals, suggestive of specific modifications in ascending thalamocortical inputs and intracortical connectivity in the ACX after visual deprivation. These results show that crossmodal sensory experience has the power to alter network circuitry and population dynamics even into adulthood, and likely plays a role in the enhancement of one sensory modality (hearing) following the loss of another (vision).

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Poster

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NIH P30 DC08369

Title: What portion of A1 receptive fields is thalamocortical?

Authors: I. INTSKIRVELI[1], A. JOSHI[1], B. J. VIZCARRA-CHACÓN[2], *R. METHERATE[1]; [1]Univ. California, Irvine, Irvine, CA; [2]Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: A common tool for distinguishing sensory-evoked response components mediated by cortical vs. thalamocortical circuits is the drug muscimol, a GABA-A receptor agonist that, when applied to the cortex, is thought to inhibit cortical circuits but not afferent inputs. Although

muscimol (1-5 mM) often is used for this purpose, recently its specificity was questioned on the grounds that it may reduce thalamocortical inputs via presynaptic GABA receptors. Here we re-examine the premise that muscimol can selectively suppress cortical circuits but not thalamocortical inputs. Using a 16-channel multiprobe in mouse A1 (urethane anesthesia), we recorded tone-evoked current-source density profiles and determined frequency receptive fields for three current sinks: input layer (fastest onset; "layer 4"), 100 μm above ("layer 2/3") and 300 μm below ("layer 5/6"). We microinjected muscimol (0.01-5 mM) into middle layers or the cortical surface and first examined the CF tone-evoked layer 4 (thalamocortical) current sink with the rationale that the ideal muscimol dose should leave thalamocortical inputs unaffected while suppressing intracortical responses. We measured sink onset latency and sink amplitude in 5 ms increments, expecting the first 5 ms to be mostly thalamocortical and subsequent 5 ms intervals to be increasingly intracortical. Muscimol at 0.01-0.05 mM had little effect on any 5-ms interval. In contrast, 0.1 mM muscimol had no effect on the first 5 ms interval but reduced longer-latency responses, implying an optimal dose that distinguishes thalamocortical vs. cortical contributions. Higher doses (≤5 mM) reduced responses at all intervals, indicating suppression of both thalamocortical and cortical circuits. Using the optimal, 0.1 mM dose, we extended the analysis across layers and to CF ± 1.5 octaves in quarter-octave steps, to estimate the laminar and spectral extent of thalamocortical inputs. Muscimol did not affect onset latency for any stimulus or current sink, implying that minimal thalamocortical projections extend broadly; however, amplitude effects at 5-ms intervals suggest that thalamocortical inputs: i) strongly drive layer 4 current sinks only from 0.25 oct. below CF to 0.5 oct. above, ii) only minimally drive the layer 2/3 current sink at any frequency, and iii) exclusively drive the layer 5/6 sink for all frequencies. The implied thalamocortical circuitry suggests that muscimol remains a useful tool, albeit at lower doses than previously used.

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Poster

814. Auditory Cortex

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 814.14/CC36

Topic: D.02. Auditory

Title: The auditory corticocollicular system in mouse: Synaptic and cellular properties

Authors: *K. STEBBINGS[1,3], B. SLATER[1], D. A. LLANO[2];
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Abstract: The neural mechanisms by which high-level information may be combined with bottom-up inputs to modulate the perception of sound are not yet known. A potential substrate is the massive set of projections from the auditory cortex to subcortical structures, such as the auditory midbrain (inferior colliculus, IC). Multiple investigators have shown that manipulation of the corticocollicular pathway can change the response properties (best frequency, best duration and minimum threshold) of targeted IC neurons. Despite the importance of the corticocollicular pathway in shaping IC response properties, basic questions about the synaptic properties of this projection and how it interacts with local IC circuitry remain unknown. To characterize the synaptic properties of the corticocollicular projections, we developed a brain slice preparation in the mouse which contains projections from the auditory cortex to the inferior colliculus. Flavoprotein autofluoresence imaging was used at the start of each experiment to asses this connectivity. In slices where connectivity was retained, the auditory cortex was stimulated using a bipolar electrode or glutamate uncaging while recording, in whole cell configuration, from individual cells in the external cortex of the inferior colliculus (ICx). We found that at least 3 of 4 types of known cells (regular, adapting, pauser) in the ICx, displaying a range of currents, including hyperpolarization-activated cation currents and rebound currents, responded to either photouncaging of glutamate in the cortex or electrical stimulation. All cells responded with relatively large EPSPs (2.5-5mV) or EPSP IPSP sequences with latencies ranging from 6-16ms. Some cells appear to show synaptic depression while others did not or showed facilitation and several responded to pulse trains up to 40 Hz. These data suggest that the mouse auditory corticocollicular pathway contains previously undescribed heterogeneity in its cellular and synaptic properties, which may help to explain the protean manifestations of manipulations of corticocollicular projections.

Disclosures: K. Stebbings: None. **B. Slater:** None. **D.A. Llano:** None.

Poster

814. Auditory Cortex

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 814.15/DD1

Topic: D.02. Auditory

Support: Burroughs Wellcome Fund

New York Stem Cell Foundation

David Mahoney Fellowship

Title: Imaging auditory and parietal cortical sound representations in mice during virtual navigation

Authors: *C. A. RUNYAN, C. D. HARVEY;
Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: The transfer of information from sensory cortex to multimodal association areas is critical for generating sensory representations of objects and appropriate behavioral responses in complex sensory environments. In the auditory system, the primary auditory cortex (A1) passes information to multiple target regions, including the posterior parietal cortex (PPC). We aimed to study the circuit-level details of the representation and transfer of sound information from A1 to PPC across behavioral contexts. First, in awake, passively listening mice, we compared population coding of sound features in A1 and PPC using two-photon calcium imaging of layer 2/3 neurons labeled with GCaMP6f. We developed a surgical preparation with two cranial windows to allow imaging of A1 and PPC neurons in mice running voluntarily on a spherical treadmill. This preparation also allows imaging in the PPC of axons originating from A1 neurons, to examine the direct transfer of signals between areas. A spatial array of four speakers surrounding the mouse's head was used to deliver sound stimuli of varying frequency, intensity, and temporal characteristics from four actual locations and an arbitrary number of "virtual" locations. During passive listening, population neuronal activity in A1 was selective across all tested simple sound dimensions, including location, frequency, and intensity. Although individual neuron tuning curves for these sound features could be obtained in A1, population activity patterns, such as multi-cell motifs or assemblies, were a more striking feature of sound-driven responses. PPC neuronal activity also contained stimulus-related activity that discriminated multiple types of sound features. Second, to investigate A1-to-PPC information transfer in the context of a navigation-based, perceptual decision task, we combined the imaging methods and sound stimulus delivery with a visual virtual reality system. We developed a behavioral task in which mice must categorize sound locations, in head-centered coordinates, to make memory-guided turns in a virtual T-maze. Mice were able to discriminate sounds within 15-degrees of azimuth and to report categorized sound locations following a ~2 second memory period. Together, this experimental framework is aimed to understand the representation of auditory information and how it is transferred to the PPC, especially in the context of guiding behavioral choices.

Disclosures: C.A. Runyan: None. **C.D. Harvey:** None.

Poster

814. Auditory Cortex

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 814.16/DD2

Topic: D.02. Auditory

Support: NSF Grant 0903622

Title: Layer-specific differences in the mouse auditory corticocollicular pathway: An anatomical study

Authors: *G. YUDINTSEV[1], A. M. H. LESICKO[1], D. A. LLANO[2];
[1]Neurosci., [2]Mol. and Integrative Physiology, Neurosci., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The auditory corticocollicular (CC) projection has recently garnered much attention due to its ability to alter response properties of cells in the inferior colliculus (IC). However, the basic anatomical organization of this pathway still remains poorly understood. CC cells emanate from two distinct bands of the auditory cortex - layer 5 and deep layer 6 - and differ in their firing properties and cellular morphology. In the present study, we sought to characterize potential anatomical differences between layer 5 and layer 6 CC cells using retrograde and anterograde tract-tracing techniques. We injected fluorogold, a retrograde tracer, into the IC of the mouse and used Neurolucida to create 3-D reconstructions of the cellular distribution of CC cells in layer 5 and 6 of the auditory cortex (AC). Reconstructions were aligned with acoustically-driven response maps obtained with transcranial flavoprotein autofluorescence imaging. We found heterogeneity in the distributions of the two layers, such that there exists a rostro-ventral area of the AC in which layer 6 corticocollicular cells were more dominant than layer 5 corticocollicular cells. To investigate potential differences in terminal size and projection patterns between layer 5 and layer 6 CC neurons, we injected dextran amine anterograde tracers into either layer 5 or layer 6 of the AC of mice. We found projections from both layer 5 and layer 6 in all subdivisions of the IC. On average, layer 5 axons were thicker than layer 6 axons and their terminals were larger and had a wider distribution of lengths than layer 6 terminals. These data further suggest that the layer 5 and layer 6 CC projections differ in their basic organization and, therefore, could serve distinct functions in auditory processing.

Disclosures: G. Yudintsev: None. **A.M.H. Lesicko:** None. **D.A. Llano:** None.

Poster

814. Auditory Cortex

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 814.17/DD3

Topic: D.02. Auditory

Title: Spatiotemporal dynamics of sub- and suprathreshold tone-evoked responses in auditory cortex

Authors: *E. NYLEN, M. GRAUPNER, A. REYES;
NYU, New York, NY

Abstract: Acoustic information is encoded in the activity of a population of neurons in primary auditory cortex (A1). During a tone stimulus, a population of neurons is recruited in a manner that depends on the frequency and intensity. The extensive local synaptic connections between the excitatory and inhibitory cells coupled with the heterogeneous firing and receptive field properties are likely to give rise to rich and complex spatiotemporal firing patterns. Here, we examine the tone-evoked responses of identified neurons in layers 2/3 and 4 of mouse A1. By sequentially documenting the responses and precise locations of excitatory and inhibitory neurons in a given area of A1, we reconstruct the spatial extent of the activated neurons, their firing patterns and underlying subthreshold synaptic events, and the temporal correlations between cell pairs. With the aid of two photon microscopy, we performed simultaneous whole-cell and/or cell-attached recordings from neuron pairs in layer 2/3 and 4 of anesthetized (ketamine/xylazine) mice. Recordings were established using targeted patching from PV and Somatotstatin interneurons expressing EGFP and from pyramidal neurons. Tone pips (50-ms duration) were presented (4 - 46 kHz in 0.1 octave steps; 0-80 dB SPL in 10 dB steps) and the membrane potentials or extracellular spikes recorded. After each recording, we documented the location of the neurons and guided one of the electrodes to another cell for cell-attached recording or repositioned a clean electrode for whole-cell recording. We were thus able to sequentially record the responses of many neurons to the same stimuli and determine the spatiotemporal firing patterns of a population of neurons. Preliminary results suggest that neurons spanning nearly one square mm area of cortex can be activated with a moderate intensity tone. Cross-correlation analyses reveal that the signal correlations between pairs of neurons are not fixed: as the tone frequency is increased, the peak of the spike time cross-correlograms shift systematically from positive (negative) time lags to zero time lag and then to negative (positive) time lags (n=57 out of 253 pairs). These observations were made from neurons separated by distances ranging from 16 to 1024 μm. Similar observations were made with spike-triggered

averages of the subthreshold membrane potentials obtained from cell-attached and whole-cell recorded neuron pairs. Hence, the flow of signals between neurons is not hardwired to the cortical circuits but can switch directions in a frequency-dependent manner.

Disclosures: **E. Nylen:** None. **M. Graupner:** None. **A. Reyes:** None.

Poster

814. Auditory Cortex

Location: Halls A-C

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Topic: D.02. Auditory

Support: NIH Grant DC005787-01A1

Alexander von Humboldt-Foundation

Title: Membrane potential correlations during spontaneous and tone-evoked activity in the auditory cortex *in vivo*

Authors: ***M. GRAUPNER**, E. L. NYLEN, A. D. REYES;
Ctr. For Neural Science, NYU, New York, NY

Abstract: Correlations in the spiking activities between neurons exist throughout cortex and have been observed under a variety of experimental conditions. In the auditory cortex, correlations may develop during acoustic stimulation (signal correlations) but may also be stimulus independent (noise correlations), arising instead from e.g. common synaptic inputs or global activity modulations. Here, we compare the noise and signal correlations in the subthreshold membrane potentials, which give rise to spike correlations, of neurons in auditory cortex during tone presentations and spontaneous activity. Using two-photon microscopy, we perform simultaneous *in vivo* whole-cell recordings from pairs of cells in layer 2/3 of the auditory cortex of ketamine/xylazine-anethetized mice (P30-45). Whole cell recordings were established in two cells using the two-photon targeted patching technique. The electrode solution contained QX-314 and cesium to block Na and K channels, respectively. Tone pips (50-ms duration) were presented (4 - 46 kHz in 0.1 octave steps; 40-60 dB SPL in 10 dB steps; 5 to 10 trials) to the contralateral ear and the membrane potentials were recorded with each electrode. To isolate excitatory and inhibitory synaptic inputs, the membrane potentials were held at their respective reversal potentials. At the conclusion of the recordings, the precise locations of the

two cells were documented and the best frequencies determined off-line. The membrane potentials of the two cells were then cross-correlated during and in between tone presentation. In the absence of tones, the membrane potentials of neighboring (<300 μm) neurons were moderately correlated (noise only correlations). During tones, the correlations (noise + signal correlations) did not change significantly from the noise only correlations. We had therefore expected that the signal only correlations obtained from the across-trial averaged potentials (to remove trial-to-trial variability) would be small. Instead, the signal only correlations were significantly larger than both the during tone correlations (signal+noise) and the in between tone correlations (noise only). Careful inspection of individually evoked synaptic potentials revealed that this unexpected result was due to the fact that tone-evoked synaptic potentials were unreliable and exhibited substantial trial-to-trial failures. In essence, the signal itself provided an additional source of stochasticity. Our results indicate that the representation of acoustic stimuli by an ensemble of neurons changes from trial-to-trial which poses an additional challenge for decoding stimuli through pooling from that ensemble.

Disclosures: **M. Graupner:** None. **E.L. Nylen:** None. **A.D. Reyes:** None.

Poster

814. Auditory Cortex

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Program#/Poster#: 814.19/DD5

Topic: D.02. Auditory

Support: NIH/NIDCD R03DC012585

NIH/NIGMS P30GM103340

Title: Synaptic circuit basis of auditory cortical modulation by prefrontal cortex

Authors: D. VUMBACO, *J. W. MIDDLETON;
Cell Biol. and Anat., LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: The ability of the Primary Auditory Cortex (A1) to encode various auditory stimuli depends upon distributed neural assemblies responding to acoustic frequencies in a spatially ordered fashion. This ordered distribution of receptive field (RF) frequency preferences is known as the tonotopy. The tonotopy in A1 exhibits stimulus dependent RF plasticity, which alters the ability of neurons to encode stimulus features, shifting the RF frequency preferences towards the

frequencies used to drive plasticity. This plastic remodeling is dependent on neuromodulation from a number of different brain regions. Recently the Prefrontal Cortex (PFC) was shown to play such a modulatory role in reshaping A1 RF distributions. However, the synaptic and circuit basis for this modulatory influence is not well understood. In order to quantify the synaptic connections from PFC to A1, neurons in the mouse PFC were transfected by adeno-associated virus (AAV) containing the genetic cassette for channelrhodopsin 2 (ChR2). This allowed us to activate PFC axons terminating in brain slices containing A1. Additionally, in the same animal, either the contralateral Auditory Cortex (CACx) or the ipsilateral Inferior Colliculus (IC) was injected with fluorescent retrograde tracers to identify long-range projection neurons. This allowed us to identify differential modulation from the PFC onto two distinct classes of A1 neurons projecting to the IC and CACx. We performed whole cell recording of pyramidal neurons in A1 slices and optogenetically activated ChR2 axon terminals to study the intrinsic, synaptic, and microcircuit properties of A1 neurons and their PFC inputs. With the aid of computer simulations of A1 networks, we show that the strong synaptic PFC inputs that we measure in experiments are able to facilitate synaptic reshaping of the model network tonotopy. Because of this powerful modulatory influence, the PFC may serve as a therapeutic target for treating chronic sensory deficits including tinnitus and presbycusis.

Disclosures: D. Vumbaco: None. **J.W. Middleton:** None.

Poster

814. Auditory Cortex

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 814.20/DD6

Topic: D.02. Auditory

Support: Del Amo Grant 2013

Title: Differential expression of parvalbumine-positive neurons in the auditory cortex of the rat due to diversity of auditory inputs

Authors: B. ROMERO-GOMEZ[1], C. FRANCES-FERRIZ[1], C. POSE-UTRILLA[1], F. CARRICONDO[1], A. MURCIANO[1], S. BAO[2], *F. PANETSOS[3,1];
[1]Univ. Complutense, Madrid, Spain; [2]Helen Wills Neurosci. Inst., Berkeley, CA; [3]IEMS, Univ. of Central Florida, ORLANDO, FL

Abstract: Sensory manipulations alter the expression of inhibitory neurons in primary sensory cortex. Recently one of us (S. Bao) showed that how experience alters cortical responses depends on the specific sensory input. They exposed rats to tones of different frequencies in different temporal orders and examined the effect of sound exposure on frequency selectivity of neurons in the primary auditory cortex (Au1): tuning of Au1 neurons is narrower if tones of the same frequency are played together is broader if tones of different frequencies are played together. Since inhibitory circuits shape frequency tuning and they are also modulated by sensory experience we hypothesized that different types of sensory experience differentially alter Au1 inhibitory circuits. Here we investigated how experience changes the expression of Au1 parvalbumine (PV)-expressing neurons. Three groups of female albino rats (single-frequency, half-range, full-range 4-32KHz) were placed with their mothers in an anechoic chamber from p9 to p35 hearing 1-s long trains of six tone pips (100 msec, 60dB SPL, one train every 2s). Tones were drawn from a logarithmically uniform frequency distribution. On p36 rats were sacrificed and histologically examined. We analyzed layers I-III, IV and V-VI separately in horizontal sections from central Au1 zones, mainly considered to respond to < 16KHz tones. Intergroup comparisons by means of ANOVA analysis and post hoc Fisher tests show intergroup differences in the number of parvalbumine-positive neurons in layers I-III in the more rostral part, putatively corresponding to neurons that mainly respond to tones between 8 and 16KHz and in layers V-VI in the more caudal part, putatively corresponding to <8KHz sensitive neurons.

Disclosures: B. Romero-Gomez: None. **F. Carricondo:** None. **A. Murciano:** None. **S. Bao:** None. **F. Panetsos:** None. **C. Frances-Ferriz:** None. **C. Pose-Utrilla:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 815.01/DD7

Topic: D.02. Auditory

Title: Identification of brain regions processing male's song in female Zebra Finch by using catFISH technique

Authors: *Y. AMADA, K. HOTTA, K. OKA;
Keio Univ. 16D-206, Yokohama, Japan

Abstract: Zebra Finch communicates each other by using songs. Male Zebra Finch learns his song from his father and practices his own song. On the other hand, female Zebra Finch recognizes male Zebra Finch's songs for mating. Therefore, Zebra Finch is one of model animals for investigating mechanism of verbal communication. We have focused on the specific function of the hippocampal formation (HF) during song recognition in female Zebra Finch. There are two types of songs: directed song and undirected song. Directed songs are directed toward a nearby female birds, and undirected songs are ones that male Zebra Finch sings alone to practice their own songs. Arc is one of immediate early genes and Arc mRNA is induced as neural activity dependent manner when female Zebra Finch is exposed to songs. A special feature of Arc mRNA is its time-dependent localization; Arc mRNA accumulates in nucleus ~5 min after auditory stimulation, and transports to post synaptic sites ~60 min after auditory stimulation. We can classify two different neuronal activities induced by sequentially presented stimuli by detecting Arc mRNA localization (cellular compartment analysis of temporal activity by fluorescent *in situ* hybridization, catFISH). We applied directed song and undirected song to female Zebra Finch sequentially, and catFISH revealed four types neurons; responded to first stimulation, responded to second stimulation, responded to both stimulations, and without responses. Then, we evaluated neuronal activities in caudomedial medial nidpallium (NCN), caudolateral mesopallium (CMM), high vocal center (HVC), and HF. We detected responding neurons not only in NCM, CMM, but also in HF and HVC. We found there are high-response sub-regions and low-response sub-regions to male's song stimulation in the HF, and density of responding neurons in high-response sub-regions is almost same as the ones in NCM and CMM. In these sub-regions, more than 50 percent of neurons responded to either directed song or undirected song, but neurons responded to both songs are few. This result shows that most of neurons in these sub-regions encode only the directed song or undirected song. We also found that the first stimulation induced neural activities in more neurons comparing to the second stimulation. Furthermore, we applied directed song twice sequentially, and found that more neurons were responded to the first stimulation of two sequential same songs (adaptation). Our results suggest that HF and HVC are associated with song recognition, and HF has specific sub-regions for recognition of male's song in female Zebra Finch.

Disclosures: Y. Amada: None. **K. Hotta:** None. **K. Oka:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 815.02/DD8

Topic: D.02. Auditory

Support: NIH DC-002266

Title: Forward masking in the superior paraolivary nucleus (SPON) of the rat

Authors: *F. GAO, A. S. BERREBI;
Otolaryngology, Sensory Neurosci. Res. Ctr., West Virginia Univ. Sch. of Med., Morgantown, WV

Abstract: Forward masking describes the phenomenon whereby the response threshold to a probe stimulus is markedly impaired when the probe is preceded by a sound (masker). Forward masking has been demonstrated at various stages of auditory processing, namely in the auditory nerve, cochlear nucleus, superior olivary complex, inferior colliculus (IC) and auditory cortex, and using psychophysical approaches. At the level of the IC, an inhibitory input active after the masker stimulus offset has been postulated to contribute to forward masking (Nelson et al., 2009), and the SPON is a candidate source for such an input. To understand how SPON neurons respond to a forward masking paradigm, and compare the features with those reported in other auditory nuclei, single-unit extracellular recordings were performed in the SPON of the rat. The forward-masking paradigm in our study used a 200-ms masker and a 20-ms characteristic frequency probe tone. The masker was presented at various intensities (20-60 dB re: threshold), durations (20-200 ms) and frequencies, and at various masker-to-probe delays (0-310 ms). The offset response thresholds of SPON units to the probe were determined under unmasked and masked conditions. Three pure tone response types were recorded in the SPON: on-offset (20/47 units), offset-transient (20/47 units) and offset-sustained (7/47 units). Almost all (19) on-offset units, 8 offset-transient units and 3 offset-sustained units showed increased probe response thresholds as masker-to-probe delays were shortened, as masker sound levels and masker durations were increased, and as the masker's frequency approached the unit's CF. Conversely, 12 offset-transient and 4 offset-sustained units showed decreased probe response thresholds as masker-to-probe delays were shortened, as masker sound levels and masker durations were increased, and as the masker's frequency approached the unit's CF. Since SPON offset responses are attributed to a post-inhibitory rebound mechanism, the effects of masker-driven inputs depend not only on the excitatory/inhibitory nature of the inputs, but also on their relative timing. Thus, increased inhibition present during the probe stimulus might contribute to the post-inhibitory rebound and thereby mediate an increased response to the probe stimulus. Conversely, slow or delayed masker-derived excitation might interfere with the post-inhibitory rebound after the end of the probe stimulus. Together these findings suggest that the SPON offset response can play a role in the generation of more pronounced forward masking in higher auditory centers, but is not itself subject to the same masking phenomenon.

Disclosures: F. Gao: None. **A.S. Berrebi:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 815.03/DD9

Topic: D.02. Auditory

Support: NIH Grant 1F32DC012449

Title: Spontaneous changes in state explain cortical and behavioral response variability

Authors: *M. J. MCGINLEY[1], S. V. DAVID[2], D. A. MCCORMICK[1];
[1]Neurobio., Yale Univ., New Haven, CT; [2]Oregon Hearing Res. Ctr., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Neuronal and behavioral responses can be highly variable, even between neighboring trials. This finding raises the possibility that the brain is intrinsically noisy, and that single neurons are unreliable indicators of sensory stimuli. However, another possibility is that an unmeasured time-varying factor contributes strongly to the sensory and behavioral response. We hypothesized that the internal state of the nervous system varies continuously and has a strong influence on decision making and cortical activity. Here, we test this hypothesis in awake-behaving mice on a cylindrical treadmill by simultaneously monitoring the internal state (as indicated by pupil diameter and cortical/hippocampal field potential), sensory-evoked responses in auditory cortical neurons, and performance in a Go/No-Go detection task. Whole cell recordings in the primary auditory cortex revealed spontaneous variability in the strength and timing of membrane potential deflections and action potential responses to complex auditory stimuli. Simultaneous monitoring of pupil diameter revealed continuous and on-going fluctuations often lasting only a few seconds. Remarkably, these spontaneous pupil changes were highly correlated with the membrane potential of cortical pyramidal and fast-spiking neurons at frequencies below ~1 Hz. Also striking, sorting the sensory responses by pupil diameter explained a substantial fraction of trial-to-trial variability in neuronal responses. The relationship between gain of sensory evoked cortical action potential responses and pupil diameter exhibited an inverted-U: responses were maximal at mid-pupil diameters. Sorting trials by walking versus still while controlling for pupil diameter revealed that much of the gain change associated with walking resulted from an increase in arousal (as indicated by the pupil). To examine if these changes in state are also reflected in behavioral performance, we trained animals to lick for a reward upon detection of a variable intensity tone embedded in complex noise. Again, sorting the trials by the pupil diameter prior to each sound substantially reduced behavioral response

variability (as measured by discrimination, bias, and lick latency). Optimal performance was achieved for mid-pupil diameters. Our results indicate that during waking, behavioral state is constantly fluctuating and strongly influences not only sensory evoked cortical responses, but also the ability of the animal to detect and respond to sensory stimuli. Thus, cortical neuronal responses to sensory stimuli, and behavior, can be "de-noised" by taking into account ongoing changes in the state of the nervous systems.

Disclosures: **M.J. McGinley:** None. **S.V. David:** None. **D.A. McCormick:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 815.04/DD10

Topic: D.02. Auditory

Support: NIH T32-DA007254-19

Title: Rapid context-dependent switching of auditory ensembles during behavior

Authors: *K. KUCHIBHOTLA, R. C. FROEMKE;
Skirball Inst., NYU Sch. of Med., New York City, NY

Abstract: Situational context helps determine the value of external stimuli. The same sensory cue can have two meanings when presented in two different environments. How do neural circuits, particularly in sensory cortex, flexibly represent the same stimuli under different conditions? Using two-photon calcium imaging in head-fixed behaving mice, we monitored neural responses in primary auditory cortex (A1) during bouts of passive hearing and active listening. In the active context, mice were trained to lick for a water reward after hearing a tone (CS+) and withhold from licking after hearing a different tone (CS-). Both tones were represented in the tonotopic field of view (e.g., 4.7 and 8 kHz). In the passive context, mice were exposed to the same two tones with no opportunity for reward. Surprisingly, the two different contexts activated distinct neuronal ensembles in all animals (n=5 mice, 1,561 cells, $p<0.001$). Both the rewarded tone (CS+) and non-rewarded tone (CS-) exhibited this feature with only 18±5% (CS+) and 16±5% (CS-) of the neurons responding under both contexts, despite the location of these neurons within the general tonotopic area encoding these sound frequencies. The change in neuronal activity patterns from active to passive may occur gradually or it might

be rapidly toggled. To answer this, we used a block-based trial design: immediately following the active context we initiated the passive context (i.e. removing the lick-tube and briefly turning on a chamber light cue). During the active context, active-only neurons responded robustly. Immediately after switching contexts, there was no tone-evoked calcium response. We measured a similar rapid switching of activity across the ensemble of active-only neurons (n = 3 mice, 664 cells, 112 responsive). Finally, we examined the functional micro-architecture of A1 in both contexts. We measured the similarity of all cell pair responses to the CS+ and CS- as a function of pairwise distance. In the passive context, neurons closest together had similar tone selectivity. The similarity in selectivity decreased linearly but remained significant out to the furthest distance in the field-of-view (250μm), as expected by the tonotopy of A1. This relationship was abolished in the active context. Neuron pairs only maintained similarity in selectivity up to 50 μm apart, indicating a breakdown in the tonotopic arrangement of the CS+ and CS-. Active engagement in a task, therefore, appears to "flatten" the tonotopic map in A1. Overall, these data demonstrate an ensemble-level switch within primary auditory cortex, allowing for flexible representations of external stimuli that depend on situational and behavioral context.

Disclosures: K. Kuchibhotla: None. **R.C. Froemke:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

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Program#/Poster#: 815.05/DD11

Topic: D.02. Auditory

Support: NSF Grant IOS-1121689

Title: Role of medio-dorsal frontal and posterior parietal neurons during auditory detection performance in rats

Authors: *M. C. WIEST, K. BOHON;
Neurosci., Wellesley Col., Wellesley, MA

Abstract: To further characterize the rat frontal and parietal cortices as models of frontal-parietal function in primate cognition, we recorded action potentials simultaneously from multiple sites in the medio-dorsal frontal cortex and posterior parietal cortex of rats while they performed a two-choice auditory detection task. We quantified neural correlates of task

performance, including response movements, perception of a target tone, and the differentiation between stimuli with distinct features (different frequencies or intensities). A minority of units - 25% in frontal cortex, 26% in parietal cortex - significantly distinguished hit trials (successful detections, response movement to the right) from correct rejection trials (correct leftward response to the absence of the target tone). Estimating the contribution of movement-related activity to these responses suggested that approximately half of these units were signaling correct perception of the auditory target, rather than merely movement direction. In addition, we found a smaller and not overlapping population of units that differentiated stimuli based on task-irrelevant details. This frontal and parietal detection-related spiking activity we observed in the rat could be a functional analog of perception-related responses reported in primate cortex.

Disclosures: **M.C. Wiest:** None. **K. Bohon:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

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Program#/Poster#: 815.06/DD12

Topic: D.02. Auditory

CIHR Grant MOP274494

Natural Sciences and Engineering Research Council of Canada (Discovery Grant)

Campbell McLaurin Chair for Hearing Deficiencies

Alberta Innovates—Health Solutions

Title: Tone suppression of thamalocortical inputs in the auditory cortex

Authors: *C. XIONG, L. KONG, J. YAN;
Dept. of Physiol. and Pharmocology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Forward masking is a common perceptual phenomenon of hearing. In the auditory cortex, temporally sequential paired tones with specific time intervals often result in attenuation and suppression of neuronal responses to the second tone. This forward masking, also known as forward suppression, continues for hundreds of milliseconds after postsynaptic inhibitory period. These findings suggest that cortical suppression induced by tone-pairs appears to be associated

with circuit level control rather than intrinsic cellular properties or direct feed-forward inhibition. One possibility is that two-tone suppression in the auditory cortex is a direct transmission from subcortical levels since this phenomenon is already observed in the auditory nerve. It remains unclear if two-tone suppression can be produced in the auditory cortex or in the thalamocortical system singly. Here we examined the tone suppression of thalamocortical inputs. We used the paired tone stimulation paradigm but substituted the second tone with electrical stimulation of the ventral medial geniculate body (ES_MBGv). We found that tone stimulus strongly suppressed the ES_MGBv-induced neuronal firings of neurons recorded in layers III and IV of the auditory cortex. The best effects were observed when the time interval between the tone and the electrical stimulation were about 80 milliseconds. This time interval appears to be related to the post-excitatory "hyperpolarization" of tone-evoked local field potential. Our data suggests that cortical and/or thalamocortical circuits have intrinsic mechanism(s) underlying paired-tone suppression.

Disclosures: **C. Xiong:** None. **L. Kong:** None. **J. Yan:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 815.07/DD13

Topic: D.02. Auditory

Support: NIH F32DC013722

NIH T32DC00046

NIH R01DC005779

CONICYT/Fulbright

Title: The laminar profile of task-dependent plasticity in spiking and local field potentials from primary auditory cortex

Authors: ***N. FRANCIS**[1], D. ELGUEDA[1], B. ENGLITZ[2], J. FRITZ[1], S. SHAMMA[1]; [1]Univ. of Maryland, College Park, MD; [2]Ecole Normale Superieure, Paris, France

Abstract: Auditory task-dependent plasticity (TDP) in primary auditory cortex (A1) is characterized by enhanced receptivity to behaviorally meaningful sounds. Because local field potentials (LFPs) are largely generated by synaptic currents that drive neuronal spiking, we hypothesized that auditory TDP should enhance LFP, as well as spike-based A1 responsiveness to behaviorally meaningful sounds. In addition, A1 laminar-specific cortical and subcortical connectivity suggests that TDP might differ across layers. To test these hypotheses, we trained ferrets on pure-tone detection and click-rate discrimination tasks, and recorded spikes and LFPs in A1 using a 24-channel laminar probe ("Plextrode"). Penetrations were made orthogonal to the cortical surface in a head-fixed preparation. Laminar electrode sites were spaced 75 μm apart, covering 1.8 mm in depth. For each recording we computed laminar profiles from the current source densities (CSDs) of low-frequency LFPs (1-80 Hz; LoLFP), the power in high-gamma LFP waveforms (80-300 Hz; HiLFP), and peristimulus time histograms (PSTHs) from spikes. We also computed spectrotemporal receptive fields (STRFs) for the LoLFP-, HiLFP- and spike-based laminar profiles. In awake, quiescent animals, we found that STRFs could be consistent across the LoLFP, HiLFP and spike bands within a given layer. In the awake, behaving animal, we observed a pattern of auditory TDP that was dynamic, largely consistent between LFPs and spiking, and that decayed quickly after the end of the behavioral session. During behavior, A1 responses were suppressed, and suppression was generally greater for the sustained response to non-target sounds. The same result was observed in LoLFPs, HiLFPs and PSTHs. Our preliminary results show TDP in layers II/III to VI, however, the laminar profile of TDP varied across stimulus onsets, steady-states and offsets. Overall, TDP was greatest in layers IV, V and VI. Again, this same result was observed in the LoLFPs, HiLFPs and PSTHs. Our results confirm the existence of TDP in A1 LFPs, complementing the demonstration of TDP in this, and previous single-unit studies. The time-dependence and laminar profile of TDP suggests that A1 cortical and subcortical inputs and outputs are strongly modulated by auditory task performance.

Disclosures: **N. Francis:** None. **D. Elgueda:** None. **J. Fritz:** None. **S. Shamma:** None. **B. Englitz:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

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Program#/Poster#: 815.08/DD14

Topic: D.02. Auditory

Support: NIH Grant R00DC010439

Title: Multiple functionally distinct circuits modulate auditory cortical activity during behavior

Authors: *S. V. DAVID;
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Abstract: Our current understanding of auditory processing is limited by behavioral manipulations that probe the effects of only a small range of behavioral states on auditory representations. However, the internal brain state that influences hearing combines many separate behavioral state variables, including selective attention, relative effort, temporal expectation, and reward associations. Each of these variables might provide distinct modulatory influence on auditory neural activity through different top-down circuits. To contrast the influence of different internal state variables on auditory representations, we developed an auditory discrimination task in which selective attention and overall effort are controlled separately. The task employs a go/no-go paradigm in which head-fixed animals are rewarded for responding to a pure tone target embedded in a continuous stream of vocalization-modulated noise (narrow-band noise modulated by the temporal envelope from a natural vocalization). On each trial, two noise streams are presented simultaneously, centered at different frequencies and from different spatial locations. To manipulate selective attention, the frequency and location of the target tone is matched to one stream and changed between trial blocks. To manipulate effort, the signal-to-noise (SNR) of the target relative to the noise is varied between blocks at a fixed frequency and location. Catch stimuli are included in each block to verify behavioral effects. We trained two ferrets to perform the task and recorded activity of single neurons in primary auditory cortex (A1) during behavior. For the selective attention manipulation, the target was fixed at the best frequency (BF) of and contralateral to the recorded neuron during one trial block. When attention was directed to BF, evoked activity was weaker than when attention was directed away from BF. Spontaneous spike rate did not change. For the manipulation of effort, on the other hand, we observed changes in evoked and spontaneous activity. Both were smaller during the difficult, low-SNR condition than during the high-SNR condition. Thus these two aspects of behavior state influence neural activity differently, selective attention modulating the gain of auditory responses and relative effort modulating overall firing rate, independent of the stimulus. Ongoing studies are comparing the effects of these behavioral state variables on filter-based models of neural activity (i.e., temporal receptive fields) measured under the different behavior conditions.

Disclosures: S.V. David: None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

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Program#/Poster#: 815.09/DD15

Topic: D.02. Auditory

Support: NIH DC003180

NIH DC013150

Title: Probing the perceptual and physiological mechanisms underlying an auditory delayed match-to-sample task in marmosets (Callithrix jacchus)

Authors: *M. S. OSMANSKI[1], X. WANG[2];
[1]Johns Hopkins Univ. SOM, Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: A fundamental question in auditory neuroscience is how activity in the cerebral cortex gives rise to perception and how changes in brain activity relate to different behavioral states. Previous studies have described important ways in which behavior modulates the response properties of neurons in primary auditory cortex, yet precisely how neural activity across auditory cortex (including secondary cortical fields such as belt and parabelt regions) is coupled with perception remains a largely untapped question. To begin to address this question, we trained four marmoset monkeys on an auditory delayed match-to-sample task ("Same/Different") in which subjects were presented with two sounds, separated by a short delay period, drawn from a large corpus of different acoustic stimuli. Animals were required to lick at a feeding tube if the sounds were different and withhold responding if they were identical. All four animals mastered this task over the course of several weeks, and were then transferred to a series of test stimulus sets comprised of a number of spectrally and temporally complex sounds, including vocalizations. These stimulus sets were designed to probe marmosets' natural perceptual categories for complex sounds. We have also begun to probe the neural responses underlying performance on this task using a 16 channel multi-electrode array covering a large portion of auditory cortex, which will allow us to examine changes in neural activity across core, belt and parabelt regions when the animal is engaged in the delayed match-to-sample task. [Supported by NIH grants DC003180 to XQW and DC013150 to MSO]

Disclosures: M.S. Osmanski: None. **X. Wang:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 815.10/DD16

Topic: D.02. Auditory

Support: NIH-NIDCD

Robert Boucai Foundation

Title: Neural and behavioral correlates of auditory scene analysis

Authors: *K. L. CHRISTISON-LAGAY[1], Y. E. COHEN[2];
[1]Perelman Sch. of Med. At the Univ. of Pennsylvania, Philadelphia, PA; [2]Otorhinolaryngology, Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA

Abstract: The environment is filled with acoustic stimuli that our brains transform from low-level sensory representations into perceptual representations that can guide behavior. These perceptual representations are the computational result of the auditory system's ability to detect, extract, segregate, and group the spectrotemporal regularities in the acoustic environment into perceptual units. Psychophysical studies have identified several of the principles and mechanisms that underlie a listener's ability to segregate and group acoustic stimuli. One important psychophysical task that has illuminated many of these principles and mechanisms is the "streaming" task, in which two alternating sequences of tone bursts are presented, while a listener reports whether the tone-burst sequences sound like one "auditory stream" (i.e., "galloping" tones) or two auditory streams. Human listeners report one stream when the frequency separation between tones is small, whereas they report two streams when the frequency separation is large. Interestingly, at intermediate frequency separations, a listener's reports become less reliable: on alternating trials, they report hearing one or two streams. In addition to manipulations of frequency separation, systematic changes to listening duration and temporal overlap of the tones alter a listener's reports. Although behavioral performance on this task is well described in humans, the initial stage of the present study was the first to explicitly test the streaming abilities of non-human animals using the methods comparable to those used in human studies. Here, we present both (1) behavioral results of the streaming task, and (2) extracellular activity from neurons in the core auditory cortex recorded while monkeys performed the behavioral task.

Disclosures: K.L. Christison-Lagay: None. **Y.E. Cohen:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

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Topic: D.02. Auditory

Support: NIDCD-NIH

Boucai Hearing Restoration Fund

Title: Relative contributions of the auditory and prefrontal cortices to auditory perceptual decision-making

Authors: *J. TSUNADA[1], A. S. LIU[1], J. I. GOLD[2], Y. E. COHEN[1];
[1]Dept. of Otorhinolaryngology, [2]Dept. of Neurosci., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Auditory perceptual decision-making requires the conversion of incoming auditory information into a categorical judgment. Previous studies suggest that the ventral auditory pathway, which begins in the auditory cortex and continues to the ventrolateral prefrontal cortex (vlPFC), plays a key role in auditory perceptual decision-making. However, the relative contributions of the auditory and prefrontal cortices in auditory perceptual decision-making have yet to be directly tested. To begin to identify these contributions, we recorded neural activity from the lateral belt regions (LB) of the auditory cortex and the vlPFC while monkeys performed a novel auditory-discrimination task. For this task, the monkeys reported whether a sequence of tone bursts contained more high-frequency tone bursts (>2 kHz) or low-frequency (<1.5 kHz) tone bursts. The monkeys could report their choice at any time during the presentation of tone bursts. By systematically changing the proportion of high-frequency and low-frequency tone bursts, we manipulated task difficulty. A trial that contained only high- (low-) frequency tone bursts was easy to discriminate as a high- (low-) frequency sequence. In contrast, a trial with a mixture of high- and low-frequency tone bursts was more difficult. We found that, as task difficulty increased, the monkeys' performance accuracy decreased and response time increased. We recorded 89 LB and 29 vlPFC neurons in two monkeys performing the task and used a multivariate regression analysis to test how neuronal activity related to the frequency content of the stimulus and the monkeys' choices on a trial-by-trial basis. These preliminary analyses indicate that a substantial proportion of LB neurons (64/89, 72%) encoded the stimulus, but fewer of these neurons (11/89, 12%) encoded choice. However, LB neurons with higher sensitivity to the stimulus tended to represent the monkeys' choices: there was a negative correlation ($r=-0.28$; $p<0.05$) between the neurometric threshold (i.e., the inverse of the neural

sensitivity to the stimulus) and choice probability. A different set of findings emerged in vlPFC. Thirty one percent of vlPFC neurons (9/29) encoded the monkeys' choices, but none of these neurons (0/29, 0%) encoded the stimulus. These results suggest that 1) LB neurons, particularly those with higher neurometric sensitivity, represent sensory information used for the frequency discrimination, and 2) vlPFC neurons represent the final choice.

Disclosures: J. Tsunada: None. **A.S. Liu:** None. **Y.E. Cohen:** None. **J.I. Gold:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

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Topic: D.02. Auditory

Support: JSPS Asian Core Program

Research Support Foundation FAP/DF

CAPES

CNPq

Title: Whole-body prepulse inhibition protocol to test sensorymotor gating mechanisms in monkeys

Authors: *P. G. SALETTI[1], R. S. MAIOR[1], E. HORI[2], H. NISHIJO[2], C. TOMAZ[1];
[1]Univ. of Brasilia, Brasilia, Brazil; [2]Univ. of Toyama, Toyama, Japan

Abstract: Acoustic startle reflex is a contraction of facial and skeletal muscles provoked by a sudden sound. This response may be reduced by a previous weak sound presented before the startle-eliciting stimulus, the prepulse inhibition phenomeon. Prepulse inhibition is an important paradigm that provides valuable information about sensorimotor gating functionality. In the present study, we aimed to identify the pattern of startle amplitude of capuchin monkeys (*Sapajus spp*.) in a whole-body protocol, and to evaluate the role of the superior colliculus in prepulse inhibition paradigm. Monkeys were tested in a whole-body protocol using a primate chamber connected to an accelerometer. Eight monkeys were employed to determine the startle amplitude and interstimuli interval. Furthermore, we tested two monkeys with bilateral superior

colliculus (SC) damage in this protocol. Results show that acoustic pulse of 115dB has induced the best startle amplitude. 120msec was the best interstimuli interval to induce inhibition of startle response. Interestingly, no difference was observed between male and female in startle amplitude. Finally, we observed a downward tendency of prepulse inhibition in SC-lesioned subjects. Our results provide the possibility of further studies with whole-body protocol in capuchin monkeys and reinforce the importance of the superior colliculus in prepulse inhibition pathway.

Disclosures: **P.G. Saletti:** None. **R.S. Maior:** None. **E. Hori:** None. **H. Nishijo:** None. **C. Tomaz:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

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Program#/Poster#: 815.13/DD19

Topic: D.02. Auditory

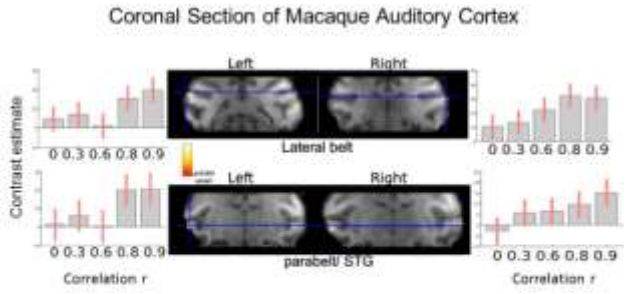
Support: Wellcome Trust grant WT091681MA

Title: Measurement of the BOLD response to acoustic spectral flux in the macaque superior temporal plane

Authors: P. DHEERENDRA[1], O. JOLY[2], S. BAUMANN[1], A. THIELE[1], *T. D. GRIFFITHS[3]; [1]Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]Exptl. Psychology, Oxford Univ., Oxford, United Kingdom; [3]Inst. of Neurosci., Newcastle upon Tyne, United Kingdom

Abstract: We measured the ensemble neural activity corresponding to spectral flux using a stimulus in which this timbral attribute can be manipulated independently of bandwidth). The stimulus can be characterized in terms of the Pearson product-moment correlation (r) between successive timeframes of the stimulus or in terms of the related time window within which any two frames show a minimum level of correlation. Previous human work) suggested that there was a relationship between r and the BOLD response in the human homologues of belt and parabelt region but not in core cortex. We sought to establish whether macaques show a similar relationship between r and BOLD response in non-core areas of the superior temporal plane (STP), and to determine whether such differences might be a physiological way of discriminating

core and belt areas. EPI images were acquired using a continuous acquisition protocol on 4.7T upright Bruker scanner on awake behaving subjects carrying out visual fixation (TR/TE = 1.3s/21ms). Fifteen blocks of stimuli were acquired in each of which there were five conditions in randomized order. In total 440 volumes per run and three runs per session were acquired. Analysis was carried out using SPM software (SPM8, www.fil.ion.ucl.ac.uk/spm). Single subject inference was carried out by applying a generalized linear model (GLM). BOLD responses from representative voxels were compared in core, belt and parabelt regions across different conditions. The figure shows the BOLD response to be an increasing function of r in belt and parabelt areas. No relationship was demonstrated in area A1 (data not shown). The data suggest that the spectral flux response is a potentially useful physiological measure to disambiguate auditory core and belt areas while avoiding interpretational difficulties that are associated with bandwidth preference used in previous studies. Reference Overath T, Kumar S, von Kriegstein K, Griffiths TD (2008) Encoding of spectral correlation over time in auditory cortex. J Neurosci 28:13268-13273



Relationship between spectral correlation (r) and BOLD response for representative voxels in belt and parabelt regions for each hemisphere

.

Disclosures: P. Dheerendra: None. **T.D. Griffiths:** None. **O. Joly:** None. **S. Baumann:** None. **A. Thiele:** None.

Poster

815. Auditory Processing: Perception, Cognition, and Action via Animal and Modeling Studies

Location: Halls A-C

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Support: Helsinki University Grant (TR)

Biotechnology and Biological Sciences Research Council, BBSRC U.K. (BB/J009849/1)

Wellcome Trust New Investigator Award (CIP; WT102961MA)

Title: Task-dependent modulations of the fMRI BOLD response in the monkey brain

Authors: T. RINNE[1], R. MUERS[2], H. SLATER[2], E. SALO[1], D. HUNTER[2], *C. I. PETKOV[2]; [1]Inst. of Behavioural Sci., Univ. of Helsinki, Helsinki, Finland; [2]Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Human brain imaging studies have shown that operations in sensory cortex are strongly modulated by active tasks. Yet, virtually all prior brain imaging studies in awake nonhuman animals were conducted under passive stimulation. To help to bridge the gap between human fMRI studies using active tasks and animal models, we trained two macaque monkeys to perform an auditory spatial task during fMRI, with and without competing visual stimuli. The monkeys were presented with pairs of "coo" vocalisation sounds that either changed in spatial location (*target*: virtual acoustic space change from -90° to +90° in azimuth) or were presented from the same location (*nontarget*: repeated location in -90° or +90°). The monkeys were rewarded for pressing a lever to auditory targets and withholding their response to nontargets. Approximately 40% of randomly selected auditory stimulus trials had competing visual stimuli (pairs of low-contrast monkey face stimuli presented either in two different spatial locations or in one location). These conditions allowed us to investigate activations to sounds presented during an active auditory task (auditory only trials) and to evaluate the influence of visual stimuli on both auditory behavioural performance and associated task-dependent fMRI modulations. After initial behavioural training, simulating the MRI scanner environment (ca. 5 daily sessions per week for >18 months with each monkey), the monkeys were scanned with fMRI at 4.7 Tesla while they performed the auditory spatial discrimination task. This resulted in 9 fMRI scanning runs (in total over 900 testing trials) for each monkey with auditory performance well above chance. As expected, sound presentation was associated with reliable activation of auditory cortex (trials with sounds compared to silent trials; cluster corrected $p < 0.05$). However, task performance strongly and significantly modulated much of auditory cortex and other brain areas (e.g., Brodmann areas 7 and 44), whereby stronger activations were associated with better performance. Moreover, we found significantly enhanced activations in a number of visual cortical areas when the monkeys' performance was influenced by visual stimulation during the audio-visual stimulus conditions. These results provide insights into how task performance modulates the nonhuman primate brain at the regional level during an active auditory spatial task with and without competing visual stimuli. * RM, ES and HS contributed equally.

Disclosures: **T. Rinne:** None. **R. Muers:** None. **H. Slater:** None. **D. Hunter:** None. **C.I. Petkov:** None. **E. Salo:** None.

Poster

816. Subcortical Visual Pathways

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 816.01/DD21

Topic: D.04. Vision

Support: NIHr01 EY09593

Title: Integration of On and Off channels in the ferret visual thalamus

Authors: ***V. SURESH**[1], U. M. CIFTCIOGLU[1], B. M. LALA[1], V. AWASTHI[1], X. WANG[2], F. T. SOMMER[3], J. A. HIRSCH[1];
[1]Biol. Sci., USC, Los Angeles, CA; [2]Salk Inst. for Biol. Sci., La Jolla, CA; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Connections from local inhibitory interneurons in the lateral geniculate nucleus of the thalamus (LGN) influence all visual information the primary visual cortex receives. Across species, relay cells in LGN have center-surround receptive fields (RFs); our work in cat has shown that this arrangement holds for RFs of interneurons as well. Furthermore, for relay cells and interneurons alike, there is a push-pull arrangement of excitation and inhibition within each RF subregion; i.e. for On center cells, where bright excites, dark inhibits. These observations suggest that a simple circuit might explain push-pull responses: push (excitation) is supplied by retinal ganglion cells with the same center sign as the postsynaptic cell whereas the pull (inhibition) is fed forward from the retina via local interneurons of the opposite sign. It is difficult to test this hypothetical circuit in cat (and in rodent), however. On and Off neurons in the LGN are intermingled, obscuring the independent contribution of each luminance channel. In ferret, while the C-layers have comingled On and Off cells, each of the main layers (A, A1) is divided into On and Off leaflets. Thus, we explored the possibility of using this species difference to gain insight into interactions between On and Off circuits, using the C-layers as a starting point. Towards this goal, we compared properties and response patterns of relay cells and interneurons in ferret using whole-cell recording *in-vivo*. In cat, interneurons and relay cells have distinct intracellular signatures. Whereas the membrane currents in interneurons are dominated by trains of unitary IPSCs, those in relay cells are marked by individual EPSCs. When we quantified these differences using an index[1] that scored the inward or outward trend of

postsynaptic currents across timescales, the two waveforms divided into separate groups. We found that this difference is preserved in ferret interneurons and relay cells. Next, we asked whether or not the spatiotemporal profile of visually-evoked responses between the two species was conserved. While ferret relay cells had stereotypical push-pull responses (as for cat), ferret interneurons had greater response heterogeneity such that input from any one interneuron could not explain the pull in relay cells. Pooled input from different classes of interneurons however, could account for the pull. We conclude that there is sufficient similarity between species to conduct a fuller investigation of On and Off integration in the ferret LGN. 1. Wang, X. et al. *Nat. Neurosci.* **14,** 224-231 (2011).

Disclosures: **V. Suresh:** None. **U.M. Ciftcioglu:** None. **B.M. Lala:** None. **V. Awasthi:** None. **X. Wang:** None. **F.T. Sommer:** None. **J.A. Hirsch:** None.

Poster

816. Subcortical Visual Pathways

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Program#/Poster#: 816.02/DD22

Topic: D.04. Vision

Support: CEN400376

R01 EY16224

Title: The impact of bursting on information and redundancy in neuronal subsets of the primate LGN

Authors: *M. CRUMILLER[1], E. KAPLAN[2], B. KNIGHT[1];
[1]Neurosci., The Rockefeller Univ., New York, NY; [2]Neurosci., The Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The mammalian Lateral Geniculate Nucleus is often labeled a “relay station,” whereby visual information from the retina is selectively filtered and relayed to the primary visual cortex. The nature of this filtering process is obscured by the many recurrent and feedback connections that result in the transfer of under half of the input spikes from the retina to the cortex. Here we investigate the role of the LGN in the processing of natural scene movies by applying a new, more convenient and accurate method of estimating information flow in populations of simultaneously-recorded neurons from anesthetized macaque monkeys.

Preliminary evidence suggests that neurons in the LGN separate naturally into groups of high and low information-per-spike rates, both of which display bursting activity_brief successions of action potentials with exceptional rapidity. By measuring information conveyed at the group level, we estimated the average levels of redundancy between neurons. Neurons in the high-information group showed little redundancy with precisely-timed, highly-informative bursts, while neurons in the low-information group displayed higher levels of redundancy with each other. Furthermore, we found that in both groups, LGN bursting activity reduces redundancy, with the first spike in each burst conveying the majority of information, and the remaining spikes in the burst reducing redundancy between cells.

Disclosures: M. Crumiller: None. **B. Knight:** None. **E. Kaplan:** None.

Poster

816. Subcortical Visual Pathways

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 816.03/DD23

Topic: D.04. Vision

Title: Anatomical connection between the superior colliculus and the amygdala

Authors: *L. MALKOVA[1], S. B. GUTHERZ[1], J. BLOCH[1], J. MCCLANE[1], R. C. SAUNDERS[2], P. A. FORCELLI[1];
[1]Dept Pharmacol & Physiol and the Interdisciplinary Program in Neurosci., Georgetown Univ. Med. Ctr., WASHINGTON, DC; [2]Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: The existence of a subcortical visual pathway that can support the transfer of visual information from the superior colliculus (SC) to the amygdala remains controversial. Several lines of evidence suggest that such a pathway may exist, projecting through a relay in the pulvinar. This idea stems from functional imaging studies in human subjects and behavioral studies in patients with blindsight, as well as diffusion tensor imaging in human subjects. In rodents, anatomical tracing studies have revealed the presence of a tectopulvinar pathway that may relay information to the amygdala. Similarly, in a lower-order primate, the tree shew, the tectopulvinar pathway appears to relay non-topographic visual information from SC through the pulvinar to the amygdala. However, it remains to be determined if a similar pathway exists in the macaque, a species in which the functional interactions between subcortical visual system and threat processing is an area of ongoing research. To address this question, we injected two pigtail macaques with retrograde tracers in the amygdala and anterograde tracers in SC. After a two-

week survival, the brains were processed. Preliminary analysis indicates a region of sparse overlap of these two projections within the medial pulvinar. This finding is consistent with those in other species and suggests that the pulvinar may be one possible subcortical route for visual information to reach the amygdala.

Disclosures: L. Malkova: None. **S.B. Gutherz:** None. **J. Bloch:** None. **R.C. Saunders:** None. **P.A. Forcelli:** None. **J. McClane:** None.

Poster

816. Subcortical Visual Pathways

Location: Halls A-C

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Program#/Poster#: 816.04/DD24

Topic: D.04. Vision

Title: Using visual flicker to estimate the temporal response profile in human subcortical nuclei

Authors: *K. DESIMONE[1], K. A. SCHNEIDER[2,3];
[1]Psychology, [2]Biol., York Univ., Toronto, ON, Canada; [3]Ctr. for Vision Res., Toronto, ON, Canada

Abstract: Introduction: The population receptive field (pRF) model has been used to map the organization of multiple cortical areas (Dumoulin & Wandell, 2008; Amano et al., 2009) and across various patient populations (Levin et al., 2010; Hoffmann 2012). More recently, this voxel-wise encoding method has been used to reveal the topographic organization of numerosity in parietal cortex (Harvey et al., 2013). We sought to extend the pRF model to the human subcortex, exploring the temporal response profiles of across multiple subcortical nuclei using visual flicker. Methods: Participants' brains were scanned with a 3T MRI scanner and a 32-channel headcoil. Standard retinotopic mapping procedures were performed (Dumoulin & Wandell, 2008). Participants performed a fixation task as slowly drifting bar apertures traversed the visual field. We varied the visual flicker of the bar by increasing and decreasing the contrast reversal rate of the checkerboard. Results: We were able to retinotopically map the multiple subcortical nuclei using the pRF model. The pRF estimates were consistent with previous imaging findings (Schneider et al., 2004; Schneider & Kastner, 2005) using a phase-encoding method. In addition, we were able to drive the subcortical nuclei differentially based on the flicker rate. Furthermore, we were able to entrain a retinotopically organized pattern of activation in the medial geniculate nucleus, the thalamic relay in the auditory pathway between the periphery and temporal cortex.

Disclosures: **K. DeSimone:** None. **K.A. Schneider:** None.

Poster

816. Subcortical Visual Pathways

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Topic: D.04. Vision

Support: Grant-in-Aid for JSPS Fellows 13J01314

Title: The similarity of receptive field properties in connections between the lateral geniculate nucleus and the primary visual cortex of the cat

Authors: ***N. SUEMATSU**, T. NAITO, T. MIYOSHI, H. SAWAI, H. SATO;
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Abstract: Stimulus-specific receptive field (RF) properties, such as spatial frequency (SF) selectivity and orientation selectivity, in the early visual system are the key features of visual information processing. Although many studies have investigated retinal, geniculate, and cortical neural functions, relatively few studies have been conducted with a particular focus on the similarity and dissimilarity of the functions between regions, such as comparison of the RF properties in retinogeniculate and geniculocortical neural pairs. We previously compared the RF structures in the cat retinogeniculate connections, and found that there were multiple types of connections which probably enhance the visual stimulus specificity. In the current study, we recorded multi-unit activity simultaneously from the lateral geniculate nucleus and the primary visual cortex (V1) of anesthetized cats. Then, we compared the RF properties obtained using the reverse correlation method with subspace stimuli between the geniculocortical local population pairs with or without the significant functional connections identified with cross correlation method. The results showed that the populations with the functional connections share more similar RF properties, especially SF selectivity but not orientation selectivity. In addition, we are conducting similar analysis on the RF properties in the cat retinogeniculate connections in order to clarify the manner of visual information transmission in the early visual system from the retina to the V1.

Disclosures: **N. Suematsu:** None. **T. Naito:** None. **T. Miyoshi:** None. **H. Sawai:** None. **H. Sato:** None.

Poster

816. Subcortical Visual Pathways

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 816.06/DD26

Topic: D.04. Vision

Support: MRC

Title: Topographic order across functional classes of retinal ganglion cells

Authors: *A. HENDRY, I. D. THOMPSON, A. S. LOWE;
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Abstract: The visual system exhibits topographically ordered maps between connected brain structures that are coherent across functional classes. Our previous work in the zebrafish has revealed two classes of motion-sensitive retinal ganglion cells (RGCs) innervating the optic-tectum that obey different rules during development (Lowe et al., 2013). Orientation-selective (OS) RGCs, which respond to motion along an axis, require visual drive during development to refine their spatial innervation patterns to distributions of four functional sub-types within a tectal lamina. Conversely, direction-selective (DS) RGCs, which respond to motion in one direction only, are not plastic during development and innervate pre-defined laminar segments for each of three sub-types. The consequences of such different developmental rules on retinotopic order are intriguing. Using a Synaptophysin-fused GCaMP3 targeted to RGC axons enabled the spatial receptive fields of axonal terminals within the optic-tectum to be mapped across cumulative populations of OS- and DS-RGCs. We will present quantitative dissections of the relative retinotopic order and precision within and across each RGC class innervating the optic-tectum.

Disclosures: A. Hendry: None. **I.D. Thompson:** None. **A.S. Lowe:** None.

Poster

816. Subcortical Visual Pathways

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Program#/Poster#: 816.07/DD27

Topic: D.04. Vision

Support: Wellcome-NIH studentship WT091467MA

NIMH IRP

Title: Response reliability of pulvinar neurons to repeated presentations of natural social movies

Authors: *A. P. MURPHY[1,2], C. DENG[1], B. E. RUSS[1], D. B. T. MCMAHON[1,3], D. A. LEOPOLD[1];
[1]Section on Cognitive Neurophysiol. and Imaging, Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom; [3]Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: The pulvinar is the largest nucleus of the primate thalamus. It is a complex structure whose various subregions exchange reciprocal connections with a wide array of visual and nonvisual cortical areas. It also receives input from subcortical structures, including the retina and superior colliculus, and sends projections to the amygdala. Despite considerable knowledge about cortical areas projecting to and from its various subdivisions, the spatial variation of functional properties across the pulvinar is relatively unexplored, particularly in the awake animal. One notable feature of the pulvinar is that, while its neurons often respond to visual stimuli, the responses are less reliable from trial to trial than in the cortical regions from which it receives input. As part of a larger study to map the functional organization of the pulvinar, we asked here whether there are regional differences in response reliability across the pulvinar. To address this question, we used an unconstrained experimental paradigm in which animals freely viewed the contents of natural social movies. This method provides rich visual stimulation whilst allowing freedom of eye movement and shifting of attention, and thus more closely resembles natural vision. We quantified variations in behavior and stimulus parameters over time in order to assess the extent to which these properties predict variations in a cell's response. We began by asking two questions. First, do single pulvinar neurons respond consistently over repeated viewings of the same movie? Second, do pairs of pulvinar neurons give similar response patterns to the same movie? Using linear multielectrode arrays in more than 42 electrode penetrations, we recorded neurons and field potentials throughout a large volume of the pulvinar while monkeys freely viewed 5 minute-long movies depicting unfamiliar conspecifics engaging in social behaviours in natural environments. Previous data from our lab has shown that neurons in the inferotemporal cortex respond in a highly consistent manner when a monkey views the same video multiple times, even if gaze patterns differ across viewings. Based on the known patterns of connectivity between the pulvinar nuclei and cortex, we predicted that certain regions sharing connections with the inferotemporal cortex, would display a similar reliability, whereas others might not. We found a high level of diversity in response reliability among pulvinar neurons that varied both within and across pulvinar subregions.

Disclosures: **A.P. Murphy:** None. **C. Deng:** None. **B.E. Russ:** None. **D.B.T. McMahon:** None. **D.A. Leopold:** None.

Poster

816. Subcortical Visual Pathways

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 816.08/DD28

Topic: D.04. Vision

Title: Optogenetic investigation of the LGN koniocellular influence on V1 activity

Authors: ***C. KLEIN**[1], H. C. EVRARD[1,2], K. SHAPCOTT[3], N. K. LOGOTHETIS[1], M. C. SCHMID[3];
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Abstract: The lateral geniculate nucleus (*LGN*) of the primate thalamus is organized into parallel parvo-, magno- and konio-cellular projection streams to primary visual cortex (*V1*). While magno and parvo cells label positive for Parvalbumin and project to layer-4 of V1, konio neurons project to the superficial layers of V1 and are positive for CamKII and Calbindin [1] [2]. Of the three systems, our understanding of the konio pathway and its contribution to vision is still very limited. Here we used optogenetics in anaesthetized macaque monkeys to investigate the influence of koniocellular LGN neurons on V1. To this end we injected the construct AAV5-CamKIIa-ChR2-eYFP into the LGN of two monkeys. Post-mortem histological and immunohistochemical analysis verified that ChR2 expression was predominantly present in the koniocellular system, which is characterized by its expression of CamKII and a focus on the LGN intercalated layers. In earlier experiments optogenetic stimulation that was applied to neurons in the LGN intercalated layers resulted in activation of the superficial layers in V1, but not layer 4, as determined from current-source-density measurements (CSD) from multi-contact laminar recordings in V1. Preliminary analysis of the cortical LFP also showed a power decrease in the beta frequency range (15-30Hz) for the superficial layers during optogenetic stimulation. In additional control experiments in one monkey, we found that electrical micro-stimulation in a parvocellular layer activated layer 4 of V1 similar to visual flicker stimulation. In contrast, electrical microstimulation in the intercalated LGN layers induced activity in superficial layers of V1 similarly to the optogenetic stimulation. In summary, we show for the first time the effective

connectivity of the koniocellular LGN projection to V1 and its influence on the LFP. Methodologically, our results demonstrate that both circuit probing approaches, optogenetics and electrical microstimulation, render results with very similar specificity. Reference 1. Hendry, S.H. and T. Yoshioka, *A neurochemically distinct third channel in the macaque dorsal lateral geniculate nucleus.* Science, 1994. **264**(5158): p. 575-577. 2. Casagrande, V.A., et al., *The morphology of the koniocellular axon pathway in the macaque monkey.* Cereb Cortex, 2007. **17**(10): p. 2334-2345.

Disclosures: C. Klein: None. **H.C. Evrard:** None. **K. Shapcott:** None. **N.K. Logothetis:** None. **M.C. Schmid:** None.

Poster

816. Subcortical Visual Pathways

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Program#/Poster#: 816.09/DD29

Topic: D.04. Vision

Support: R01 EY018251

Title: Long lasting modulation of excitability and synaptic dynamics in the awake geniculocortical system

Authors: *C. R. STOELZEL[1], J. HUFF[1], Y. BERESHPOLOVA[1], J. ZHUANG[1], X. HEI[1], J.-M. ALONSO[2,1], H. A. SWADLOW[1,2];
[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Biol. and Visual Sci., SUNY Optometry, New York, NY

Abstract: Both psychophysical and physiological experiments show that the visual system adapts to prolonged intense visual stimulation at multiple levels. Most single neuron studies have revealed adaptation effects that last from tens of milliseconds to a few minutes, while some psychophysical experiments have revealed longer lasting effects. Here, we describe a novel, experience-dependent modification of neurons in the lateral geniculate nucleus (LGN) of awake rabbits that persists for > one hour. We recorded single unit activity from concentric and directionally selective LGN neurons and, after recording baseline spontaneous firing rates, we presented a high-contrast flickering spot or drifting grating over the receptive field center for 10 - 30 minutes, generating a stimulus-induced elevation in spike rate. During the period following this strong visual stimulation, we found that all cells showed a severe reduction in spontaneous

activity lasting > one hour that was due to the prior visually induced activity, was associated with increased bursting activity, and was independent of alert vs. non-alert brain state. We explored two additional phenomena that accompanied this post-stimulation period of reduced spontaneous activity: (1) These cells showed a lasting reduction in contrast response gain to visual stimulation and an increase in the ratio of visually evoked to spontaneous firing, and (2) the action potentials of affected LGN cells generated stronger post-synaptic currents in primary visual cortex. Lastly, following prolonged visual stimulation with suppressive stimuli, we observed the opposite effect, with spontaneous firing rates remaining elevated for 20 minutes or more, showing that such modulations are bi-directional.

Disclosures: C.R. Stoelzel: None. **J. Huff:** None. **Y. Bereshpolova:** None. **J. Zhuang:** None. **X. Hei:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

816. Subcortical Visual Pathways

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Program#/Poster#: 816.10/DD30

Topic: D.04. Vision

Support: UC LEADS

Title: Extreme binocular plasticity and dynamic strategy implementation sustains visual prey capture in cephalopod cuttlefish

Authors: *V. CHEUNG, O. J. MULLINS, P. L. NGUYEN, A. D. HUBERMAN;
Dept. of Neurosciences, Univ. California San Diego, La Jolla, CA

Abstract: A fundamental goal in neuroscience is to understand how neurons cooperate to mediate and support goal-directed behaviors. Cephalopods, which include squid, octopi, and cuttlefish, are highly visual creatures with complex nervous systems. The cuttlefish in particular is anecdotally reported to undergo large shifts in eye position during its transition from prey to predator. Here, we quantitatively evaluated if ocular convergence occurs in response to prey detection. We also asked whether such convergence is necessary for depth perception leading to successful prey capture. Finally, we explored how stereopsis deficient cuttlefish adapt to conditions of altered stereopsis, when survival (eating) was contingent on successful prey capture. Cuttlefish were hatched and maintained in our lab. Their eye movements during prey-capture behavior were analyzed via dual-angle, high-speed imaging (1000 frames per second)

and semi-automated eye tracking software. 3D positional coordinates were generated and subsequently used to calculate angle change between eyes throughout prey-capture. These recordings were also used to analyze tentacle dynamics (speed, angle and distance of ballistic attack). Necessity of binocularity/stereopsis for efficient prey-capture was tested by transiently blinding cuttlefish in one eye, and filming their hunting episodes to evaluate changes in prey-capture dynamics. Hunting episodes were also filmed after recovery of vision to the deprived eye. Multiple parameters of prey-capture were measured and compared to control animals. Control cuttlefish with sight in both eyes displayed 100% prey-capture success rates. Temporary suture of one eye significantly reduced the success rate to ~60%. Analysis of tentacle dynamics showed that cuttlefish deprived of vision in one eye tended to miscalculate prey distance, thus failing to capture their target. Removal of sutures restored sight in the deprived eye and the full ability to capture prey. Results from these experiments indicate binocular convergence and stereopsis are necessary for cuttlefish to accurately and efficiently capture prey. Interestingly, unilateral lid suture caused emergence of novel head movements during prey capture, which may sustain motion parallax and the remaining prey capture successes. We also discovered that cuttlefish can employ distinct prey distraction tactics depending on prey orientation during the stalking phase. Together these data indicate cuttlefish implement highly dynamic, rapidly updated strategies for successful visual prey capture. We are starting to make headway on the underlying neural circuits that support this extreme plasticity.

Disclosures: V. Cheung: None. **O.J. Mullins:** None. **P.L. Nguyen:** None. **A.D. Huberman:** None.

Poster

816. Subcortical Visual Pathways

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Program#/Poster#: 816.11/DD31

Topic: D.04. Vision

Support: Deutsche Forschungsgemeinschaft SFB 936/A2

Deutsche Forschungsgemeinschaft SPP 1665 EN 533/13-1

Title: Functional connectivity between superior colliculus and cortex during ongoing activity in the anesthetized ferret

Authors: *I. M. STITT, E. GALINDO-LEON, F. PIEPER, G. ENGLER, A. K. ENGEL; Dept. of Neurophysiol. and Pathophysiology, Hamburg, Germany

Abstract: In the absence of sensory stimulation or motor output, the brain exhibits complex spatiotemporal patterns of intrinsically generated neural activity. This form of activity is thought to reflect the underlying functional architecture of large-scale neuronal networks in the brain. Here, we investigate the spontaneous interaction between the superior colliculus (SC) of the midbrain and the posterior cortex in the anesthetized ferret. Cortical field potentials were recorded using a 64 contact micro electrocorticogram (μECoG) that was custom-designed for the ferret posterior cortex. At the same time, broadband neural activity from all SC layers was recorded with a 2 X 16 dual-shank linear silicon probe (NeuroNexus Technologies). Data from both μECoG and linear probes were sampled simultaneously with an AlphaLab SnRTM recording system (Alpha Omega Engineering). Spontaneous corticotectal interaction was characterized by correlated fluctuations in the amplitude of extracellular fields in the 120-300Hz frequency band. Correlated corticotectal activity typically occurred between recording sites located in superficial and intermediate SC layers, and μECoG recording sites spanning visual and suprasylvian cortical areas. Brief fluctuations in the power of high frequency oscillations in the cortex were highly correlated to spiking activity in the SC, suggesting that coincident corticotectal power fluctuations reflect correlated spiking activity. Correlated SC and cortical activity was strongly locked to the phase of the cortical slow oscillation (~0.7Hz). SC spiking activity was additionally locked to the phase of spindle oscillations in the cortex (~10Hz, $p < 0.01$). Finally, ketamine anesthesia enhanced the power of the slow cortical oscillation, and indirectly increased the intensity of correlated high frequency corticotectal activity. Collectively, our findings reveal that spontaneous activity in the SC is tightly coupled to the state of cortex under anesthesia.

Disclosures: I.M. Stitt: None. **E. Galindo-Leon:** None. **F. Pieper:** None. **G. Engler:** None. **A.K. Engel:** None.

Poster

816. Subcortical Visual Pathways

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Program#/Poster#: 816.12/DD32

Topic: D.04. Vision

Support: NIMH IRP

Title: Systematic mapping of basic visual responses across the pulvinar of the awake macaque

Authors: *C. DENG[1], A. P. MURPHY[1,2], D. A. LEOPOLD[1];
[1]NIMH/NIH, Bethesda, MD; [2]Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: The pulvinar is a region of the posterior thalamus that is particularly large in primates and composed of functionally distinct areas. As it receives most of its input from the dorsal and ventral pathways of the visual cortex, it is thought to be an important hub for integrating cortical signals and likely coordinating them with visually guided actions. It is also the target of prominent projections from other regions of the cortex, as well as from the superior colliculus, and receives direct, albeit notably weaker, input from the retina, primarily from the contralateral eye (Cowey, 1994; O'Brien, 2001; Warner, 2010). These various inputs are thought to shape responses in different subregions of the pulvinar. Although previous studies in the anesthetized monkey have described the pulvinar's retinotopic organization (e.g. Gattas, 1979; Bender, 1981), and studies in the awake animal have studied the pulvinar's role in aspects of cognition (Petersen, 1987; Wilke, 2009; Komura, 2013) and eye movement (Robinson, 1991), the regional diversity of receptive field properties has not been systematically mapped in the awake animal. In this study we measured basic visual response properties of neurons at known 3-D positions throughout the pulvinar of fixating animals, determining the spatial extents, ocularity, and basic feature selectivity of each neuron's receptive field. We acutely penetrated the dura using a multi-channel linear probe consisting of 24 contacts spaced over approximately 7 millimeters, recording both single unit and local field activity. Over several months, we recorded neural activity during 25 penetrations, and were thus able to assess the basic functional properties from over 400 spatial positions throughout the volume of the pulvinar and adjacent structures. For basic receptive field mapping, we presented small, moving random dot patterns, averaged over at least 20 interleaved trials for each of 54 tested locations spanning 25 degrees of visual space around the fixation spot. Unlike in previous studies in the awake monkey, we presented these stimuli separately to the two eyes. Feature selectivity was also assessed for each position by using both flashed images and natural videos. Linking receptive field properties to the recording position, we created detailed maps of both sensory and oculomotor responses. Receptive field structure, feature selectivity, and eye movement responses were strongly regionalized. Preliminary data suggests a fraction of neurons in the retinorecipient zone of the inferior pulvinar are driven primarily, or even exclusively, by the contralateral eye.

Disclosures: C. Deng: None. **A.P. Murphy:** None. **D.A. Leopold:** None.

Poster

816. Subcortical Visual Pathways

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Topic: D.04. Vision

Support: JSPS KAKENHI Grant Number 25290005

Title: Face categorization by population activity patterns of the monkey pulvinar neurons

Authors: M. N. NGUYEN[1], J. MATSUMOTO[1], A. H. TRAN[1], T. ONO[1], *H. NISHIJO[2];
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Abstract: The pulvinar nuclei appear to function as the subcortical visual pathway that bypasses the striate cortex, detecting fundamental information of face. We recorded neuronal responses from monkey pulvinar during a delayed non-matching-to-sample (DNMS) task, in which monkeys were required to discriminate 5 categories of visual stimuli (photos of human faces, cartoon faces, face-like patterns, eye-like patterns, and simple geometric patterns). Facial stimuli consisted of 35 facial photos (profiles or frontal faces with different gaze directions) of 5 human models. Of 401 neurons recorded, 165 neurons responded differentially to the visual stimuli. Neuronal responses to the 35 facial photos were analyzed by Multidimensional scaling (MDS) analysis. MDS results indicated that the pulvinar neurons could classify some aspects of facial information such as orientation, gender and identity of the models into specific groups. Interestingly, the identities were better discriminated in the frontal faces as compared with the profiles. These results suggest that pulvinar neurons participate in fundamental facial processing and provide neurophysiological evidence for pulvinar involvement in social cognition.

Disclosures: M.N. Nguyen: None. **H. Nishijo:** None. **T. Ono:** None. **J. Matsumoto:** None. **A.H. Tran:** None.

Poster

816. Subcortical Visual Pathways

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Topic: D.04. Vision

Support: NIH Grant EY018251

Title: Brain state modulation of directional selective neurons in the awake LGN

Authors: *X. HEI[1], C. R. STOELZEL[1], J. ZHUANG[1], Y. BERESHPOLOVA[1], J. HUFF[1], J.-M. ALONSO[2,1], H. A. SWADLOW[1,2];
[1]Psychology Dept., Univ. of Connecticut, Storrs, CT; [2]Biol. Sci., SUNY State Col. of Optometry, New York, NY

Abstract: The shift between alert and non-alert states in awake rabbits is correlated with profound changes in the behaviors of various cell types in the dorsal lateral geniculate nucleus (LGN). When alert, concentric neurons in the LGN exhibit higher spontaneous firing rate, lower burst rate, broader temporal tuning and stronger and more sustained responses than when not alert (Bereshpolova et al. 2011; Bezdudnaya et al. 2006; Cano et al. 2006; Swadlow and Weyand, 1985). However, the manner in which alert and non-alert brain states affect the response properties of direction selective (DS) neurons in the rabbit LGN has not been studied. Here, we identified the LGN DS neurons in awake rabbit and show that their preferred directions cluster around the four cardinal directions and their responses are strongly modulated by brain state. Like LGN concentric neurons, LGN DS neurons increased their spontaneous firing rates and lowered their burst rates during the alert state. Alertness also enhanced the response gain to stimulation in the preferred direction and enhanced the response suppression to stimulation in the null direction. The response suppression to stimulation in the null direction was ~ 2 times greater in the alert than non-alert states. Finally, we developed a vector-based-population-coding model to investigate how the main two effects of alertness (enhanced responding in the preferred direction, and increased suppression in the null direction) could influence the speed at which stimulus direction could be inferred from the responses of DS neurons. We simulated four DS cells, each with a preference for one of the four cardinal directions. We then used their average population tuning curve to extract their firing rates to a particular stimulus direction and generate spike trains by Poisson process. Then, the spike rates were integrated over different time windows and fed to a vector sum detector which yielded the predicted stimulus direction. We found that the integration time needed to obtain a reasonable prediction error (the angle difference between the stimulus direction and predicted direction) was shorter in the alert than non-alert state. Moreover, both effects of alertness (enhanced response in preferred direction and suppression in null direction) contributed significantly (and approximately equally) to the faster direction detection. The simulation also predicted that stimuli moving closer to a cardinal direction could be detected faster by populations of LGN DS cells than stimuli moving in other directions.

Disclosures: X. Hei: None. **C.R. Stoelzel:** None. **J. Zhuang:** None. **Y. Bereshpolova:** None. **J. Huff:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

816. Subcortical Visual Pathways

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Topic: D.04. Vision

Support: NEI R01EY017699

Title: Functional and anatomical connectivity between the pulvinar and temporal cortex

Authors: *M. J. ARCARO, M. PINSK, S. KASTNER;
Princeton Univ., Princeton, NJ

Abstract: Anatomical studies in non-human primates have shown that the pulvinar contains extensive, reciprocal connections with large portions of ventral temporal cortex (Benevento & Rezak 1976; Webster et al. 1993). Despite this established connectivity, little is known about the relation of these connectivity patterns to the functional organization of ventral temporal cortex. Using neuroimaging, we investigated functional and anatomical connectivity between the pulvinar and functionally defined temporal regions in humans and macaques. Retinotopic mapping and category localizer tasks were used to functionally identify cortical areas in individual subjects. Functional connectivity was assessed in humans from data collected during states of rest in which subjects were instructed to either maintain a central fixation, or keep their eyes closed. Functional connectivity was assessed in macaques from data collected during anesthetized states. Correlation-based analyses were employed to assess functional connectivity. Anatomical connectivity was assessed using probabilistic tracking analyses on diffusion tensor imaging data. In both species, temporal cortex was most strongly linked with the ventral pulvinar. Individual areas were connected with focal regions in the ventral pulvinar. There was a broad topography of connections between temporal areas and the pulvinar. Posterior temporal areas were most strongly connected with the rostro-lateral pulvinar and anterior temporal areas most strongly connected with the caudo-medial pulvinar. Connectivity patterns of neighboring areas overlapped in the pulvinar. In general, the organization of temporal connectivity within the pulvinar appeared to reflect anatomical cortical distance more than functional similarity. Functional and anatomical cortical connectivity patterns within the pulvinar were similar, though notable differences were observed. Overall, these connectivity patterns are consistent with prior anatomical connectivity studies in the macaque. We are currently exploring the functional response properties within regions of the pulvinar that showed strong connections with temporal cortex.

Disclosures: **M.J. Arcaro:** None. **M. Pinsk:** None. **S. Kastner:** None.

Poster

816. Subcortical Visual Pathways

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Topic: D.04. Vision

Support: NIH EY 021575

Title: Changes in temporal structure of spike trains in the early visual pathway

Authors: ***A. R. CASTI**[1], E. KAPLAN[2], Y. XIAO[3];
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Abstract: A single extracellular electrode in the lateral geniculate nucleus (LGN) can capture both LGN output spikes as well as the primary retinal ganglion cell (RGC) input in the form of slow synaptic potentials (S potentials). Previously, we have exploited this type of recording to make a direct comparison of the information processing capabilities of an LGN cell relative to its retinal input. We found that the LGN, despite its relay cells firing far fewer spikes than their retinal drivers, nonetheless transmits a higher amount of Shannon information per spike than does the retina. This shows clearly that the thalamic transformation of retinal input is nontrivial. The earlier analysis, however, did not afford any insight into the thalamic circuitry that effects the temporal structure of the spike train, which can be quantified by the spike train complexity, i.e. the size (bits) of the minimal program required to reproduce the spike train. In this work we explore various measures of complexity to attain additional insight into the temporal structure of RGC and LGN spike trains and the underlying thalamic circuitry (involving cortical feedback and input from local interneurons) that is responsible for the LGN's ability to transmit information to cortex more efficiently. To quantify the complexity of RGC and LGN spike trains, we initially chose a popular measure of signal complexity - the Lempel-Ziv (LZ) complexity - which also serves as a lower bound on the entropy rate. For our retinogeniculate data we found that the LZ complexity does not afford any more insight into the complexity of RGC/LGN spiking than does the firing rate. LZ complexity increases monotonically with the randomness of the signal, so, in effect, a higher firing rate translated directly into higher entropy. For example, we found that artificially generated LGN spike trains, obtained from random

deletions of the input RGC spike trains, had approximately the same LZ complexity under a variety of stimulus conditions. This stands in contrast to the fact that deletions of incoming RGC inputs performed by the real LGN are far from random, as revealed by real LGN autocorrelation functions compared to artificially generated ones. Other complexity measures, such as lattice complexity, permutation entropy, and Causal State Models, scale differently with the degree of randomness in the signal and are therefore more appropriate quantifiers of complexity for our purposes. We compute and compare these alternative complexity measures to draw conclusions about RGC and LGN temporal complexity, and how it is affected by a wide range of visual input conditions, such as size, contrast, and spatiotemporal stimulation.

Disclosures: **A.R. Casti:** None. **Y. Xiao:** None. **E. Kaplan:** None.

Poster

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Topic: D.04. Vision

Support: NRF-2010-0003717

Title: Immunocytochemical localization of calbindin D28K, calretinin, and parvalbumin in bat superior colliculus

Authors: *S.-J. JEONG[1,2], H.-G. KIM[2], Y.-N. GU[2], C.-J. JEON[1,2];
[1]Biol., Kyungpook Nat'L Univ., Daegu, Korea, Republic of; [2]KNU Creative BioResearch Group (BK21), Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: The purpose of this study was to investigate the localization of cells containing the calcium-binding proteins (CBPs) calbindin D28K (CB), calretinin (CR), and parvalbumin (PV) in the superior colliculus (SC) of the bat using immunocytochemistry. CB-immunoreactive (IR) cells formed a laminar tier within the upper superficial gray layer (SGL), while CR-IR cells were widely distributed within the optic layer (OL). Scattered CR-IR cells were also found within the intermediate gray, white, and deep gray layers. By contrast, PV-IR cells formed a laminar tier within the lower SGL and upper OL. Scattered PV-IR cells were also found throughout the intermediate layers, but without a specific laminar pattern. The CBP-IR cells varied in size and morphology: While most of the CB-IR cells in the superficial layers were small round or oval cells most CR-IR cells in the intermediate and deep layers were large stellate cells. By contrast,

PV-IR cells were small to large in size and included round or oval, stellate, vertical fusiform, and horizontal cells. The average diameters of the CB-, CR-, and PV-IR cells were 11.59, 17.17, and 12.60 μm, respectively. Double-immunofluorescence revealed that the percentage of co-localization with GABA-IR cells was 0.0, 0.0, and 10.27% of CB-, CR-, and PV-IR cells, respectively. These results indicate that CBP distribution patterns in the bat SC are unique compared with other mammalian SCs, which suggests functional diversity of these proteins in visually guided behaviors.

Disclosures: **S. Jeong:** None. **H. Kim:** None. **Y. Gu:** None. **C. Jeon:** None.

Poster

816. Subcortical Visual Pathways

Location: Halls A-C

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Title: Functional hierarchy in shape transformed apparent motion activates zebrafish tectal neurons revealed by two-photon calcium imaging

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Abstract: When a visual object has shape changed while jumping across space within a reasonable distance, human perceived a continuous motion. Previous human studies showed that this form transformed apparent motion has a functional hierarchy of perception and performance, in some ways corresponding to the geometrical stability of the shapes. That is, when an object changes from square to ring, the shape stability is disrupted in a higher order than when the changing is between square and parallelogram. Functional MRI studies demonstrated that it is more about form perception and object continuity, with larger brain activations during higher order transforming. Zebrafish tectal neurons are essential for object detection and recognition, in which processing of shape information is important. Following the approach enlightened by fMRI studies, the zebrafish tectal neurons become a good candidate to explore the neural

correlates of the form transformed apparent motion at the single neuron level. In the current study, we recorded *in vivo* calcium signals with two-photon microscope from nacre larval zebrafish expressing GCaMP5G under the evalv3 promoter. The calcium images were acquired continuously while a single slice was scanned repeatedly for 580 times at 0.91 Hz with a 40x lens at 2.5 zoom factor. The fish larva was embedded in agarose between two screens on which visual stimuli may delivered by two miniature projectors. The shape transformed apparent motion was delivered in a hierarchical manner, changing from square to parallelogram, to trapezoid, to disc and to ring, respectively and repeat 3 times. After detecting region-of-interest (ROI) that showed calcium transients by an automatic procedure, timeseries were extracted for each ROI. For each ROI, or tectal neuron, the calcium transient ($\Delta F/F$) was compared between different levels of shape changes. We found that the number of the significantly activating neurons increased systematically, following the hierarchy of the shape changes. When compared with no shape change, shapes changing from square to parallelogram activated 16.6 neurons on average ($n = 20$) in the scanning field of view, and 22.7 neurons to trapezoid, 26.7 neurons to disc and 28.6 neurons to ring, respectively. A repeated measure general liner model analysis revealed a significant effect of the shape hierarchy on the number of activated neurons ($F(3, 57) = 7.592$, $P < 0.001$, Greenhouse-Geisser corrected). This result consists with previous human fMRI study and may provide a good window to explore the neural circuitry of a general neural architecture for shape transformed apparent motion perception.

Disclosures: Z. Liu: None. **M. Ma:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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EY021016

EY012716

EY016155

Title: Ultrastructural and optogenetic dissection of V1 corticotectal synapses in the mouse

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[1]Anatom. Sci. and Neurobio., [2]Psychological and Brain Sci., Univ. of Louisville, Louisville, KY

Abstract: The superior colliculus (SC) is a major site of sensorimotor integration in which sensory inputs are processed to initiate orienting movements of the eyes, head, and body. In order to understand how inputs originating from the visual cortex (V1) affect the circuitry of the SC, we investigated corticotectal (CT) terminals using electron microscopy and *in vitro* optogenetic techniques. To examine the ultrastructure of CT terminals we injected biotinylated dextran amine into V1 to label corticotectal terminals via anterograde transport. Using electron microscopy, we then examined labeled terminals in tissue stained for gamma amino butyric acid (GABA) via post embedding immunocytochemical labeling techniques. The staining density required to identify GABAergic profiles was standardized in tissue from transgenic mice that express green fluorescent protein in neurons that contain glutamic acid decarboxylase. At the ultrastructural level, CT terminals (n = 215) were observed to be small profiles (0.44 ± 0.27 μm2) that contained densely packed round vesicles. CT terminals contacted small (0.51 ± 0.69 μm2) non-GABAergic dendrites and spines (93%) and a few small GABAergic dendrites (7%). To activate CT terminals in SC slices maintained *in vitro*, we induced V1 neurons and their axon projections to express the channelrhodopsin variant Chimera EF with I170 mutation (ChIEF) and a red fluorescent protein (tdTomato) via injection of an adeno-associated viral vector. Whole cell recordings were obtained from SC cells in regions of the stratum griseum superficiale and stratum opticum that contained tdTomato-labeled CT axons. Biocytin was included in the pipette to subsequently reveal the morphology of the recorded neurons. ChIEF-expressing terminals were photoactivated with 1-10ms blue light pulses and monosynaptic connections were identified by excitatory postsynaptic potentials/currents (EPSPs/EPSCs) that exhibited a fixed latency and amplitudes that increased in proportion to light intensity. These monosynaptic responses were abolished in the presence of 1 μM tetrodotoxin (TTX), but they could be elicited in the presence of 1 μTTX when paired with 1mM 4-aminopyridine. We found that a subset of SC neurons responded to activation of V1 CT terminals with large amplitude monosynaptic EPSPs that depress when stimulated at frequencies > 5Hz. Our results indicate a high degree of specificity in CT synaptic targets and additionally indicate that V1 CT inputs may strongly influence the activity of a subset of SC neurons.

Disclosures: S.P. Masterson: None. **B.K. Akers:** None. **W. Dang:** None. **N. Zhou:** None. **G. Govindaiah:** None. **W. Guido:** None. **M.E. Bickford:** None.

Poster

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Title: The organization and development of cholinergic brainstem input to the mouse visual thalamus

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Abstract: The dorsal lateral geniculate nucleus (dLGN) of the thalamus serves as the principal relay of visual information from retina to visual cortex. However, only 10% of the synapses onto dLGN relay cells come from the retina, while the rest arise from non-retinal sources. One of the largest originates from cholinergic neurons in the mesopontine tegmentum region of the brainstem. While such input has been shown to affect the gain of retinogeniculate signal transmission in a state dependent manner, the cellular basis of such modulation remains poorly understood. To visualize the pattern and developmental timing of cholinergic innervation of dLGN, we utilized the transgenic mouse ChAT-Cre x Ai9 to express the red fluorescent protein tdTomato (tdT) in cholinergic neurons. At early postnatal ages, robust cellular tdT labeling was seen in pedunculopontine tegmentum (PPTg), laterodorsal tegmentum (LDT), and parabigeminal nucleus (PBG). Beginning at about postnatal day 5-7, cholinergic axons from these nuclei were found to travel along the optic tract and begin to innervate the dorso-lateral portion of dLGN. Even by natural eye opening (P14), cholinergic innervation of dLGN was restricted to the dorso-lateral tier and antero-medial pole of dLGN. By P30, the entire nucleus was innervated. In order to determine the primary source of cholinergic input to dLGN, the retrograde tracer CTB was injected into the adult dLGN. In cases where injections were restricted to dLGN, a dense plexus of CTB labeled cells was seen in the contralateral PBG. Additionally, sparse and somewhat weakly labeled cells were detected in the PPTg and LDT. To begin to understand the functional role of these inputs to dLGN, we used an adeno-associated virus to express the light-gated ion channel ChIEF, fused with tdT, in cholinergic brainstem neurons. This approach allowed for the direct and selective photo activation of brainstem cholinergic terminals in dLGN while recording from relay cells *in vitro*. Optogenetic activation of ChIEF-expressing PBG terminals in dLGN with either short (1ms) or long (100 ms) pulses of blue light evoked both excitatory and inhibitory postsynaptic activity in relay cells. In most cells, photo-stimulation with a 100 ms single pulse in the presence of bath-applied glutamate and GABA antagonists led to a slow rising depolarization that peaked roughly 25 ms after the onset of light, followed by a slow sustained 1 mV hyperpolarization that lasted about 70-100 ms. Taken together these studies provide new insight about the development, structural organization, and functional role of ascending cholinergic input to subcortical visual structures.

Disclosures: **G. Sokhadze:** None. **N. Zhou:** None. **M.E. Bickford:** None. **W. Guido:** None.

Poster

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Support: NIH 1R01EY023337-01

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Title: Development of receptive field properties in mouse dLGN is accompanied by changes in excitatory and inhibitory retinal convergence

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Abstract: The dorsal lateral geniculate nucleus (LGN) of the thalamus serves as an important model for understanding activity-dependent synaptic development and refinement. Previous studies indicate an age-related pruning in retinal convergence from as many as a dozen separate retinal afferents at early postnatal ages, to just one or a few inputs a few weeks after natural eye opening. However, it is not known how these changes in convergence translate into the maturation of receptive field properties. To address this we conducted both *in vivo* and *in vitro* recordings at the time of eye opening (P14) and after one week of visual experience (P21). For *in vivo* assessment of receptive fields, we performed multisite extracellular recordings in LGN in awake mice viewing a repertoire of visual stimuli. We discovered very large receptive fields at eye opening that undergo significant spatial refinement during the first week. Average spot size preference was 32 degrees after eye opening and 10 degrees after one week of visual experience. This spatial refinement was accompanied by functional changes, including a 2-fold increase in surround suppression. To begin to understand the underlying circuitry that accompany these changes in receptive field properties, we recorded the excitatory and inhibitory synaptic responses *in vitro* in an acute thalamic slice preparation. By varying the intensity of optic tract simulation we generated amplitude by stimulus intensity plots to obtain estimates of retinal convergence onto dLGN cells. Because retinal afferents form synapses with inhibitory interneurons, which in turn form feed-forward inhibitory connections with relay cells we were

also able to examine the degree of inhibitory convergence. Between P14 and P21 there was about a 2.5 fold decrease in excitatory convergence (from 5-2 inputs) with little change in peak EPSC amplitude (1nA). IPSCs exhibited a 2-fold increase (2 to 4 inputs) in convergence with no change in the peak amplitude. Thus, these *in vitro* measurements showing a pairing of decreased excitatory convergence with increased inhibitory convergence provide a potential mechanism to explain the *in vivo* measurements demonstrating a decrease in receptive field size and increase in surround suppression.

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Poster

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Title: Nuclei-specific differences in nerve terminal distribution, morphology, and development in mouse visual thalamus

Authors: G. CARRILLO[1], A. MONAVARFESHANI[1], S. HAMMER[1], G. GUBBI GOVINDAIAH[2], W. GUIDO[3], *M. A. FOX[4];
[1]VTCRI, Roanoke, VA; [2]Univ. Louisville, Louisville, KY; [3]Univ. of Louisville, Louisville, KY; [4]Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Mouse visual thalamus has emerged as a powerful model for understanding the mechanisms underlying neural circuit formation and function. Three distinct nuclei within mouse thalamus receive retinal input, the dorsal lateral geniculate nucleus (dLGN), the ventral lateral geniculate nucleus (vLGN), and the intergeniculate nucleus (IGL). However, in each of these nuclei retinal inputs are vastly outnumbered by non-retinal inputs that arise from cortical and subcortical sources. Although retinal and non-retinal terminals associated within dLGN circuitry have been well characterized, we know little about nerve terminal organization, distribution and development in other nuclei of mouse visual thalamus. Immunolabeling specific subsets of synapses with antibodies against vesicle-associated neurotransmitter transporters or

neurotransmitter synthesizing enzymes revealed significant differences in the composition, distribution and morphology of non-retinal terminals in dLGN, vLGN and IGL. For example, inhibitory terminals are more densely packed in vLGN and cortical terminals are more densely distributed in dLGN. Overall, synaptic terminal density appears least dense in IGL. Similar nuclei-specific differences were observed for retinal terminals using immunolabeling, genetic labeling, axonal tracing and serial block face scanning electron microscopy: retinal terminals are smaller, less morphologically complex, and more densely distributed in vLGN than in dLGN. Since glutamatergic terminal size often correlates with synaptic function, we used *in vitro* whole cell recordings and optic tract stimulation in acutely prepared thalamic slices to reveal that excitatory postsynaptic currents (EPSCs) are considerably smaller in vLGN and show distinct responses following paired stimuli. Finally, anterograde labeling of retinal terminals throughout early postnatal development revealed that anatomical differences in retinal nerve terminal structure are not observable as synapses initially formed, but rather developed as retinogeniculate circuits mature. Taken together, these results reveal nuclei-specific differences in nerve terminal composition, distribution, and morphology in mouse visual thalamus. These results raise intriguing questions about the different functions of these nuclei in processing light-derived information, as well as differences in the mechanisms that underlie their unique, nuclei-specific development.

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Title: The mouse lateral posterior nucleus links the extrastriate visual cortex with the striatum and amygdala: An optogenetic investigation

Authors: *N. ZHOU, S. P. MASTERSON, G. GOVINDAIAH, W. GUIDO, M. E. BICKFORD;
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Abstract: The lateral posterior nucleus (LPN) of the mouse dorsal thalamus receives input from the superior colliculus and projects densely to the striatum, lateral amygdala and the lateral extrastriate (LES) cortex. However, it is unclear whether these circuits are interconnected. To determine the cortical cell types activated by the LPN, we used a viral vector to induce the expression of the channel rhodopsin variant Chimera EF with I170 mutation (ChIEF) and a red fluorescent protein (TdTomato) in LPN cells and their axon projections. Coronal slices of the LES were prepared and whole cell recordings were obtained in regions that contained TdTomato-labeled thalamocortical axons (distributed primarily in layers I and IV). ChIEF-expressing terminals were photoactivated with 1-10ms blue light pulses and monosynaptic connections were identified by excitatory postsynaptic potentials/currents (EPSPs/EPSCs) that exhibited a fixed latency and amplitudes that increased in proportion to light intensity. These monosynaptic responses were abolished in the presence of 1 μM tetrodotoxin (TTX), but could be elicited in the presence of 1 μTTX when paired with 100 μM 4-aminopyridine. We found that most cells (87%) in layer IV of the LES (n = 70) responded to activation of LPN-LES terminals with large amplitude monosynaptic EPSPs that depress when stimulated at frequencies > 5Hz. Biocytin was included in our pipettes to reveal the morphology of the recorded cells. Of 53 filled responsive cells we found that 29 (55%) were pyramidal cells, 15 (28%) were spiny stellate cells and the remainder did not clearly fall into either category. To further characterize the cell types targeted by LPN-LES terminals, we paired injections of cholera toxin subunit B conjugated to Alexafluor 488 (CTB-488) in the striatum and amygdala with virus injections in the LPN. This resulted in a dense distribution of CTB-labeled cells within the LES that overlapped the distribution of LPN-LES terminals. In slices of the LES, whole cell recordings were obtained from CTB-labeled cells and surrounding ChIEF-expressing terminals were activated with blue light pulses. The majority of CTB-labeled cells (26/32 or 81%) responded to activation of LPN terminals with large amplitude monosynaptic postsynaptic potentials. Responsive filled striatum/amygdala projecting cells were pyramidal cells that exhibited apical and basal dendritic tufts that overlapped the distribution of LPN terminals. Our results indicate that the LPN and LES are highly interconnected with the striatum and amygdala. These circuits may be particularly important for the visual guidance of movement.

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EY016155

Title: Convergence of "driver-like" inputs in the direction-selective zone of the mouse visual thalamus

Authors: *M. E. BICKFORD[1], N. ZHOU[1], T. E. KRAHE[2], G. GOVINDAIAH[1], W. GUIDO[1]; [1]Anatom. Sci. and Neurobio., Univ. Louisville Sch. Med., Louisville, KY; [2]Anat. and Neurobio., Virginia Commonwealth Univ. Med. Ctr., Richmond, VA

Abstract: The mouse dorsal lateral geniculate nucleus (dLGN) contains a number of functional subdivisions, including a direction-selective zone (DSZ), which is specifically innervated by direction-selective retinal ganglion cells and the superior colliculus (SC). To examine DSZ circuitry and understand how such inputs are organized, we combined anatomical, physiological and optogenetic techniques. To label and activate tectogeniculate (TG) terminals, we injected a viral vector into the SC to induce the expression of TdTomato (a red fluorescent protein) and the channel rhodopsin variant ChIEF (a cation channel activated by blue light). Using electron microscopy, we found that TdTomato-labeled TG terminals constitute a new class of dLGN synaptic profiles that converge with retinogeniculate (RG) terminals to innervate the proximal dendrites of neurons in the DSZ of the dLGN. To examine the postsynaptic responses elicited by activation of these convergent inputs, whole-cell recordings were obtained within the DSZ of the dLGN maintained *in vitro*. ChIEF-expressing TG terminals were activated with short pulses of blue light and RG terminals were activated via electrical stimulation of the optic tract. Individual DSZ neurons responded to activation of both TG and RG terminals with large amplitude excitatory postsynaptic currents that depressed when stimulated at frequencies greater than 2 Hz. To determine whether these neurons project to the superficial layers of V1, we placed filter paper infused with a fluorescent retrograde tracer on the surface of V1, and virus injections in the SC. Layer I-projecting cells were confined to the DSZ, precisely overlapping the distribution of TG terminals. When we targeted our recordings to layer I projecting cells we found that they display "W-like" morphology and receive TG input. Finally, we examined the synaptic contacts of geniculocortical terminals in V1 layer I and found that the majority (85/87 or 98%) contacted

nonGABAergic dendrites, likely arising from lower layer pyramidal cells which extend their apical dendritic tufts within layer I. Taken together these results suggest that W-like cells in the DSZ receive convergent "driver-like" input from both the retina as well as SC. Moreover, the circuitry of the DSZ forms a segregated channel linking retinal direction-selective inputs with SC inputs to influence subsets of V1 cells. Future experiments to reveal the specific cortical cell types innervated by DSZ neurons should help to shed further light on how this channel affects cortical circuits.

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Poster

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EY022871

Title: Functional organization of the dorsolateral tier in the lateral geniculate nucleus of the mouse

Authors: G. PANGENI[1], G. GOVINDAIAH[2], B. G. BORGHUIS[2], *M. A. MCCALL[1], W. GUIDO[2];
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Abstract: Recent studies indicate that the mouse dorsal lateral geniculate nucleus (dLGN) is segregated into different visual channels that represent retinal input from the two eyes or that serve as primary recipient zones for different classes of retinal ganglion cells (RGCs). One such domain the dorsolateral tier, which resides just below the optic tract, receives input from several well-described classes of direction selective RGCs. To better understand the functional organization of this region we conducted a series of anatomical as well as *in vivo* and *in vitro* electrophysiological recording experiments in the adult pigmented mouse. Using single unit

extracellular recording techniques in an *in vivo* anesthetized mouse preparation we found that neurons in the dorsolateral tier had ON, OFF, and ON/OFF responses, and large receptive fields that represented the upper contralateral hemifield. The majority of cells (>60%) in this region showed strong preferences to the direction and orientation of drifting gratings. We found variable tuning widths in all cardinal directions of movement. To begin to understand the underlying circuitry and dLGN cell types that reside in the dorsolateral tier we made whole cell recordings with biocytin filled electrodes in an acute thalamic slice preparation that preserves the retinal afferent connections to relay cells within dLGN. We recorded the excitatory postsynaptic responses evoked by the electrical stimulation of the optic tract and generated estimates of retinal convergence for identified relay cells that were filled with biocytin. We found that virtually all dLGN cells in the dorsolateral tier had a W-like cell morphology (e.g., small soma and hemispheric dendritic architecture) and received input from only 1-2 RGCs. Our reconstructions further revealed that the dendritic fields of these W-like cells overlapped substantially with the retinal terminal fields of at least two classes of DS cells that respond to posterior motion, as well as non-retinal input that arises from the superficial layers of the superior colliculus. Preliminary *in vivo* studies using optogenetics are underway to understand how input from the superior colliculus contributes to the receptive field properties of cells in the dorsolateral tier of dLGN. Taken together, these studies suggest that the dorsolateral tier is a highly specialized division of dLGN that contributes to a visual channel designed to process the direction of motion.

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Poster

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Support: EY012716

Title: Optogenetic activation of inhibitory synapses in the dorsal lateral geniculate nucleus of the mouse

Authors: *G. GUBBI, N. E. CHARALAMAKIS, W. GUIDO;
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Abstract: In the dorsal lateral geniculate nucleus (dLGN), inhibition plays an important role in shaping the receptive field properties of relay cells as well as setting the gain of retinogeniculate transmission. However the synaptic mechanisms underlying such modulation has been difficult to study, in part because of the complex circuitry and diverse cell types involved. To overcome this we adopted an optogenetic approach to selectively activate the inhibitory terminals in dLGN arising from two sources, neurons of the thalamic reticular nucleus (TRN) and intrinsic interneurons within dLGN. In mice, we made stereotaxic injections of an adeno-associated virus containing the channelrhodopsin variant ChIEF fused with tdTomato into TRN or dLGN. We then made *in vitro* whole cell recordings in an acute thalamic slice preparation and stimulated with blue light, inhibitory terminals in dLGN that expressed the light-gated ion channel, ChIEF. In TRN, direct photo-stimulation of ChIEF expressing neurons led to strong excitation that was sufficient to evoke a train of action potentials that followed the rate of stimulation (5-20 Hz). Photo-stimulation of ChIEF expressing TRN terminals in dLGN evoked strong GABAA-receptor mediated inhibitory post-synaptic currents (IPSCs) in relay cells, but not in interneurons. Repetitive blue light stimulation (1-50 Hz) evoked IPSCs that decreased in amplitude with each successive light pulse. Such depression was also frequency dependent, and greatest when high temporal rates were used. (>20Hz.) A similar pattern of inhibitory activity was evoked by photo-activation of intrinsic interneurons. Finally, we tested the impact of TRN mediated inhibition during retinogeniculate signal transmission, by photo-activating TRN terminals during the delivery of electrical stimulation of the optic tract. We found that activation of TRN input onto relay neurons suppressed retinogeniculate signaling in an activity-dependent manner. Retinally evoked spiking and EPSP activity was greatly attenuated especially during the early phases of TRN activation. Taken together these data suggest that the degree of inhibitory modulation occurs in an activity dependent fashion, and has its greatest impact during the initial phase of activation. Such a pattern may help gate retinogeniculate transmission during highly depolarized and sustained states.

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Poster

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Topic: D.04. Vision

Title: Orientation columns in the mouse superior colliculus

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Abstract: Over twenty retinal ganglion cell (RGC) subtypes and their axons form mosaics in the retina and downstream visual structures that are postulated to ensure uniform sampling of the visual field and thereby prevent blind spots. We applied two-photon calcium imaging to explore how input from RGCs drives computations in the superior colliculus (SC), also known as the optic tectum, the principal retinorecipient area in most vertebrates. We found that neighboring neurons in the mouse SC have similar orientation preferences, in sharp contrast to the mosaic arrangement observed in the retina. This observation was extended with wide-field imaging to reveal an arrangement reminiscent of the columnar organization of cat and primate visual cortex. Unlike the cortical feature, SC columns are larger than the projective field of a single point in space, suggesting that the SC does not uniformly process the visual field. The discrepancy between the observed local tuning for orientation and the retinotopic arrangement of RGC inputs suggests that local circuits shape response tuning in the superficial SC.

Disclosures: E.H. Feinberg: None. **M. Meister:** None.

Poster

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Title: Variation in visual receptive field properties in subdomains of the superior colliculus of Islet2-EphA3 knock-in mice

Authors: *R. B. KAY[1], J. W. TRIPLETT[1,2];
[1]Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [2]Pediatrics, Pharmacol. & Physiol., George Washington Univ., Washington, DC

Abstract: Complex visual representations in higher processing centers are hypothesized to arise through precise connectivity of converging inputs from subtypes of neurons that monitor simpler aspects of the visual scene, beginning in the retina. Retinal ganglion cells (RGCs) send all visual

information to the brain and have diverse receptive fields, which can be broadly grouped into center-surround and direction-selective RGCs (DSGCs). A major target of retinal output is the superior colliculus (SC), where neurons have receptive fields distinct from those of RGCs, including orientation-selective (OS) cells. From what inputs these OS receptive fields are constructed remains unclear. Previously, we and others demonstrated that *Islet2-EphA3* knock-in mice ($Isl2^{EphA3/EphA3}$) have typically developed retinal wiring, but RGC subtypes connect to the SC in a unique fashion. In these mice, disparate sets of RGCs project to two independent subdomains of the SC. $Isl2^{+}$ RGCs, which are mostly non-DSGCs, terminate in the anterior half of the SC, while $Isl2^{-}$ RGCs terminate posteriorly. Prior intrinsic signal optical imaging experiments suggested functional differences in these subdomains, possibly due to altered properties of SC neurons. To test this hypothesis, we made extracellular recordings with 16-channel silicon electrode arrays from each of the two subdomains in the SC of $Isl2^{EphA3/EphA3}$ mice. We presented anesthetized mice with drifting gratings of variable orientation, spatial frequency and temporal frequency to determine direction- and orientation-selectivity. We were able to isolate an average of 6 units per penetration. We found that approximately 63% of visually-responsive neurons in the anterior subdomain were OS, while only 14% of neurons in the posterior were OS. Importantly the OS neurons were similar to those previously described in wild-type SC in regards to spatial and temporal frequency tuning, as well as firing rate. These preliminary data show a concentration of OS cells in the anterior subdomain of the SC in $Isl2^{EphA3/EphA3}$ mice. Since this subdomain receives input from $Isl2^{+}$ RGCs, which are predominantly non-DSGCs, these data suggest that innervation by DSGCs is not necessary to establish OS receptive fields in the SC.

Disclosures: **R.B. Kay:** None. **J.W. Triplett:** None.

Poster

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Title: Two-photon Calcium imaging of visual response properties of neurons in the mouse superior colliculus

Authors: *S. INAYAT, J. BARCHINI, J. CANG;
Neurobio., Northwestern Univ., EVANSTON, IL

Abstract: The superior colliculus (SC) is a subcortical structure important for vision in mice. Here, we used two-photon calcium imaging to study the visual response properties of neurons in the superficial layers of SC of urethane anesthetized mice. Part of the left SC anterior to the lambda suture was exposed and injected with the calcium sensitive dye Cal-520TM AM (AAT Bioquest Inc). 1-2 hours post injection, neurons in the superficial SC were imaged using a two-photon laser scanning microscope (Ultima Bruker), while visual stimuli (sinusoidal drifting gratings and light or dark spots on a gray background) were presented to the contralateral eye. The acquired data were analyzed with custom Matlab scripts. Responses of the imaged SC neurons were calculated from the relative change (baseline to stimulus) in the fluorescence signal obtained from manually selected regions of interest identified as neuronal somata. ~64% and ~49% of neurons showed significant calcium transients in response to spot stimuli and drifting gratings of certain size, respectively. For each responsive neuron, its receptive field size, location, ON and OFF structure, and selectivity for stimulus direction and orientation were calculated. These experiments thus allow us to determine the spatial organization of receptive field properties in the mouse SC.

Disclosures: S. Inayat: None. **J. Barchini:** None. **J. Cang:** None.

Poster

817. Subcortical Visual Pathways: Rodents

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 817.12/EE18

Topic: D.04. Vision

Support: HHMI

Title: Shared and distinct sources of retinal input to the mouse dorsal lateral geniculate nucleus and superior colliculus

Authors: G. GAUVAIN, B. SIVYER, *G. J. MURPHY;
Janelia Farm Res. Campus / HHMI, Ashburn, VA

Abstract: Signals frequently diverge from one region of the central nervous system to multiple downstream targets. How does information conveyed to one target differ from that sent to

another? Mouse retinal ganglion cells (RGCs) project to two principle retinorecipient areas: the dorsal lateral geniculate nucleus (dLGN) and the superior colliculus (SC). Some individual RGCs provide synaptic input to neurons in both brain nuclei and, therefore, convey the same information to different areas; others are likely to project exclusively to the SC or dLGN. The fraction and functional properties of RGCs that terminate in the SC, LGN, and both the SC and dLGN remain poorly constrained; as a consequence, the information downstream visual areas can and cannot inherit from the periphery remains speculative. To resolve these ambiguities we systematically characterized RGCs retrogradely labeled from the SC, dLGN, and SC+dLGN. Stereotaxic injection of virus coding for the expression of fluorescent proteins, or latex microspheres ('beads') or cholera toxin conjugated to fluorescent dyes, labeled a high density of RGCs - i.e., ~2000 cells/mm^2. In these areas, ~1/3 of RGCs were labeled from the SC and dLGN. Qualitatively similar fractions of RGCs were labeled exclusively from the SC or dLGN, raising the possibility that visual information conveyed to the two areas could differ significantly. To determine the functional properties of RGCs we made targeted injections of GCaMP6f into the SC and beads into the dLGN (or vice versa) and used multiphoton excitation fluorescence microscopy to measure Ca2+-dependent fluorescence signals as a function of several stimulus parameters; complementary experiments employed electrophysiological recordings from RGCs retrogradely-labeled from one or both retinorecipient areas. These approaches revealed several projection-specific differences. For example, roughly equal fractions of cells exhibiting On, Off, and On-Off responses comprised the population of SC-projecting RGCs; by comparison, On cells were enriched, and Off and On-Off cells were less common, in the population of RGCs retrogradely labeled from the dLGN. More than half of the On cells projecting to the SC also innervated the dLGN, while less than a quarter of SC-projecting Off and On-Off RGCs projected to the dLGN as well. Together, these data suggest that (1) many of the On RGCs projecting to the SC also project to the dLGN and (2) a substantial fraction of Off and On-Off RGCs target the SC but not the dLGN.

Disclosures: **G. Gauvain:** None. **B. Sivyer:** None. **G.J. Murphy:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 817.13/EE19

Topic: D.04. Vision

Support: Centre for Integrative Neuroscience (DFG Exec 307)

Researcher grant from the European Research Council (project acronym: PERCEPT)

Title: The influence of cortical feedback on visual information processing in mouse dorsolateral geniculate nucleus

Authors: *A. VAICELIUNAITE[1,2], S. ERISKEN[1], O. JURJUT[1], M. FIORINI[1], S. KATZNER[1], L. BUSSE[1];
[1]Ctr. for Integrative Neurosci., Univ. of Tuebingen, Tuebingen, Germany; [2]Neurobio. - Biophysics, Vilnius Univ., Vilnius, Lithuania

Abstract: Visual information processing is mediated not only by feedforward inputs from lower visual centers but also profoundly modulated by feedback connectivity. Whether cortico-thalamic feedback substantially changes the representation of visual information at the level of the dorsolateral geniculate nucleus (dLGN) remains an open question, as investigations of feedback-related effects in dLGN have yielded inconsistent results. Here, we used the mouse model to test how temporal and spatial processing of dLGN neurons is affected by cortico-thalamic feedback. To study the role of cortico-thalamic feedback on neural responses in dLGN we performed extracellular recordings with multisite linear silicon probes in awake head-fixed mice. During recordings, we transiently silenced visual cortex by optogenetically stimulating parvalbumin-positive (PV+) inhibitory interneurons. To assess feedback-related changes of temporal response properties, we first examined spontaneous activity. We found that during global inactivation of V1, firing rates decreased abruptly. After ~200 ms of cortical inactivation, dLGN neurons transitioned into burst mode firing, leading to a 3-fold increase in the burst-tonic firing ratio. To assess feedback-related changes in spatial response properties, we measured size tuning in the dLGN by presenting gratings of different diameter. We found that numerous neurons in mouse dLGN exhibit surround suppression, and that we could alter this suppression by optogenetically silencing visual cortex. In dLGN neurons, transient inactivation of visual cortex led to an expansion of RF center size (22.3%) and a reduction of surround suppression (21.8%). We observed many neurons in which the decrease in surround suppression during cortical inactivation seems driven by two effects: a reduction of dLGN responses to stimuli up to the optimal size and an increase of dLGN responses to stimuli exceeding the optimal size. We conclude that, similar to what has been reported for higher-order mammals, both temporal response properties and spatial integration in mouse dLGN are at least partly shaped by cortico-thalamic feedback circuits.

Disclosures: **A. Vaiceliunaite:** None. **S. Erisken:** None. **O. Jurjut:** None. **S. Katzner:** None. **L. Busse:** None. **M. Fiorini:** None.

Poster

817. Subcortical Visual Pathways: Rodents

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 817.14/EE20

Topic: D.04. Vision

Title: Neural signals in the superior colliculus during innate defensive behavior

Authors: *M. AGROCHAO[1,2], M. MEISTER[1];
[1]Caltech, Pasadena, CA; [2]Champalimaud Neurosci. Programme, Lisbon, Portugal

Abstract: The superior colliculus is strongly implicated in behaviors that serve for defense from predators. Three supporting lines of evidence for this are: 1) Studies showing that neurons in the upper layers of the superior colliculus are sensitive to visual displays of approaching objects (Liu et al, 2011), 2) Electrical stimulation in the deeper layers can trigger freezing and escape behaviors (Sahibzada et al, 1986) and, 3) Lesions of the superior colliculus sharply reduce defensive behaviors (Blanchard et al., 1981). What is missing is an account of neural population activity in the superior colliculus in the course of innate defensive behaviors. To tackle this, we developed an approach for population recording of the superior colliculus of the rat, using a multi-tetrode array and a wireless transmitter. As a complement to the freely-moving preparation, we also trained rats to tolerate head-fixed recording while on a circular treadmill, which allows for precise control of visual stimuli while retaining some of the mobility of the animal. Early recordings focused on sensory responses in the visual layers of the superior colliculus. At the most superficial positions we recorded large post-synaptic events (juxtazonal potentials) that appear triggered by a class of retinal ganglion cell sensitive to predator features. Further, we confirmed that many superior colliculus neurons respond more strongly to expanding stimuli (mimicking approaching objects, that trigger defensive behaviors in rodents) than to contracting stimuli (receding objects). Given the literature suggesting that there is an anatomical segregation in the superior colliculus of neurons involved in defense and those involved in approach behaviors (Westby et al, 1990), we expect to record from neurons in the deeper layers of the superior colliculus involved in one type of behavior, but not the other. Liu Y-J et al (2011): Neuronal responses to looming objects in the superior colliculus of the cat. Brain Behav Evol 77:193–205 Sahibzada N et al (1986): Movements resembling orientation or avoidance elicited by electrical stimulation of the superior colliculus. The Journal of Neuroscience 6(3): 723-733 Blanchard D.C. et al (1981): Taming of wild Rattus norvegicus by lesions of the mesenphalic central gray. Physiological Psychology 9:157-163 Westby G.W.M et al (1990): Output pathways from the rat superior colliculus mediating approach and avoidance have different sensory properties. Exp Brain Res 81:626-638

Disclosures: **M. Agrochao:** None. **M. Meister:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Topic: D.04. Vision

Support: Knights Templar Eye Foundation (OSD)

NIH R01-EY022157-01 (ADH)

Pew Charitable Trusts (ADH)

Title: Genetic mechanisms that establish connectivity and function of parallel optic pathways

Authors: *O. S. DHANDE[1], T. A. SEABROOK[1], R. N. EL-DANAF[1], A. H. PHAN[1], J. T. WANG[2], P. L. NGUYEN[1], A. D. HUBERMAN[1];
[1]Dept. of Neurosciences, Univ. California San Diego, La Jolla, CA; [2]Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Features such as visual motion, contrast and luminosity are first encoded in the retina and conveyed to the brain by retinal ganglion cells (RGCs), the output neurons of the eye. RGC projections to the brain are divided into multiple parallel pathways, each serving a distinct function by linking specific subtypes of RGCs and central targets (Dhande and Huberman, Curr Opin Neurobiol, 2014). Here we addressed how these parallel pathways are established and how the activity of the different RGC subtypes within them relates to visual perception and behavior. We discovered by mRNA and protein expression analyses that several T-box family transcription factors are highly enriched in intrinsically photosensitive RGCs that comprise the non-image forming (NIF) pathways; the same transcription factors are near absent in other RGC subtypes. We then generated mice lacking these transcription factors specifically in NIF RGCs using the Cre/loxP system. Selective deletion of either of these T-box family members in NIF RGCs resulted in a massive reduction in the number of intrinsically photosensitive RGCs, as measured by numbers of these cells in the eye and the density of their axonal central projections to the brain. In order to explore whether these factors are also sufficient for endowing RGCs with features characteristic of NIF RGCs, we are now performing gain-of-function/misexpression experiments. The basic design is to introduce these T-box factors in RGC subtypes that normally do not express them in the developing or mature retina using *in vivo* retinal electroporation (Dhande et al., J Neurosci. 2011), and then evaluate the structural, physiological, and behavioral consequences of that misexpression. We have also established a suite of visual behaviors to probe the consequence of removing or altering RGC identities on the functional integrity of the

visual system (e.g., Dhande et al., J Neurosci. 2013). These experiments allow us to better understand how parallel visual pathways are sculpted during development and how their unique wiring patterns influence visual processing.

Disclosures: O.S. Dhande: None. **T.A. Seabrook:** None. **R.N. El-Danaf:** None. **A.H. Phan:** None. **J.T. Wang:** None. **P.L. Nguyen:** None. **A.D. Huberman:** None.

Poster

817. Subcortical Visual Pathways: Rodents

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: Fondecyt 1110281

Title: The complex morphology and interconnected circuitry of neurons in the pretectum and ventral thalamus of the chicken (Gallus gallus)

Authors: *T. VEGA-ZUNIGA[1], A. HARTMANN[1], E. PLANITSCHER[1], C. GONZALEZ[2], G. MARIN[2,3], H. LUKSCH[1];
[1]Technische Univ. Muenchen, Freising, Germany; [2]Univ. de Chile, Santiago, Chile; [3]Facultad de Medicina, Univ. Finis Terrae, Santiago de Chile, Chile

Abstract: In the avian brain, the pretectal and ventrothalamic nuclei such as the tectal gray (GT), the nucleus lentiformis mesencephali (LM) and the nucleus geniculatus lateralis pars ventralis (GLv), are prominent retinorecipient structures implicated in optic flow sensing and visuomotor control. Previous work has shown reciprocal connections between these structures, however, the detailed morphology and neurochemical identity of the neurons involved remain undetermined. Using intracellular cell filling technique we identified two different cell types in GT: one with a dorsal t-shaped axon and a ventral dendritic process; and a second with a ventral axon with two dendritic processes extending dorso-ventrally. The first population projects laterally through the deep tectal layer 13, and medially upon LM and GLv. The second population projects ventrally to LM and GLv via the optic tract. *In situ* hybridization essays, using mRNA probes specific for different vesicular transporters (VGLuT2, VIAAT, VGLIT and VAChT), revealed that most neurons in tectal gray express VGLUT2, indicating that these neurons are predominantly glutamatergic. The cells located in LM showed multipolar cells with an axon extending dorsally without ramification. The dendrites extend through the whole dorso-

ventral axis. At the level of mRNA, large multipolar neurons scattered in the external part of LM showed strong VGLuT2 expression, along with fewer, smaller neurons in the medial part of LM. The cells located in the lamina interna of GLv were multipolar cells with two highly ramified dendritic fields: one that extends throughout the ventrolateral thalamus (VLT) and gives rise to the projecting axon; and a second that ramifies in the GLv-neuropile, and sends a neurite that terminates in LM. *In situ* hybridization experiments showed that virtually all cells in GLv-lamina interna along with scattered cells in GLv-neuropile strongly expressed VIAT mRNA without expressing GLiT mRNA, suggesting a GABAergic identity for these neurons. Our results show that the pretectal and ventrothalamic nuclei are highly interconnected by neurons displaying large dendritic and axonal fields that span several nuclei. These nuclei contain, predominantly, either glutamatergic or GABAergic neuronal profiles. We suggest that this complex cytoarchitecture and connectivity form the anatomical substrate for the functional dynamic subserving optic flow sensing and visuomotor control.

Disclosures: T. Vega-Zuniga: None. **G. Marin:** None. **A. Hartmann:** None. **E. Planitscher:** None. **H. Luksch:** None. **C. Gonzalez:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Topic: D.04. Vision

Support: NSF GRFP Fellowship DGE 0228243

NIH Grant R01 EY012793

Title: Retinal inputs to the lateral habenula: A new pathway for light aversion

Authors: *P. M. FOGERSON, M. AKASAKO, L. E. QUATTROCHI, E. NGUYEN, D. M. BERSON;
Neurosci., Brown Univ., Providence, RI

Abstract: Background Intrinsically photosensitive retinal ganglion cells (ipRGCs) express the photopigment melanopsin, which enables them to transduce light information directly, without input from the outer retina. To date, 5 different types of ipRGCs have been documented, each distinguishable by dendritic structure, receptive field properties, and level of melanopsin

expression. ipRGCs are best known for entraining circadian rhythms to the day-night cycle and driving the pupillary light reflex via axonal projections to the suprachiasmatic nucleus and olivary pretectal nucleus, respectively. Interestingly, ipRGCs also send a small but specific projection to the extreme lateral border of the lateral habenula (LHb) - a region associated with mood, reward, and sleep. We sought to determine which types of RGCs, including ipRGCs, synapse in this "retinorecipient" portion of the LHb. We also aimed to reveal downstream targets of LHb neurons receiving retinal input to infer behavioral roles associated with this ipRGC output. **Methods** A BAC-transgenic mouse line expressing GFP under the *Opn4* (melanopsin) promoter was used to label ipRGC axons, including those targeting the LHb. Stereotaxic deposits of anterograde or retrograde tracer were made in the retinorecipient LHb. Retrolabeled RGCs were classified by dendritic morphology and melanopsin expression revealed by tyramide-amplified melanopsin immunohistochemistry. Injection sites and retrograde and anterograde labeling were evaluated by fluorescence microscopy. **Results** When retrograde tracer deposits were limited to the habenular complex, ipRGCs were the only ganglion cells labeled. Of these, nearly all (26/29; 90%) were M1 cells; the rest were M3 cells. Other RGC types (including non-ipRGCs) were labeled when injection sites spread beyond the LHb to include the rostral pretectum. Anterograde injections into the retinorecipient zone of the LHb revealed projections mainly to the ventrolateral periaqueductal grey. Sparse fibers were also found in the habenular commissure. Well-known targets of the LHb such as the substantia nigra and rostromedial tegmental nucleus lacked fiber labeling when deposits were restricted to the retinorecipient LHb. **Conclusion** ipRGCs are the only retinal ganglion cells that project to the extreme lateral margin of the LHb. M1 cells dominate this projection, and a few M3 cells also contribute. The retinorecipient zone of the LHb projects preferentially to the ventrolateral periaqueductal grey (PAG), a region associated with pain, protective reflexes, and arousal. This pathway may contribute to ipRGC-associated functions such as light aversion and negative masking.

Disclosures: **P.M. Fogerson:** None. **M. Akasako:** None. **L.E. Quattrochi:** None. **E. Nguyen:** None. **D.M. Berson:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Topic: D.04. Vision

Support: NIHr01 EY09593

Title: A physiological and anatomical comparison of relay cells in the ventral and dorsal divisions of the murine lateral geniculate nucleus of the thalamus

Authors: *U. M. CIFTCIOGLU[1], V. SURESH[1], B. M. LALA[1], W. A. SMITH[1], F. T. SOMMER[2], J. A. HIRSCH[1];
[1]Biol. Sci., USC, Los Angeles, CA; [2]Universiy of California, Berkeley, Berkeley, CA

Abstract: The lateral geniculate nucleus of the thalamus comprises two subnuclei. The dorsal division (dLGN) processes and transmits retinal information to the primary visual cortex. The evolutionarily older ventral division (vLGN) connects with many subcortical structures and serves diverse roles ranging from coordinating movement to maintaining circadian rhythms[1]. Indeed, the vLGN receives input from functionally heterogeneous sets of ganglion cells including intrinsically photosensitive[2] and weakly direction selective types[3]. In higher mammals, the dLGN dwarfs the vLGN, but in the rodent the subnuclei occupy comparable territory. The relatively large size and accessibility of the vLGN in the mouse, coupled with recent insights from transgenic animals[2,3] provide an opportunity to study this often overlooked station in the visual pathway. Past physiological studies of the vLGN in cat[4] and in rat[5] reported that most cells had large, undifferentiated receptive fields (including On, Off and On-Off types) and limited feature selectivity. By contrast, most neurons in the dLGN have variously small to large receptive fields with a center-surround structure and many are tuned for orientation or direction[6]. To explore the basis of differences between the subnuclei, we recorded visual responses from the mouse LGN using whole-cell recordings with dye-filled electrodes. Thus we were able to link physiology with anatomy. As for other species, most receptive fields in the mouse vLGN were large, ~25 -60°, On, Off or On-Off and lacked the center-surround structure characteristic of the dLGN. Further, the shapes of the synaptic currents recorded during visual stimulation in the ventral vs. dorsal divisions were different. Large, unitary retinogeniculate EPSCs were prominent in records from relay cells in the dLGN[7]. On the contrary, it was often difficult or impossible to resolve single stereotyped events in records from the vLGN. There were commensurate anatomical differences between relay cells in the two divisions as well. Dendrites of relay cells in the vLGN were sparse but long, spanning one or both axes of the nucleus. Dendritic arbors of cells in the dLGN are denser and spatially compact[8]. Thus, it is possible that the large receptive fields in the vLGN are built by small contributions from many inputs.
References: 1) Harrington. (1997) *Neuro Bio Rev.* **21,** p705; 2) Hattar et al. (2006) *JCN.* **497,** p326; 3) Rivlin-Etzion et al. (2011) *J. Neurosci.* **31,** p8760; 4) Spear et al. (1977) *J. Neurophys.* **40,** p390; 5) Sumitomo et al. (1979) *Exp. Neurol.* **66,** p721; 6) Piscopo et al. (2013) *J. Neurosci.* **33,** p4642; 7) Wang et al. (2011) *Nat. Neurosci.* **14,** p224; 8) Krahe et al. (2011) *J. Neurosci.* **31,** p17437.

Disclosures: U.M. Ciftcioglu: None. **V. Suresh:** None. **B.M. Lala:** None. **W.A. Smith:** None. **F.T. Sommer:** None. **J.A. Hirsch:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Program#/Poster#: 817.19/EE25

Topic: D.04. Vision

Support: Grant-in-Aid for Young Scientists （KAKENHI)(B)

Title: The effect of the electrical stimulation of optic nerve on rat brain

Authors: *K. NISHIDA[1], H. SAKAGUCHI[1], M. KAMEI[1], H. SAWAI[2], T. MIYOSHI[2], R. KAWASHIMA[1], T. FUJIKADO[1], K. NISHIDA[1];
[1]Ophthalmology, Osaka Univ. Grad. Sch. of Med., Suita/Japan, Japan; [2]Integrative Physiol., Osaka Univ. Grad. Sch. of Med. & Fronteir Biosci., Suita, Japan

Abstract: Purpose: To determine the effect of the electrical stimulation of optic nerve on brain. Methods: The AV-DONE electrode (Artificial Vision by Direct Optic Nerve Electrode) was implanted in a 40-week-old Royal College of Surgeon's rats'optic nerve. Electrical stimulations were applied for one hour every week. The reference electrode was located on rat's paw at each session. ERG (Electroretinography), VEPs (Visual Evoked Potentials), and EEPs (Electrical Evoked potential) were measured after one month of implantation. In addition, immunohistologic studies of rat's brain were also performed after the final session. Results: Although ERGs and VEPs were non-recordable, but the EEPs were measured. The implicit times of the EEPs were 3.6 ± 0.5 ms with the amplitudes of 18.2 ±5.5 μV. We found that the astrocytes in colliculus superior were widely activated. Conclusions: AV-DONE could elicit the EEPs in RCS rats without light perception. Optic nerve electrical stimulation seems to activate the astrocytes in colliculus superior.

Disclosures: K. Nishida: None. **H. Sakaguchi:** None. **M. Kamei:** None. **H. Sawai:** None. **T. Miyoshi:** None. **R. Kawashima:** None. **T. Fujikado:** None. **K. Nishida:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Topic: D.04. Vision

Support: NIH Grant EY020950

NIH Grant EY023060

HHMI International Student Fellowship

Title: Looming-evoked responses in the mouse superior colliculus and the influence of corticotectal pathway

Authors: *X. ZHAO, M. LIU, J. CANG;
Northwestern Univ., Evanston, IL

Abstract: The superior colliculus (SC) is a conserved computation center for visually-guided behaviors in vertebrates. In order to understand how visual information is processed in the SC, we studied the response properties of mouse superficial SC (sSC) cells to looming stimulus, which has been shown to effectively evoke innate defensive behavior in this species. Extracellular recordings were made in sSC in both awake and urethane-anesthetized mice. Under both conditions, sSC cells displayed fast and robust responses to looming stimuli. Most cells preferred fast looming speed within the tested range (5-160°/s), with this trend slightly more prominent in awake mice. In contrast, cells in the primary visual cortex showed relatively slow and poorly tuned responses to the looming stimuli, which perhaps reflects distinct behavioral functions of the two visual centers. sSC receives both feedforward retinal input and top-down cortical input through the corticotectal pathway. We thus sought to reveal the influence of the cortical input on the sSC by optogenetically silencing the visual cortex. Silencing cortex did not alter sSC responses in anesthetized mice, but reduced the sSC's response magnitude at every stimulus speed by almost half in awake mice. Speed tuning and response time course, however, were not altered by silencing cortex. Furthermore, the regulation of sSC responses by the cortical input is organized retinotopically. Visual cortex may thus modulate SC-mediated visual behaviors by regulating the response gain in the sSC. Finally, we performed intracellular recording *in vivo* to investigate the cellular and network computations underlying looming-evoked responses in the sSC.

Disclosures: X. Zhao: None. **M. Liu:** None. **J. Cang:** None.

Poster

817. Subcortical Visual Pathways: Rodents

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Program#/Poster#: 817.21/EE27

Topic: D.04. Vision

Support: NIH

Title: Organization of neuronal subtypes and retinal inputs in the mammalian superior colliculus

Authors: *D. L. ROUSSO;
Harvard Univ., Cambridge, MA

Abstract: In the retina, ~30 retinal ganglion cell (RGC) subtypes analyze discrete visual features, such as contrast, color, and movement. They all send axons to the brain for further processing; the main retinorecipient structure in the brain of most mammals is the superior colliculus (SC). RGC axons innervate the SC with spatial retinotopy, such that neighboring RGCs terminate in close proximity within the SC. An orderly map of the visual world is thus transferred to the SC surface. Within this map, RGCs of different subtypes are restricted to distinct SC layers. Less is known, however, about the organization of SC neuron (SCN) subtypes comprising retinocollicular circuits; such information is required to learn how these circuits form and function. Here I establish molecular and genetic tools to address these questions. First, I developed a panel of markers that distinguish SCNs restricted to specific layers. Conventional histology distinguishes 7 layers in the rodent SC. I identified combinations of molecular markers (including calcium-binding proteins, transcription factors and neuropeptides) that label cells restricted to each of the 7, and in some cases substrata within these layers. Some of the identified cells correspond to previously characterized SCN types, while others are novel. Next, I examined the spatial arrangement of SCNs within layers. In deep layers, which are multisensory, markers identify periodically arranged vertical clusters of SCNs that may correspond to previously characterized “honeycombs” (Chevalier and Mana, J. Comp. Neurol, 2000). In the superficial layers, which are exclusively visual, I found that cell bodies of at least one SCN type have a 3-dimensional lattice-like arrangement, with the distance between neighboring cells being highly non-random. This arrangement is reminiscent of retinal mosaics, which are a widely accepted criterion for defining a retinal neuron subtype. Such patterning has not previously been described in parts of the brain outside the retina. Finally, I asked how terminal arbors arising from specific RGC subtypes are arranged with respect to SC layers and columns. I confirm previous studies showing distinct laminar terminations of several subtypes (Hong et al., J. Comp. Neurol. 2011). Additionally, I show that the terminal fields of functionally related RGC subtypes are distributed heterogeneously within molecularly defined layers and appear to be vertically in register with the multisensory columns in the deep layers. Taken together, these data provide an anatomical framework for analyzing the development and function of visual circuits in the SC. (Supported by NIH.)

Disclosures: D.L. Rousso: None.

Poster

818. Striate Cortex: High-Level Factors

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 818.01/EE28

Topic: D.04. Vision

Support: NSF GRFP

Title: V1's fast network time constant predicts features of its frequency response

Authors: *K. REINHOLD[1], M. SCANZIANI[2];
[1]Neurosciences, Univ. of California At San Diego, San Diego, CA; [2]UCSD, San Diego, CA

Abstract: A trade-off exists, fundamentally, between appropriate temporal filters for integrative processes and temporal filters for fast following. The second type may better characterize neural circuits required to reproduce input timing with high fidelity, for instance, in primary sensory brain areas. Primary visual cortex (V1), for example, shuts off very rapidly following the optogenetic removal of thalamic input, implying a fast temporal filter for fast dynamics. We call this property of the V1 circuit a fast "network time constant" (NTC), in analogy to linear systems. In this poster, we use electrophysiology and optogenetics in mice to investigate the relationship between the fast NTC of V1 and the ability of this region to follow high-frequency input, both at the level of the network in terms of multi-unit activity and at the level of isolated single units across cortical layers. We compare the frequency response profiles of dorsal lateral geniculate nucleus (dLGN), the main thalamic input to V1, and V1 itself. We suggest that the NTC of V1 predicts features of the frequency transformation between thalamus and cortex, and we hypothesize that V1's fast temporal filter may help V1 to relate the precise timing of visual inputs to ongoing cognitive processes and brain state.

Disclosures: K. Reinhold: None. **M. Scanziani:** None.

Poster

818. Striate Cortex: High-Level Factors

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Topic: D.04. Vision

Support: NIH 5T32NS007220-32

Title: Contextual modulation in mouse primary visual cortex

Authors: *M. S. CAUDILL, M. SCANZIANI;
CNCB, Univ. California San Diego, San Diego, CA

Abstract: Context is everything. The perception of sensory information depends on context: a car horn in a cacophony of street sounds, an aroma in a stew, or a colorful painting on a drab wall. In the visual system, neural responses, like our perceptions, are also modulated by context. In cats and monkeys, numerous studies over the last 50 years have demonstrated that the activity of pyramidal cells in the primary visual cortex (V1) in response to a visual stimulus depends on the context within which the stimulus is presented. For example, the response of a stimulus confined within the receptive field (RF) can be enhanced or suppressed if simultaneously presented with a 'context' stimulus falling outside the RF. Here using in-vivo imaging of GCAMP6, we show that layer 2/3 pyramidal cell responses in the visual cortex of the mouse to a grating (alternating light and dark bars) confined to the RF are suppressed when surrounded by a context grating of the same orientation and enhanced when surrounded by a context grating of the orthogonal orientation. Thus pyramidal cell responses to a visual stimulus in V1 of the mouse, like cats and monkeys, depend on the context in which the stimulus is presented.

Disclosures: M.S. Caudill: None. **M. Scanziani:** None.

Poster

818. Striate Cortex: High-Level Factors

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DFG Exec 307

Title: Learning improves robustness of spiking activity in mouse primary visual cortex

Authors: *O. JURJUT, S. ERISKEN, A. VAICELIUNAITE, L. BUSSE, S. KATZNER;
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Abstract: In visual cortex, robustness of stimulus-driven activity is thought to depend on the behavioral relevance of sensory information. Selective attention, for instance, is known to reduce trial-to-trial variability and shared fluctuations in neuronal pairs (Cohen & Maunsell, 2009; Mitchell et al., 2009). The behavioral relevance of any stimulus is typically learned over time. Does learning progress affect robustness of neuronal activity in mouse primary visual cortex (V1)? In head-fixed mice, we used a classical conditioning paradigm, in which the presentation of one of two orthogonal grating orientations was immediately followed by a fluid reward. We measured licks occurring before reward delivery and assessed learning progress by comparing lick rates during stimulus presentation to baseline lick rates before stimulus onset. In individual animals (n=7), quantitative analysis of behavioral performance revealed step-like changes in learning progress. First, lick rates were unaffected by the presentation of either orientation ('naïve stage'). Second, an unspecific conditioned response appeared abruptly, with lick rates higher than baseline for both grating orientations. Finally, the conditioned response became specific, such that increases in lick rates became strongest for the rewarded orientation ('well-trained stage'). To examine if learning progress affected robustness of neuronal responses, we used multi-contact silicon probes recording simultaneously from multiple single units. First, for individual neurons (n=503), we analyzed trial-to-trial variability in spiking responses (Fano factor). We found that, during stimulus presentation, Fano factors were smaller for well-trained compared to naïve learning stages. Next, we analyzed population activity by computing, in 200-ms time windows, shared variability in spike counts between pairs of neurons to repeated presentations of the same stimulus (noise correlations). During spontaneous activity measured before stimulus onset, noise correlations did not differ between naïve and well-trained stages. During stimulus presentation, however, noise correlations were strongly reduced in well-trained compared to naïve stages. We conclude that progress in orientation discrimination across distinct learning stages is associated with an increase in robustness of sensory representations already at the level of V1.

Disclosures: O. Jurjut: None. **L. Busse:** None. **S. Erisken:** None. **S. Katzner:** None. **A. Vaiceliunaite:** None.

Poster

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Topic: D.04. Vision

Support: ERC Grant 281886 Percept

DFG Exec 307

Title: Reward-related modulation of sensory responses in mouse primary visual cortex

Authors: *A. WAL, O. JURJUT, L. BUSSE, S. KATZNER;
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Abstract: A large body of literature has documented effects of attention throughout the visual system, including primary visual cortex (V1). In contrast, few studies have investigated how V1 activity is shaped by other top-down influences, such as reward expectancy (e.g., Stanisor, 2013). We asked whether reward expectancy can affect spiking responses of single neurons in mouse V1. Using head-fixed mice on a spherical treadmill, we presented stimuli that provided identical sensory stimulation, but differed in reward contingencies. We used a single drifting grating, which was presented either behind a square or diamond aperture. Only one of these two stimuli could be used to earn a fluid reward. Mice started a trial by moving forward on the treadmill, which triggered the presentation of a randomly selected stimulus. In case of the rewarded stimulus, mice could earn a fluid reward by continuing to run for an additional 3 s. At any point in time, mice could abort a trial by stopping and thereby immediately request a new trial. After the mice had reached a stable level of performance, we used multi-contact silicon probes to record extracellular activity from multiple V1 neurons during task performance. Analyses of running behavior showed robust effects of reward. For stimuli associated with reward, mice ran until reward delivery in most of the trials yielding low abortion rates (19 %, average across 5 sessions). Complementary, for unrewarded stimuli, average trial abortion rates were high (68 %). In addition to the effects on behavior, reward expectancy modulated firing rates of individual V1 neurons. During a time window of 1.5 s after stimulus onset, firing rates were higher for rewarded than for unrewarded stimuli. Across the population of recorded neurons, firing rates increased by about 13%. These reward-related modulations of firing rates could not be caused by an unequal sensory drive provided by the different grating shapes, as sensory control measurements revealed largely similar firing rates. We conclude that, in mice, expectation of reward can enhance firing rates already at the level of V1.

Disclosures: A. Wal: None. **L. Busse:** None. **S. Katzner:** None. **O. Jurjut:** None.

Poster

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DFG Exec 307

Title: Temporal expectancy modulates gamma oscillations in mouse primary visual cortex

Authors: *S. VISWANATH, N. A. DEL GROSSO, O. JURJUT, L. BUSSE, S. KATZNER; Ctr. for Integrative Neurosci., Tuebingen, Germany

Abstract: A large body of behavioral literature has documented that rodents can form precise representations of the temporal statistics of events (e.g. Gallistel & Gibbon, 2000). In contrast, only few studies (e.g. Shuler & Bear, 2006; Lima et al., 2011) have investigated how temporal expectancy can influence neural responses in early sensory areas, such as primary visual cortex (V1). We asked whether temporal expectancy could affect network activity in mouse V1. To manipulate temporal expectancy, we relied on a classical trace conditioning paradigm. On a given trial, we presented either of two gratings drifting in orthogonal directions for a duration of 1.5 s. One of the gratings signaled a short delay (1 s), the other one a long delay (3 s) until the delivery of a fluid reward. We measured licks occurring before reward delivery, and quantified temporal expectancy by comparing lick rates during the delay period to baseline lick rate before grating onset. After a few weeks of training, the animals learned to use the temporal information provided by the stimuli. Anticipatory lick rates typically increased during the delay periods relative to baseline, and the latency of this increase was smaller for short-delay compared to long-delay conditions. Once the animals reached a stable level of behavioral performance, we recorded extracellular activity with multi-contact silicon probes spanning all layers of V1. To examine whether temporal expectancy affects network activity in mouse V1, we focused on the local field potential and analyzed oscillations in the gamma frequency range (40-90 Hz). During the 1-s delay after stimulus offset, average gamma power was higher in short-delay compared to long delay-conditions. This increase in gamma power was comparable across recording sites, suggesting a global, unspecific modulation throughout the cortical column. In a control analysis, we performed the same comparison for an identical window before stimulus onset, which yielded no difference in power. Together, our findings indicate that V1 network activity, in the absence of a visual stimulus, can reflect the timing of reward. We conclude that, in mice, classical trace

conditioning paradigms can be used to manipulate temporal expectancy, and that expectancy-driven modulation of local field potentials is present already at the level of V1.

Disclosures: S. Viswanath: None. **N.A. Del Grosso:** None. **O. Jurjut:** None. **L. Busse:** None. **S. Katzner:** None.

Poster

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Title: Factors determining narrowband gamma oscillation in mouse visual cortex

Authors: *M. KRUMIN, A. B. SALEEM, K. D. HARRIS, M. CARANDINI;
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Abstract: Gamma oscillations are found throughout the cortex, and have been suggested to be involved in cortical functions such as visual attention and feature binding. Gamma oscillations are thought to be generated by resonance in inhibitory circuits: recordings *in vitro* and computational models suggest that gamma can occur in response to tonic driving of interneurons even without excitatory transmission (Whittington et al, Nature 1995; Wang & Buzsaki, J Neurosci, 1996). In visual cortex, in addition to the more commonly described broadband oscillations, a gamma oscillation with a particularly narrow band close to 60 Hz has been reported in humans (Muthukumaraswamy et al, PNAS, 2009) and mice (Niell and Stryker, Neuron 2010). Is this narrowband oscillation an artifact? And if not, what are the factors that determine its strength and frequency? Preliminary experiments measuring local field potentials with glass pipettes in mouse visual cortex revealed a gamma oscillation in a narrow band between 60 and 65 Hz in awake but not anesthetized mice (Haider et al, 2013). Similar gamma oscillations were seen using silicon electrodes in a virtual reality environment. This oscillation was not an artifact of line noise (50 Hz, in the United Kingdom), and did not reflect entrainment of visual cortex by the monitor's refresh rate (60 or 75 Hz). Indeed, visual stimulation with a flickering LED induced cortical oscillation at narrowband gamma frequency at 60-65 Hz regardless of the LED's flicker rate (55 - 85 Hz). The amplitude and frequency of the narrowband gamma oscillation depended on visual stimulation and on the animal's behavior.

First, the oscillation appeared to increase with light level, and was absent in darkness. Second, it increased with an animal's running speed and decreased when animals slowed down and licked a water spout for reward. Power and frequency were highest at the moments of running onset. To test the hypothesis that gamma is generated by resonance in inhibitory networks, we used ramp light stimuli in mice expressing ChR2 in PV+ interneurons. Contrary to expectation, this stimulus suppressed, rather than increased the narrowband gamma oscillation, suggesting that this oscillation is not generated exclusively by this inhibitory network. In fact, laminar analysis indicated that the narrowband gamma oscillation was strongest in layer 4, suggesting that it might originate from thalamic inputs. We conclude that narrowband gamma activity in mouse visual cortex represents a genuine, brain-generated oscillation. We speculate that this oscillation is driven not by networks of inhibitory neurons but rather by coherent rhythmic inputs from thalamus.

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Poster

818. Striate Cortex: High-Level Factors

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Title: Correlates of anticipation and movement in mouse visual cortex

Authors: ***C. P. BURGESS**[1,2], A. RANSON[1], J. F. LINDEN[2], K. D. HARRIS[3], M. CARANDINI[1];
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Abstract: The activity of the sensory cortex is determined not only by afferent sensory stimuli, but also by behavioural context factors such as movement, attention, expectation, and reward. To investigate the impact of these factors, we measured the activity of primary visual cortex (V1) in mice performing a visually guided task. We trained mice to turn a wheel with their forepaws to

indicate the location of visual gratings that could appear to their left or to their right. The mouse initiated trials by holding the wheel stationary for a 2-3 s interval. Mice typically learned the task in 2-3 weeks, producing high-quality psychometric curves parametric on stimulus contrast, with 75% accuracy at contrasts as low as 8%. In the same mice, we injected a virus in V1 to express GCaMP6, so we could perform two-photon calcium imaging of neural populations in superficial layers while the animals performed the task. Calcium imaging revealed a steady build up in trial-averaged activity during the 2-3 s stationary period preceding a new stimulus. This build-up in activity was present in many visually responsive neurons as well as neuropil elements, and reflected an increasing probability of network-wide bursts when examined at a single-trial level. The subsequent appearance of a stimulus in the contralateral hemifield caused a further increase in calcium activity that was typically ~5 times larger than the build-up activity. This further increase was visually-driven, as it depended on stimulus contrast and was not seen with stimuli in the ipsilateral hemifield. Conversely, the build-up activity was rapidly truncated when the animal turned the wheel in response to an ipsilateral stimulus or made premature wheel movements (thus delaying trial initiation). This decline in build-up activity is unlikely to represent a direct suppressive effect of bodily movement on V1 activity. Indeed, in control experiments where mice ran on a treadmill we found that running was positively correlated with fluorescence. These results suggest that the build-up activity is due to stimulus anticipation, perhaps related to increases in alertness or to the allocation of attentional resources. We conclude that activity in superficial layers of V1 are modulated by non-sensory variables, such as expectation of stimuli. We speculate that this modulation reflects activity from higher cortical areas.

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Poster

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Title: Active engagement induces stimulus-specific modulation of population activity in visual and parietal cortex of mice

Authors: *G. N. PHO, M. J. GOARD, B. CRAWFORD, M. SUR;
MIT, Cambridge, MA

Abstract: Two individuals presented with similar sensory input may process the incoming material in very different ways depending on their level of engagement with the presented information. Attentional engagement is thought to improve encoding of sensory inputs, but the underlying neural mechanisms are not well understood. Studies in non-human primates have shown that attention can modulate neural response amplitude and between-cell correlations, with stronger attentional effects in higher cortical regions compared to primary sensory areas. However, the specific circuit mechanisms by which stimulus-selective enhancement is achieved have been difficult to elucidate in primates. Here we investigate the effects of active behavioral engagement using large-scale two-photon calcium imaging in mice trained on a go/no-go visual discrimination task. Population activity from hundreds to thousands of neurons in primary visual cortex (V1) and posterior parietal cortex (PPC) were measured using the genetically encoded calcium indicator GCaMP6s and a novel volumetric imaging approach. Using a retractable lick spout, we alternated blocks of "engaged" behavior with blocks of "passive" viewing of identical stimuli, and compared the neural responses across the two conditions. We find that V1 neurons show subtle but significant modulation with engagement in a stimulus-specific manner, with enhanced responses to target (go) stimuli and suppressed responses to nontarget (no-go) stimuli. Additionally, engagement results in a decrease in between-cell noise correlations for both target and nontarget-selective neurons. In PPC, however, we find a dramatic enhancement of responses to target stimuli during engagement, reminiscent of the enhanced attentional effects observed in higher cortical areas in primates. Lastly, we have used the VGAT-EYFP-ChR2 mouse line (which express ChR2 in all inhibitory neurons), to test the causal role of each region during the task with high spatiotemporal precision. Optogenetic inhibition of either V1 or PPC during the stimulus period of the task completely disrupts performance. Ongoing work will investigate the underlying circuit connectivity that enables behavioral modulation of information flow from lower to higher cortex.

Disclosures: G.N. Pho: None. **M.J. Goard:** None. **B. Crawford:** None. **M. Sur:** None.

Poster

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Title: A neural model of kinetic border-ownership

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Abstract: Camouflaged animals that have very similar textures to their surroundings are difficult to detect when stationary. The survival of many animals depends on the detection of predators or prey when they break camouflage. Successful camouflage is characterized by mimicry of color, texture, and reflectance properties of the surrounding environment, and so the visual system often relies on relative motion to perceive a figure moving against its background. Although the processing of motion is classically believed to take place in dorsal brain areas of primates, such as MT, recent evidence indicates that areas V2 and V4 in the ventral stream demonstrate selectivity to kinetically defined shapes and edges, which are characteristic of camouflage-breaking stimuli. We present a model that demonstrates how the primate visual system can perform figure-ground segregation in extreme cases of breaking camouflage based on motion alone. Border-ownership signals arise at kinetic edges in V2 as an emergent property of the model, which explains the salient percept of depth ordering at the boundary of the kinetic shape. Motion sensitivity develops in model V1 through the spatio-temporal correlations in convergent signals from LGN that arrive according to biologically realistic conduction delays. We predict that neurons in V4 and MT sensitive to kinetically defined figures play a crucial role in determining whether the foreground surface accretes, deletes, or produces a shearing motion with respect to the background.

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Title: Task dependent changes in stimulus representations in mouse visual cortex during learning

Authors: *J. POORT[1], A. KHAN[2,1], M. PACHITARIU[3], A. NEMRI[1], I. ORSOLIC[2], J. KRUPIC[1], M. BAUZA[1], G. KELLER[4], M. SAHANI[3], T. MRSIC-FLOGEL[2,1], S. B. HOFER[2,1]; [1]Univ. Col. London, London, United Kingdom; [2]Biozentrum, Univ. of Basel, Basel, Switzerland; [3]Gatsby Computat. Neurosci. Unit, UCL, London, United Kingdom; [4]Friedrich Miescher Inst., Basel, Switzerland

Abstract: Throughout life, animals need to learn the significance of sensory stimuli in their environment. How do cortical representations of such stimuli change as they become behaviourally relevant? We studied this question in mouse primary visual cortex (V1) by using repeated two-photon calcium (GCaMP6) imaging to follow neural populations in head-fixed behaving animals during acquisition of a visually-guided task. Mice were trained to discriminate between two gratings with different orientations presented on the walls of a virtual corridor through which they ran, by selectively licking in response to only one of the gratings to obtain a reward. Within a week, mice learned this Go/No-go task with >90% accuracy. Bilateral optogenetic silencing of V1 in PV-ChR2 mice demonstrated that V1 was required for the visual discrimination task. Increasing light intensity gradually reduced task performance, rendering animals unable to perform the task at the highest intensity, while performance in an odour discrimination task was unaffected. With learning, neural population responses to the rewarded and non-rewarded stimuli became progressively more distinguishable. The time course of this increase in neuronal stimulus discriminability was correlated with the day-to-day improvement in behavioural discrimination in each animal. This learning effect was stimulus and task specific because (i) there was no increase in discriminability of responses to other, irrelevant visual features in the virtual corridor, and (ii) the same cells showed substantially different responses, with an overall decrease in the neural discriminability, when presenting the same stimuli to trained mice when they were anesthetised. Moreover, these learning effects could not be explained by the changes in running behaviour that occurred across learning. Taken together, our results show that as the stimulus acquires behavioural relevance this leads to progressive, task-dependent changes in its representation already at early stages of visual processing.

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Max Planck Institute for Brain Research

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Title: Interhemispheric connections modulate single-unit and assembly activity driven by figure-ground stimuli in early visual areas

Authors: *S. CONDE-OCAZIONEZ[1], T. WUNDERLE[2], T. S. ALTAVINI[3], D. ERIKSSON[2], K. E. SCHMIDT[3];
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Abstract: The activity of neurons in early visual areas can be modulated by stimulation outside their classical receptive field (CRF). In agreement, V1 spiking already reveals correlates of complex visual tasks as, for example, figure-ground segmentation. It is assumed that long-range lateral connections are involved in early figure-ground segmentation and feedback connections in later processes (i.e attention). It remains understudied whether figure processing is reflected also in the coordinated activity of populations of neurons (neuronal assemblies). For studying context-dependent modulation of unit spiking and assembly activity, we recorded multiunit activity and local field potentials (LFPs) from 48 electrodes in parallel in areas 17 and 18 of anesthetized cats (n=9). We stimulated the population with moving high-contrast gratings and natural scenes in two conditions: Either, all CRFs were part of a foreground patch of orthogonal orientation or opposite movement direction to its background, or, of the corresponding homogenous whole-field (WF). In order to modulate lateral connectivity we removed

interhemispheric input of the contralateral visual areas by reversible cooling deactivation. In the population, both response increases and decreases were found when a foreground patch compared to the background WF stimulated CRFs. However, on average, the patch condition evoked 9.8% less firing rates and 29.7% less assembly activations, i.e. events of coincident firing of neuronal groups within a 5 ms window, than the WF grating condition. During natural scene stimulation, decreases for figure contrast (patch) as opposed to WF were even more frequent, reaching 19.2% lower firing rates and 39.8% lower assembly activations. When removing interhemispheric input, patch responses decreased stronger than WF responses for both gratings (14.3% lower firing rates and 49.4% lower assembly activations) and natural scene stimulation (20.1% lower firing rates and 58.7% lower assembly activations). This indicates a stronger supporting role of the intact network for responses to the foreground patch. A phase-locking analysis revealed that, assembly activity was more locked to the low gamma phase during patch than during WF condition. This phase-relationship was strengthened when deactivating the contralateral hemisphere. In conclusion, our findings support the idea of a figure-ground segmentation process reflected in early visual areas not only in firing rates of single cells but also in coordinated responses of neuronal populations, and that this process can be modulated by long-range lateral connections (e.g. via the corpus callosum).

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Poster

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Title: Task-linked ongoing hemodynamics suffices to explain negative BOLD in awake monkey V1

Authors: M. M. B. CARDOSO[1,2], B. LIMA[1,3], *A. DAS[1];
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Abstract: In functional brain imaging (e.g. fMRI) the response to sensory stimulation is characterized by local increases in hemodynamic activity (e.g. increased BOLD signal) in the stimulated brain region. But this increase is often accompanied by significant decreases or 'negative BOLD' in distal locations, typically attributed to decreased local neural activity. While 'negative BOLD' is also used in describing decreases occurring across distinct brain areas, or across hemispheres in motor cortex, here we focus specifically on sensory stimulus-triggered decreases occurring within the same cortical area (Shmuel et al., 2002). We propose that in this 'local' case, negative BOLD may simply reflect phase mismatches between stimulus-evoked and ongoing, i.e. task-related hemodynamic responses, disconnected from changes in local spiking. As an example of local negative BOLD, robust but spatially restricted visual stimulation is reported to evoke, in V1 (primary visual cortex), both positive BOLD in the stimulated location and negative BOLD in distal locations. Further, the negative BOLD is accompanied by a suppression of local neural responses below baseline (Shmuel et al., 2006). We have shown, however, that the V1 imaging response in task-engaged subjects contains a robust task-related signal ('TRS') that entrains to task timing independent of visual input (Sirotin & Das, 2009). Notably, the TRS is not predicted by local spiking or field potentials. The temporal phase of the TRS is different from that of the visually evoked response. We thus wondered whether the negative BOLD within V1, as seen in alert subjects, could be explained by the TRS which, due to its phase difference, appears as a negative-going signal when visually evoked responses are positive. Our aim was to ask the following: 1: When showing spatially localized visual stimuli to alert monkeys performing a periodic fixation task, can we obtain a residual reduction in blood volume in distal locations even after correcting for the TRS? 2: In the same situation, is there a corresponding distal reduction of spiking? We recorded simultaneous multi-unit spiking and intrinsic-signal optical images from alert monkeys performing a periodic fixation task, while we presented visual stimuli ranging systematically in size, location and intensity. We failed to see reliable reduction in neural spiking below baseline at locations distal to the stimulation. We did see distal hemodynamic activity phased negative to stimulus-evoked responses, but that could largely be accounted for by the TRS. We thus propose that the 'local' V1 negative BOLD recorded in alert, task-engaged subjects may largely be a reflection of TRS.

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Poster

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Support: Cognitive Neuroscience of the Korea Ministry of Education, Science, and Technology

Title: Temporal expectation in the primate V1 during visually guided saccades

Authors: *K. KIM, C. LEE;
Dept. of Psychology, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Temporal expectation of upcoming events is known to shorten detection latency (Coull and Nobre, 1998) and enhance sensitivity of stimulus discrimination (Rohenkohl et al., 2012; Cravo et al., 2013). However, cellular mechanisms mediating temporal expectation remain largely unknown. The spike activity of the primate V1 is suppressed before a burst of discharge in response to a visual stimulus used as a saccade target (Lee et al., 2013). The initial suppression occurred before the onset of target presented either in the receptive field (RF) or in the hemifield opposite to RF, suggesting that the suppression of spike activity at the time of target onset reflects temporal expectation of target onset. In the current study, we examined whether the level of spontaneous discharge is modulated in a behavioral paradigm in which the level of expectation of the timing of target was directly controlled. Monkeys were trained to fixate at a central target and then to make a saccadic eye movement toward an eccentric Gabor stimulus. When the fixation target was a square in green, the saccade target appeared after a fixed central fixation of 800ms. When it was a circle in red, the target appeared after a variable duration of central fixation (400-1300ms). These two trial types, ‘high’ and ‘low’ expectation conditions, were randomized within a block. The saccade latency was significantly reduced in the ‘high’ with respect to ‘low’ expectation condition, indicating that the level of expectation was controlled. The V1 activity was simultaneously recorded while the subject performed the task. The size and orientation of the Gabor stimulus matched to the RF of a cell under study, and it was presented at the RF or in the hemifield opposite to the RF. The spontaneous discharge of V1 neurons was suppressed during fixation period in the ‘high’ expectation condition, whereas it was not in the ‘low’ expectation condition. This was true for both saccade targets at the RF and contralateral to the RF. Thus, temporal expectation made the state of V1 different at the time of saccade target onset. These results are consistent with the previous studies that the level of spontaneous activity of V1 plays roles in modulating perceptual processing of visual information by downstream neurons (Supèr et al., 2003; Lee et al., 2013).

Disclosures: **K. Kim:** None. **C. Lee:** None.

Poster

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Title: Higher visual areas contribute to orientation dependent contextual modulation in mouse primary visual cortex

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Abstract: Neurons in primary visual cortex (V1) of cats and primates are driven by visual input within their receptive fields, but this activity can be modulated by contextual information presented outside their receptive fields. One example of such contextual modulation is orientation tuned surround suppression, in which the response of V1 neurons elicited by an oriented stimulus in the receptive field is suppressed more by a stimulus in the RF-surround with the same orientation than by a surround stimulus with a different orientation. The mouse could be a valuable model to investigate the cortical circuitry behind orientation tuned surround suppression. However, the mouse visual cortex lacks orientation columns that are present in

primate and carnivore primary visual cortex, and the question therefore arises whether mouse V1 is modulated by orientation-context. To address this question we made laminar electrophysiological recordings in awake and anesthetized mice. We found that neurons in mouse V1 are more suppressed by an iso-oriented surround than by a cross-oriented surround. This effect was strongest in layer 4 and the superficial layers. In a first step towards unravelling the circuitry for orientation tuned surround suppression, we investigated the contribution of the superficial layers to surround suppression in layer 4, by silencing the superficial layers through topical lidocaine application to the cortical surface. Interestingly, layer 4 neurons still showed orientation tuned surround suppression, even with strongly reduced neural activity in superficial layers. Second, we tested the involvement of higher visual areas in Gad2Cre mice with cre-dependent ChR2 expression in inhibitory neurons of higher visual areas. Silencing higher visual areas lead to a reduction in the level of orientation-tuned suppression in V1, suggesting that part of this effect is due to feedback from higher areas. Our results demonstrate that the mouse is a useful model for the study of orientation tuned surround suppression, in spite of the absence of orientation columns. Our optogenetic experiments, uniquely feasible in mice, reveal that feedback from higher visual areas to V1 contributes to orientation-tuned surround suppression.

Disclosures: **J.A. Lorteije:** None. **M.W. Self:** None. **J. Vangeneugden:** None. **U. Schnabel:** None. **E. van Beest:** None. **C. Levelt:** None. **J.A. Heimel:** None. **P.R. Roelfsema:** None.

Poster

818. Striate Cortex: High-Level Factors

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Support: IRP NINDS, NIH

IRP NIMH, NIH

Title: Neural responses to naturalistic movies in the common marmoset using electrocorticography

Authors: ***J. DAY-COONEY**[1], C.-C. HUNG[1,2], B. E. RUSS[1], J. CIUCHTA[2], A. C. SILVA[2], D. A. LEOPOLD[1];
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Abstract: The common marmoset (Callithrix Jacchus) is a New World monkey whose visual system, based upon cytoarchitecture and anesthetized electrophysiology, has many ostensible homologous areas to humans and Old World monkeys. However, the functional responses to more complex and naturalistic stimuli of the marmoset visual cortex need to be studied to accurately determine what homologies are present. The use of naturalistic movies is a rich paradigm that promises to provide new perspectives on brain activity. Here, we investigated cortical visual responses to dynamic movies under free viewing conditions in the marmoset. In two animals, we subdurally implanted pairs of 32-channel surface electrocorticographical (ECoG) arrays covering a large, uninterrupted swath of the marmoset's occipitotemporal cortex. Post-op anatomical scans confirmed the locations of the anterior array, covering much of ventral STS and TE, and the posterior array, covering much of the occipital and posterior temporal lobe, including V2, V3, V4, and TEO. Each animal viewed 15 one-minute movies of animals' social interactions while ECoG and eye position signals were recorded. Subjects viewed each movie approximately 20 times across multiple sessions. Electrodes were locally referenced to the average signal of each array and band-pass filtered. We calculated the signal power in beta (10 to 30 Hz) and gamma (50 to 150 Hz) frequency ranges as animals watched the videos. Superimposed upon an overall decrease in the beta range and an increase in the gamma range, there were consistent temporal modulations of the power driven by the events in the movie. Dividing the data for each movie into odd and even runs demonstrated that, across the population, the modulations in the beta band were movie-specific (within-movie r = 0.382 +/- 0.01; between-movie r = 0.015 +/- 0.01). Analyzing the similarity of responses to all movies across different channels revealed that channels covering the earlier visual areas tended correlated with each other, whereas those in higher visual areas were more diverse in their temporal response patterns. Taken together, these findings demonstrate reliable and differentiated ECoG responses along the occipitotemporal pathway of the marmoset, paving the way for future studies using this paradigm.

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Poster

818. Striate Cortex: High-Level Factors

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Title: Stimulus-specific adaptation induced by the oddball paradigm in rat visual cortex

Authors: *K. VINKEN, G. VAN DEN BERGH, R. VOGELS, H. P. OP DE BEECK;
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Abstract: The oddball paradigm is an experimental protocol where two stimuli are presented at different probability in a train of stimulus presentations. This paradigm is often used to elicit a mismatch negativity (MMN) in evoked potentials, which refers to a stronger response to the stimulus that is presented at low probability (deviant) compared to the one that is presented at high probability (standard). Based on recordings in cat auditory cortex it has been suggested that the mechanism behind the MMN is stimulus-specific neural adaptation to the standard rather than a surprise effect in the form of enhanced activity to the deviant (Ulanovsky et al., 2003, Nature Neuroscience). We used the oddball paradigm to investigate whether presentation probability dependent adaptation or a surprise effect can be observed in rat visual cortex. Specifically, we recorded from single neurons (SN) and multi-unit clusters (MU) in the primary visual cortex (V1) as well as the extrastriate latero-intermediate area (LI). Three Long-Evans rats were used: one for V1 recordings only (26 SN and 28 MU), one for LI recordings only (12 SN and 19 MU), and one for both V1 and LI recordings (29 SN and 25 MU in V1; 26 SN and 24 MU in LI). While recording, rats were passively viewing blocks of randomly interleaved trains of 100 stimulus presentations. Each presentation lasted 300 ms with an inter-stimulus interval of the same duration. During the oddball condition, the standard was presented 90 times while the deviant was presented 10 times. An equiprobable reference condition was included where each of 10 stimuli (i.e. the deviant and the standard from the oddball condition as well as 8 other stimuli) was presented 10 times. To summarize the results, we report the median increase or decrease in response strength relative to the reference, averaged across rats. In both areas, we observed clear adaptation to the standard in the SN and the MU responses. Moreover, adaptation in extrastriate area LI was stronger than in V1: -29% for the SN and -44% for the MU responses in V1, versus -68% for the SN and -71% for the MU responses in LI. Evidence for a surprise effect in terms of a stronger response to the deviant in the oddball condition compared to that same stimulus in the reference condition was very inconsistent. There was no evidence in V1, and in LI there was an effect in the MU data (+39%) that was not supported by the SN data. We conclude that the oddball paradigm can be successfully used to investigate stimulus-specific adaptation dependent on frequency of stimulus occurrence both in V1 as well as extrastriate area LI.

Disclosures: K. Vinken: None. **H.P. Op de Beeck:** None. **G. Van den Bergh:** None. **R. Vogels:** None.

Poster

818. Striate Cortex: High-Level Factors

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Support: CNRS

ANR 06-neuro-025-01

Title: Slow adaptation, but not MT feedback inactivation, reduces surround suppression in area V1 of the anesthetized marmoset

Authors: *L. G. NOWAK[1], J.-B. DURAND[1], N. PICARD[2], L. RENAUD[1], P. GIRARD[1]; [1]Cerco Lab. - UMR 5549, TOULOUSE, France; [2]Dept. of Neurol., Boston Children's Hosp., Boston, MA

Abstract: Stimulus size influences the response of a majority of V1 neurons in a nonlinear fashion: when stimulus size increases, the response first increases up to a peak that corresponds to the receptive center, then decreases below this peak due to the presence of surround suppression. Importantly, studies showed that the response as a function of stimulus size (size tuning) depends on stimulus contrast: when contrast decreases, the apparent center size increases while surround suppression decreases. We examined whether contrast-dependent size tuning in area V1 depends on feedback input from area MT. Area V1 receives a direct feedback input from area MT. In addition, MT neurons display high contrast sensitivity. Finally, the divergence of the feedback from MT corresponds well with the dimension of the surround of V1 neurons. These features suggest that MT may contribute to the increase in center size when contrast decreases and/or to the strengthening of the surround when contrast increases. Extracellular recordings were performed in area V1 of the anesthetized marmoset monkey while MT was inactivated by cooling or by GABA injection. Stimuli were drifting gratings with 8 different diameters (0.5 to 15 deg) and 3 contrast levels representing low, medium and high values in the range of effective contrasts for each neuron. These 8×3 stimuli represent 1 block of stimuli. Two control conditions (Cont1 and Cont2), of 7 blocks each, were performed prior to the inactivation condition (Inac) that also consisted of 7 blocks of stimuli. Size tuning curves were computed for each condition and each contrast and were fitted with ratio of Gaussians from which we extracted the following parameters: peak response (PR), center diameter, surround diameter, response for a 15 deg wide grating (P15), and surround suppression index (CSI) calculated as 1-P15/PR. The effects of

contrast on size tuning in the control conditions were comparable to those described in previous studies: when contrast decreased, the center size increased while the CSI decreased. Among the 5 parameters extracted from the size tuning curves, only the CSI showed consistent differences between conditions: in comparison to Cont1, the CSI at medium and high contrast was significantly lower (less suppression) during Inac. However, a comparable difference was already present when comparing Cont2 with Cont1 whereas comparison of Cont2 and Inac revealed no significant difference. We conclude that, 1) in our experimental conditions, the feedback from MT had little influence on size tuning and its contrast dependency in area V1; 2) the mechanism responsible for surround suppression seems to adapt over a time course of several minutes.

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Poster

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Title: Pupil dilation reveals fast switching of cortical states during quiet wakefulness

Authors: *J. REIMER[1], E. FROUDRAKIS[1], C. CADWELL[1], D. YATSENKO[1], G. DENFIELD[1], A. S. TOLIAS[2,3];
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Abstract: Neural responses to external stimuli are strongly modulated by the brain's internal state, which can vary with arousal, attention and behavioral context. In mice, neural responses to visual stimuli are increased and cortical neurons are desynchronized during exploratory behavior compared to periods of quiet wakefulness, but these quiescent periods between bouts of activity have not been well studied. Here, we performed whole-cell patching and calcium imaging in awake mice and focused our analysis on quiet periods between epochs of running and whisking. During these periods, we observed small spontaneous fluctuations in pupil diameter on a timescale of 1-2 seconds, which were linked to cortical state changes in both visual (V1) and somatosensory cortex (S1). Dilating corresponded to a desynchronized and depolarized membrane potential in whole-cell recordings, while constricting was linked to a synchronized state dominated by low-frequency oscillations. In the desynchronized state, the reliability and selectivity of visual responses was increased, while correlated activity across neural ensembles was reduced. Measurements of the pupil have been used for more than 50 years to index alertness and attention in humans and other primates, but this study is the first to link changes in intracellular membrane potential and neural correlations to this phenomenon in any species. Desynchronization and enhanced sensory responses are hallmarks of covert attentional modulation. Our results demonstrate that a similar process may be studied in mice - including mouse models of human disease - and emphasize the utility of the pupil as a proxy for non-invasively monitoring internal cortical states.

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Poster

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Title: A saccadic view of the "silent surround" of visual cortical receptive fields

Authors: F. GERARD-MERCIER[1,2], P. CARELLI[3], *M. PANANCEAU[1], P. BAUDOT[1], X. TRONCOSO[1], Y. FREGNAC[1];
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Abstract: Horizontal connections (HCs) in primary visual cortex (V1) have been hypothesized to underlie long distance modulatory interactions between the discharge field center and the "silent" surround of visual receptive fields (RFs). A distinctive feature of HCs is their speed of information transmission (0.1-0.3 m/s), which matches that of the retinal image shift during saccades. This match raises the possibility that during the oculomotor scanning of natural scenes, the feedforward and horizontal drives may bind in phase, allowing both local feature processing and global motion integration. To test this hypothesis, we carried out intracellular recordings of cat V1 cells (n=48) and systematically explored the "silent" surround of their RFs. This exploration consisted in the presentation of randomized interleaved sequences of 1, 2 or 3 strokes of an oriented Gabor patch (GP) optimized for the subthreshold RF. One-stroke stimuli were static, while two- and three-stroke stimuli induced apparent motion (AM) at saccadic speed. To explore all the possible effects across the visual field, we varied the position, the orientation of the static GPs, the direction of the AM, and the orientation of the GPs relative to the AM axis (either co-aligned or orthogonal). The one-stroke GPs revealed a very large synaptic integration zone, whose anisotropy is dependent on the orientation of the GP. This static association field is dominantly excitatory and extends maximally for iso-oriented GPs along the cell's preferred orientation (main) axis. Two- and three-stroke stimuli revealed that V1 neurons integrate saccadic speed motion across long distances (>10°). This integration can locally be strongly non-linear and is generally strongest for collinear centripetal AM stimuli along the cells' main axis. A small subset of cells showed isotropic AM-sensitive responses across the visual field, as expected from motion flow collectors. These results suggest the involvement of V1 long-range HCs in binding local iso-feature preference and global motion flow in the "silent surround". When the horizontal and feedforward drives are recruited in a precisely time-ordered manner, V1 cells can perform contour integration along their main axis. The functional expression of the "silent surround" is thus modulated both spatially and temporally. We propose the existence of a new kind of adaptive computation in V1, where the ever-changing spatio-temporal properties of the natural visual flow continuously reconfigure the dynamic association field surrounding the classical RF.

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Poster

819. Striate Cortex: Plasticity

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Title: Neuroserpin limits experience-dependent plasticity in adult visual cortex

Authors: *N. BUKHARI[1], P. BURMAN[2], M. DEMARS[3], A. HUSSEIN[3], H. MORISHITA[3]; [1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Icahn SOM at Mount Sinai, New York, NY

Abstract: Reshaping of cortical connections by sensory experience declines with age. Permissive proteolytic activity regulated by a serine protease, tissue plasminogen activator (tPA), becomes equally restricted in adult brain. Understanding the mechanism for this loss of proteolytic activity is, therefore, a key link for improving function in adult brains. Using mouse visual cortex as a model, we identified that an endogenous inhibitor of tPA, called Neuroserpin remains elevated in the adult visual cortex in mark contrast to the juvenile period when neuroserpin reduces in an experience-dependent manner. Strikingly, the removal of this proteolytic brake in the adult unmasked robust experience-dependent cortical plasticity, and synergistic reduction of perineuronal net, a plasticity brake enwrapping parvalbumin interneurons. At the upstream of Neuroserpin, an increase of another plasticity brake Lynx1 in the adult act as a gate to limit the experience-dependent Neuroserpin reduction and subsequent molecular and functional plasticity. These results suggest a novel role of Neuroserpin as a brake for plasticity, and a proteolytic homeostasis as a key target for functional recovery in the adult brain disorders.

Disclosures: **N. Bukhari:** None. **P. Burman:** None. **M. Demars:** None. **A. Hussein:** None. **H. Morishita:** None.

Poster

819. Striate Cortex: Plasticity

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Friedman Brain Institute

MCHDI

Knights Templar Eye Foundation

Title: Regulation of dendritic spine dynamics in adult visual cortex by lynx1

Authors: *M. SAJO[1,2,3], G. C. R. ELLIS-DAVIES[2], H. MORISHITA[1,2,3];
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Abstract: The rate of synapse turnover decreases as a function of age to consolidate synaptic networks. A clinically central issue is that this in turn limits the potential of adult plasticity to recover from brain disorders and injuries. Identification of the specific mechanisms that regulate developmental decline in spine turnover would provide promising targets to unmask the potential of adult plasticity for interventions. We hypothesized that the robust spine turnover in the adult brain is masked by the increased expression of Lynx1, an endogenous brake for nicotinic acetylcholine receptors, recently identified to limit experience-dependent plasticity in the adult visual cortex. To test this, the spine turnover rate in adult visual cortex of Lynx1 knock-out (KO) mice was measured by chronic *in vivo* imaging using two-photon microscopy. Dendrites from layer 2/3 and 5 pyramidal neurons were sparsely labeled by mating Lynx1KO mice with Thy1-GFP M line mice. Cranial windows were chronically implanted over the visual cortex. Dendrites in layer 1 of male mice were subjected to longitudinal fluorescent imaging every four days. We found an overall increase in both gain and loss of spines in the Lynx1KO mice compared to WT mice. This result suggests that the adult cortex does have a potential for robust synapse turnover, but it is effectively masked by Lynx1.

Disclosures: M. Sajo: None. **G.C.R. Ellis-Davies:** None. **H. Morishita:** None.

Poster

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Mindich Child Health and Development Institute (H.M.)

Friedman Brain Institute (H.M.)

Title: Nicotinic modulator Lypd6 regulates plasticity in adult visual cortex

Authors: *M. DEMARS[1], P. BURMAN[1], C. GALLEGO[1], A. ZIMMER[5], H. MORISHITA[1,2,3,4];
[1]Psychiatry, [2]Freidman Brain Inst., [3]Ophthalmology, [4]Mindich Child Hlth. and Develop. Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Univ. of Bonn, Bonn, Germany

Abstract: The ability of the neocortex to adapt based on experience greatly declines after a developmental phase known as the critical period. Recent findings revealed that Lynx1, a protein that inhibits signaling through nicotinic acetylcholine receptors (nAChR), is a brake, limiting visual plasticity following the critical period via increased expression. However, the role of other Lynx family members is unknown. Another member of the Lynx family, Lypd6, has been shown potentiate calcium currents through nAChRs Here, we seek to elucidate the role of Lypd6 in visual plasticity. Using mouse visual cortex (V1) as a model, genetic and pharmacologic manipulations were combined with biochemical and functional analysis *in vivo*. We show that Lypd6 expression declines across critical period in visual cortex and is predominantly localized to lower layer somatostatin interneurons. Further, transgenic over-expression of Lypd6 leads to a persistence of visual plasticity in adult mice. Interestingly, the transgenic mice have increased somatostatin-parvalbumin connectivity potentially underlying the phenotype. These results suggest a novel role of Lypd6 in somatostatin interneurons as a positive regulator of cortical plasticity.

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Poster

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Title: Deficits of synaptic plasticity in visual cortex of NARP -/- mice

Authors: *C. L. LANTZ, E. M. QUINLAN;
Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: NARP (neuronal activity regulated pentraxin) is an AMPAR-binding protein that is enriched at excitatory synapses onto fast-spiking parvalbumin positive interneurons (FS (PV) IN). NARP-/- mice have reduced excitatory drive onto FS (PV) INs, and deficits in several types of homeostatic and Hebbian plasticity, as seen in both hippocampus and visual cortex. Here I characterize several additional properties of the NARP-/- visual system. Electroretinograms (ERG) from NARP -/- mice reveal subtle deficits in retinal responses, with increased A-wave amplitudes in response to high luminance stimulation (269 lux; NARP = 69.5 μV ± 13.8, WT = 48.2 μV ± 7.6), increased duration of the ERG and an increased failure rate at high (269 lux, NARP = 6.8% ± 3.5, WT = 0%) intermediate (86 lux, NARP = 25% ± 7.5, WT = 2.3% ± 2.2) and low luminance (32 lux, NARP = 36% ± 6.2, WT = 6.8% ± 3). Despite the mild deficit in retinal processing; visual processing and stimulus selectivity in the primary visual cortex appear normal, with single unit responses retaining normal orientation selectivity (OSI; WT = 0.48±0.27, NP = 0.53 ± 0.22) and orientation tuning (curve half width; NARP=11.04° ± 0.39, WT = 11.97° ± 0.35). Using vertical microelectrode arrays spanning the depth of the cortex in awake-head fixed subjects, I examined the expression of two robust forms of synaptic plasticity in the primary visual cortex of NARP-/- mice: stimulus-selective response potentiation (SRP) observed after repetitive visual stimulation and the shift in ocular dominance in response to monocular deprivation (MD). 4 hours and 24 hours after repetitive visual stimulation NARP -/- mice show no increase in visually evoked spike frequency or the amplitude of the visually evoked potentials (VEP) in any cortical layer. Similarly, 5 days of MD does not reduce the contralateral bias of VEPs (CBI range: 0.3 - 0.08 before MD, 0.2 - 0.01, after 5 days), nor does it shift the distribution of single unit ocular preference in any cortical layer (CBI range: 0.35 to -0.1 before MD, 0.29 to -0.02 after 5 days of MD). Paradoxically, following repetitive visual stimulation, we see a transient shift in the orientation preference of the majority of single units away from the repeated orientation (56% ± 15 units), indicating the persistence of orientation tuning plasticity in NARP -/- mice. Together this demonstrates that deficits in NARP-dependent recruitment of inhibition induce widespread deficits in synaptic plasticity in the visual cortex, without impairing the development or plasticity of stimulus selectivity.

Disclosures: C.L. Lantz: None. **E.M. Quinlan:** None.

Poster

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Title: Rescue of ocular dominance plasticity in NARP -/- mice by NRG1

Authors: *Y. GU[1], E. QUINLAN[2];
[1]Biol., [2]NACS, Univ. of Maryland, College Park, MD

Abstract: NARP (neuronal activity-regulated pentraxin) is an immediate early gene which is highly expressed at excitatory synapses onto fast-spiking interneurons. We have previously shown that genetic deletion of NARP decreased the excitatory drive onto FS INs, and retained the visual cortex in a hyper-excitable state that does not express ocular dominance plasticity (Gu et al., 2013). To rescue ocular dominance plasticity in NARP -/- mice, we systemically delivered neuregulin 1 (NRG1; 10ng/kg, i.p., 2X/day, 3 days), a neurotrophic factor shown to enhance excitation onto FS INs. NRG1 treatment increased evoked firing rates of fast-spiking neurons (NARP -/- 5.57±0.16, NARP -/- + NRG1 6.43±0.30 spikes/s) and decreases evoked firing rates of regular-spiking neurons (NARP -/- 4.34±0.27, NARP -/- + NRG1 3.09±0.20 spikes/s). 3 days of monocular deprivation, which is ineffective in NARP -/- mice, induces a robust shift in ocular dominance when administered concurrently with NRG1 ($H(2)=18.38$, $p<0.001$, Kruskal-Wallis test). To ask if NRG pathway can be harnessed to promote ocular dominance plasticity in the hypo-excitable adult visual cortex, we systemically delivered an inhibitor of the NRG1 receptor (ErbB4; PD 198780; 10mg/kg, i.p., 2X/day, 3 days). ErbB4 inhibition decreased evoked firing rates of fast-spiking neurons (WT 7.52±0.34, WT + PD 158780 6.05±0.13 spikes/s) and increased evoked firing rates of regular-spiking neurons (WT 2.38±0.20, WT + PD 158780 3.65±0.11 spikes/s). 3 days of monocular deprivation, which is ineffective in adult WT mice, induced a robust shift in ocular dominance when administered concurrently with the ErbB4 inhibitor ($H(2)=15.24$, $p<0.001$, Kruskal-Wallis test). Importantly, the ocular dominance plasticity that is reactivated in the adult visual cortex promotes the recovery from chronic monocular deprivation. cMD (from P14 to P90) induces severe amblyopia that does not recover spontaneously following reverse deprivation (5 days). However, reverse deprivation concurrent with inhibition of ErbB4 resulted in a significant recovery of ocular dominance ($H(2)=15.37$, $p<0.001$, Kruskal-Wallis test). Together these results suggest that manipulation of the NRG1-ErbB4 pathway can maintain excitability in the visual cortex in the optimal range for the expression of ocular dominance plasticity.

Disclosures: Y. Gu: None. **E. Quinlan:** None.

Poster

819. Striate Cortex: Plasticity

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Title: Visual processing in human striate cortex as a result of congenital deafness

Authors: *A. K. VILLWOCK, D. BOTTARI, B. RÖDER;
Biol. Psychology and Neuropsychology, Univ. of Hamburg, Hamburg, Germany

Abstract: Event-related potential (ERP) research on the impact of congenital human deafness on visual processing has shown consistent signs of intramodal plasticity as early as 110 ms after the onset of a visual stimulus (in the P1). This result suggests that within the visual system extrastriate cortices show evidence of neural plasticity as a result of auditory deprivation from birth. In the present study we investigated visual processing at the level of the striate cortex (V1) in a group of congenital deaf adults and matched hearing controls. To this aim we investigated the C1 response - a negative wave peaking in the ERP between 80 and 90 ms from stimulus onset - which is assumed to represent an electrophysiological marker of striate cortex activity. Horizontally oriented Gabor patterns were presented in the upper and lower visual field with respect to fixation. Participants were asked to detect an infrequent (p=0.2) Gabor pattern (target) characterized by having a vertical orientation. The EEG was recorded from 73 scalp electrodes (referenced to the nose) using the 10-20 System montage. We analyzed the non-target stimuli (horizontal Gabor patterns) which were presented in the lower visual field. ERPs to these stimuli allowed us to clearly separate the C1 and P1. The deaf group and the control group did neither significantly differ with respect to C1 latency nor in peak amplitude. These results suggest that the timing of bottom up processing in striate cortex as well as the involved neural representations do not seem to change as a result of auditory deprivation from birth.

Disclosures: A.K. Villwock: None. **D. Bottari:** None. **B. Röder:** None.

Poster

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MEXT

NIMH Silvio Conte Center 1P50MH094271

Title: Parvalbumin cell-specific transcriptional networks underlying epigenetic induction of adult cortical plasticity

Authors: *Y. KOBAYASHI[1,2], Y. NORO[3], N. K. LEE[1], A. E. TAKESIAN[2], M. SALIMULLAH[3], T. KUJI[4], A. ASAKA-OBA[4], M. NAKAMURA[1,4], C. PLESSY[3], M. FAGIOLINI[2,4], P. CARNINCI[3], T. K. HENSCH[1,2,4];
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Abstract: Experience-dependent brain plasticity is typically limited to specific time windows, or 'critical periods' in early postnatal life. Both in humans and rodents, inhibiting histone deacetylases (HDACs) can reopen such plasticity in adulthood, but the molecular mechanisms remain unknown. We explored genome-wide dynamics of transcription in two defined cortical cell-types - excitatory pyramidal or inhibitory parvalbumin (PV)-positive neurons - upon treatment with HDAC inhibitors. Mice expressing fluorescent proteins in either pyramidal (Thy1-YFP) or PV-cells (PV-GFP) were treated with either Trichostatin A (TSA) or Valproic acid (VPA) for 2 hours or 2 days to parallel plasticity induction. Intrinsic firing properties of PV-cells were rapidly moderated at 2 hours and their mIPSCs enhanced for the duration of HDAC inhibitor treatment. Transcription start site usage was analyzed by nano-Cap Analysis of Gene Expression (nanoCAGE) with next generation sequencing after FACS. Modeling expression dynamics using predicted cis-regulatory sites (Motif Activity Response Analysis; MARA) revealed different transcriptional networks across conditions in the two neuronal types. Notably, a Sp1 motif was identified as consistently up-regulated within PV-cells at all timepoints by both HDAC inhibitors. Co-administration of the Sp1 inhibitor, Mithramycin A, abolished the ectopic loss of visual acuity in response to monocular deprivation induced in adulthood by VPA. These data indicate that Sp1 activation in PV-cells plays a pivotal role in reopening plasticity induced by HDAC inhibition.

Disclosures: **Y. Kobayashi:** None. **Y. Noro:** None. **N.K. Lee:** None. **A.E. Takesian:** None. **M. Salimullah:** None. **T.K. Hensch:** None. **T. Kuji:** None. **A. Asaka-Oba:** None. **M. Nakamura:** None. **C. Plessy:** None. **M. Fagiolini:** None. **P. Carninci:** None.

Poster

819. Striate Cortex: Plasticity

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R01AG034606

R01 EY014882

Title: Associative Hebbian synaptic plasticity in primate visual cortex

Authors: *S. HUANG[1], C. ROZAS[1], M. TREVINO[1], J. CONTRERAS[1], S. YANG[1], L. SONG[1], H.-K. LEE[1,2], A. KIRKWOOD[1,2];
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Abstract: In primates, the functional connectivity of adult primary visual cortex is susceptible to be modified by sensory training during perceptual learning. It is widely held that this type of neural plasticity might involve mechanisms like long-term potentiation (LTP) and long-term depression (LTD). NMDAR-dependent forms of LTP and LTD are particularly attractive because in rodents they can be induced in a Hebbian manner by near coincidental presynaptic and postsynaptic firing, in a paradigm termed spike timing-dependent plasticity (STDP). These fundamental properties of LTP and LTD, Hebbian induction and NMDAR dependence, have not been examined in primate cortex. Here we demonstrate these properties in the primary visual cortex of the rhesus macaque (*Macaca mulatta*), and also show that, like in rodents, STDP is gated by neuromodulators. These findings indicate that the cellular principles governing cortical plasticity are conserved across mammalian species, further validating the use of rodents as a model system.

Disclosures: S. Huang: None. **C. Rozas:** None. **M. Trevino:** None. **J. Contreras:** None. **S. Yang:** None. **L. Song:** None. **H. Lee:** None. **A. Kirkwood:** None.

Poster

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Support: NSF BCS-1028584

Title: Four days of visual contrast deprivation reveals limits on neuronal adaptation

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Abstract: Neurons in sensory cortex are highly adaptable, altering their function depending upon recent input. But how do they adjust to more permanent changes in the environment? Adaptation to altered visual contrast is probably the best understood form of sensory adaptation, but prior studies have only lasted tens of minutes. We measured perceptual effects of cortical adaptation in seven human subjects who viewed a world lacking vertical information for four days continuously. Subjects wore a video camera, and viewed its feed on a head-mounted display (HMD) after filtering in real-time to remove 85% of vertical contrast. Subjects performed many everyday activities while wearing the HMD, and were blindfolded nights and during rest breaks. Adaptation was measured twice daily with two tasks: a contrast appearance task, where observers adjusted the contrast of a horizontal pattern to match the apparent contrast of a weak vertical one (5% contrast), and an orientation judgment in which subjects adjusted the relative tilts of two 45 deg diagonal patterns to make their intersections appear square. As expected, visual adaptation strengthened during the first day. Apparent contrast of the 5% vertical grating increased to over 8%. Orientations that appeared 45 deg shifted to about 47 deg, a version of the tilt aftereffect (TAE). Both measures of adaptation, however, then showed a surprising drop in strength ($p < 0.05$) the next testing session. Over days 2 through 4, apparent contrast gained strength steadily, while the orientation measure rose, but then declined again (both $p < 0.05$). Short-term contrast adaptation alters the gain and tuning of neurons in primary visual cortex (V1). The decline of effects after day 1 indicates that these changes may not endure; neurons likely shifted back toward their initial response properties, even while the adapting environment remained present. The different patterns for the two tasks over the last 3 days is consistent with effects arising in later visual cortex, since changes in V1 would be expected to affect both tasks. The appearance effects are beneficial, as they restore the world to its unfiltered state. The TAE, on the other hand, is an illusion in which the world is perceived non-veridically, due to changes earlier in the visual hierarchy producing errors at later stages (the “coding catastrophe”). Our results suggest that the visual system can reduce this cost of adaptation over time.

Disclosures: **S.A. Engel:** None. **K. Haak:** None. **E. Fast:** None. **M. Bao:** None.

Poster

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Support: CRSNG

FRQ-NT

Title: Plasticity in the visual cortex: Emergence of new orientation maps

Authors: ***L. BACHATENE**[1], V. BHARMAURIA[1], S. CATTAN[1], N. CHANAURIA[1], J. ROUAT[2], S. MOLOTCHNIKOFF[1];
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Abstract: Within the mammalian primary visual cortex, neurons are sensitive to oriented stimuli thus forming cortical domains. This organization rests upon the principle that cells sharing similar orientation-selectivity are co-located based on the segregation of inputs. Such selectivity is believed to be rigid in the adult brain. However, it has been well established that neurons modify their optimal properties following brief or long periods of stimulus exposure (visual adaptation) resulting in a new cortical map (Cattan et al., 2014*). Thus, a key question arises: how a group of non-adapted neurons modifies its selectivity in relation to an adapted group of neurons, i.e. how the entire orientation map is reprogrammed following visual adaptation within a specific circumscribed receptive field. In other words, does an orientation hole appear after adaptation? In the present study we recorded spiking activity of neurons from layer 2/3 of the primary visual cortex in conventionally prepared and anaesthetized adult cats in order to investigate the impact of long stimulus exposure away from the receptive field of recorded neurons. After the determination of the respective orientation-tuning-curves of two groups of cells belonging to two distinct sites, a prolonged visual adaptation was performed locally over one site. No simultaneous stimulation was carried out. The stimuli were presented in isolation alternatively in each site. We provide evidence that an orientation module in the primary visual cortex functionally remaps itself in relation to other columns to maintain the hypercolumn-organization contingent upon the input. Indeed, neurons whose receptive fields

were located remotely from the adapted-site also exhibit new optimal selectivity to orientation. In addition, we show that this reorganization extends up to a distance of 15 deg. between both receptive fields. Our results indicate a robust domino-effect leading to remapping of the cortical orientation domains suggesting that orientation columns transcend anatomy, and are almost strictly functionally dynamic. *S. Cattan, L. Bachatene, V. Bharmauria, J. Jeyabalaratnam, C. Milleret, S. Molotchnikoff, Comparative analysis of orientation-maps in areas 17 and 18 of cat primary visual cortex following adaptation. European Journal of Neuroscience (In press).
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Disclosures: L. Bachatene: None. **V. Bharmauria:** None. **S. Cattan:** None. **N. Chanauria:** None. **J. Rouat:** None. **S. Molotchnikoff:** None.

Poster

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CIHR

Title: Darkness promotes a constant rate of anatomical recovery from different durations of monocular deprivation

Authors: *K. DUFFY, J. C. BOWDRIDGE, D. E. MITCHELL;
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Abstract: Monocular visual deprivation (MD) beginning at the peak of the critical period in kittens causes severe vision impairment in the deprived eye, and pronounced shrinkage of deprived-eye neurons in the lateral geniculate nucleus (dLGN). We recently demonstrated that immersion in complete darkness for 10 days following one week of MD was sufficient to promote rapid and full recovery of visual acuity, as well as a complete recovery of neuron soma size in the dLGN. In the current study we examined the effectiveness of 10 days of darkness to promote anatomical recovery in four groups of animals that were monocular deprived for either 1, 2, 3, or 6 weeks beginning at PND30. The cross-sectional soma size of neurons in the dLGN was measured using the nucleator stereology probe, and all measurements were made blind to

rearing condition. In one of our recovery conditions we also examined the activity of calpain, an intracellular calcium-dependent cysteine protease that has been implicated as a regulator of long-term synaptic potentiation (LTP). Comparison of the soma size of deprived and non-deprived neurons in the dLGN revealed that the extent of neuron atrophy increased with longer durations of MD. There was substantial recovery of neuron soma size in all deprivation durations after 10 days of darkness. Complete and near complete recovery was observed for the 1- and 2-week deprivation conditions, respectively. Though recovery measured for the 3- and 6-week deprivation durations was substantial, it remained incomplete (70% and 60% recovery, respectively). Measurement of the rate of recovery across the four conditions indicated that, despite differences in the magnitude of deprivation effects prior to darkness, the rate of recovery was the same: 2% growth of deprived neurons per day spent in darkness. Measurement of calpain activity in the primary visual cortex of kittens monocularly deprived for 2 weeks that then spent 10 days in darkness revealed elevated μ-calpain relative to control, while there was no difference in m-calpain activity compared with controls. We conclude that 10 days of darkness can produce substantial recovery of dLGN cell size even from a long duration of MD begun at the peak of the critical period. The extent of recovery appears to be limited by the rate of growth, which remained constant irrespective of deprivation effect magnitude. This indicates that longer durations spent in darkness may be required to remediate the larger deficits observed with long durations of visual deprivation. The elevated μ-calpain activity observed in the context of darkness may represent an aspect of the recovery response that promotes reconnection and growth of deprived dLGN neurons.

Disclosures: **K. Duffy:** None. **D.E. Mitchell:** None. **J.C. Bowdridge:** None.

Poster

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Support: PA Department of Health Formula Grant SAP#4100062201

Title: Binocular deprivation produces sustained decrease of visual responsiveness in parvalbumin expressing GABAergic interneurons, in-vivo

Authors: *B. D. FEESE, R. ZHANG, S. J. KUHLMAN;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The goal of this project is to develop a paradigm for the identification of molecular mechanisms that couple visual experience to postnatal maturation of parvalbumin (PV) expressing interneuron response properties. To this end we binocularly deprived (BD) mice during the critical period for 1, 2, or 3 days. We found deprivation significantly decreased PV responsiveness (one-way ANOVA $P<0.001$). Post-hoc analysis revealed that PV response properties reverted back to an immature state in as little as 24 hours ($P<0.05$, control: 23.5 ±2.5 Hz, n=12 cells; 1 day BD: 13.2 ±1.8 Hz, n=22). Notably the decrease in firing rate was sustained (2 day BD: 11.3 ± 1.4 Hz, n=13; 3 day BD: 11.4 ±2.3 Hz, n=7). To measure PV response properties we used 2-photon guided imaging to do targeted cell attached recordings of fluorescently labeled PV interneurons. This sustained decrease in PV responsiveness differs from reports using monocular deprivation in which it was shown that PV neuron firing rate returns to control levels by 3 days (Kuhlman et al. 2013, Hengen et al. 2013). Thus, the presence of open eye input is required for recovery of PV responsiveness during monocular deprivation. The BD-PV recording paradigm will now be used to test molecular mechanisms; for example, we are currently testing the hypothesis that the neuregulin/ErbB4 signaling pathway is a critical signaling mechanism for the maturation of PV interneurons.

Disclosures: B.D. Feese: None. **R. Zhang:** None. **S.J. Kuhlman:** None.

Poster

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Topic: D.04. Vision

Support: NIH Grant AA022455

Title: Concerted action of SRF and CREB on the regulation of visual cortex plasticity

Authors: *N. S. PULIMOOD[1], P. TRINDADE[2], W. S. RODRIGUES[2], A. E. MEDINA[2]; [1]Pediatrics, Univ. of Maryland Baltimore, Baltimore, MD; [2]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: The transcription factors SRF (Serum Response Factor) and CREB (cAMP Response Element Binding factor) play critical roles in neuronal plasticity. SRF and CREB can act alone or in concert by triggering the expression of distinct or overlapping pools of genes. In recent years, SRF and CREB have been implicated in LTD and LTP respectively. LTD and LTP are crucial

for the depression and potentiation components of ocular dominance plasticity (dc-ODP and pc-ODP respectively), however the role of SRF and CREB in these *in vivo* processes is poorly known. Here we hypothesize that SRF and CREB have opposite and complimentary functions in visual cortex plasticity, being crucial for dc-ODP and pc-ODP respectively. We predict that blockade of SRF expression will disrupt dc-ODP but will spare pc-ODP. In contrast, blockade of CREB will disrupt pc-ODP but spare dc-ODP. To elucidate the role of SRF and CREB in depression and potentiation in separate, we infected animals with a Herpes Simplex viral (HSV) vector expressing dominant negative forms of SRF (SRF-DN) or CREB (CREB-DN). We then chronically implanted electrodes in the binocular zone of the mouse visual cortex, to record visually evoked potentials (VEPs) in awake animals before and after 5 days of monocular deprivation. This 5 day period allows us to distinguish the contributions of dc-ODP and pc-ODP to visual cortex plasticity. In a naïve animal, a 5-day monocular deprivation causes visual cortical responses from the deprived eye to depress (dc-ODP) and responses from the open eye to potentiate (pc-ODP). In SRF-DN infected animals, we see that dcODP is reduced dramatically, but pc-ODP is not affected. In CREB-DN infected animals, we see that dc-ODP remains intact, but pc-ODP is affected. This supports the complimentary role of SRF and CREB in the depression and potentiation components of ocular dominance plasticity.

Disclosures: N.S. Pulimood: None. **P. Trindade:** None. **W.S. Rodrigues:** None. **A.E. Medina:** None.

Poster

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FWO Flanders Research Grant

Title: Aberrant structure and functionality of the visual cortex of MMP-3 deficient mice

Authors: *S. VREYSEN, J. AERTS, L. VAN DEN BOER, L. ARCKENS;
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Abstract: Matrix metalloproteinase-3 (MMP-3, stromelysin-1) is a Zn^{2+}-dependent endopeptidase that regulates cell behavior by targeting the extracellular matrix, adhesion molecules, growth factors, receptors and an array of intracellular substrates. It does not only play an important role during the development of the CNS, but also in plasticity and regeneration after CNS injury. Moreover, uncontrolled MMP-3 activity appears to negatively contribute to several neurodegenerative disorders. Using the activity reporter gene *zif268* as a read-out, we recently observed perturbed open-eye potentiation in the visual cortex of adult MMP-3 deficient (MMP-$3^{-/-}$) mice in response to monocular enucleation. To further characterize this phenotype at the functional level we performed extracellular multi-unit electrophysiology in the binocular primary visual cortex (V1b) of MMP-$3^{-/-}$ and wild-type (WT) control mice. Our recordings revealed differences in binocularity. MMP-$3^{-/-}$ mice appear to have more binocular driven neurons in V1b, yet lack ipsilaterally dominated neurons, which may explain the perturbed open-eye potentiation. We also found specific differences in the tuning bandwidth characteristics of spatial frequency and temporal frequency. Visual acuity of MMP-$3^{-/-}$ mice was comparable to that of WT mice. At the structural level, Golgi-Cox stainings revealed an aberrant structural architecture of infragranular pyramidal neurons, including a significant reduction in apical dendrite length and the presence of more mature spines in $MMP3^{-/-}$ mice. WGA-HRP tracer injections in the eye also hint at atypical wiring in the brain of MMP-$3^{-/-}$ mice. Together, this dataset demonstrates that genetic ablation of MMP-3 has a profound impact on the structural and functional integrity of the mouse visual system.

Disclosures: S. Vreysen: None. **J. Aerts:** None. **L. Van Den Boer:** None. **L. Arckens:** None.

Poster

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Topic: D.04. Vision

Support: NIH Grant EY023374

Title: Resetting the interocular inhibitory-and-excitatory balance with a novel video game based on the push-pull perceptual learning protocol

Authors: T. L. OOI[1], *Z. HE[2], Y. R. SU[3];
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Louisville, Louisville, KY; [3]Dept. of Basic Sci., Pennsylvania Col. of Optometry, Salus Univ., Elkins Park, PA

Abstract: Unbalanced inhibitory-and-excitatory cortical interaction between visual signals from the two eyes causes poor binocular vision. For example, an amblyope who exhibits a larger inhibition from the strong eye to the weak eye does not have good stereo depth perception. To correct the interocular imbalance, and thus improve binocular vision, we previously designed a push-pull protocol that employs attention cueing and binocular rivalry display as training stimulation to specifically tap on the interocular inhibitory network. While it effectively reduces the strong eye's inhibition of the weak eye and improves the excitatory network of the weak eye (Xu et al, 2010; Ooi et al, 2013), the stimulus design is unsuitable for observers with strabismus who also have unstable binocular fusion ability. Here, we report a new push-pull stimulus design, in the form of a video game, which obviates the binocular fusional challenge. The video game uses a binocular rivalry training stimulus that has boundary contour only in the weak eye while the strong eye views an image without boundary contour at corresponding retinal areas. Since the boundary contour in the weak eye readily suppresses the strong eye, there is no need for attention cueing. Significantly, the stimulus design reliably causes suppression of the strong eye regardless of the stability of binocular alignment for fusion. We implemented the new video game push-pull protocol on four adult observers in their twenties. S1 had strabismus with VA (visual acuity) of RE=20/25 and LE=20/20 on the logMAR chart. S2 had anisometropia-and-strabismus with VA of RE=20/63 and LE=20/16. S3 and S4 were non-amblyopes with significant interocular imbalance but equal VA in each eye (S3=20/16; S4=20/20). Post-training tests revealed (i) improved strength of the weak eye, as evidenced by an increased predominance ratio of the weak eye to strong eye; (ii) reduced stereo thresholds; and (iii) shortened stereo response time. Furthermore, the amblyopic eye's VA for S1 and S2 improved to 20/20 and 20/50, respectively. These findings lead to the conclusion that the new video game design based on the push-pull principle can successfully recalibrate the inhibitory-and excitatory balance to improve vision. The video game format has the advantage of being (1) top-down attention grabbing, (2) interactive since the observer has to make perceptual, cognitive and motor responses while playing, and (3) fun.

Disclosures: T.L. Ooi: None. **Z. He:** None. **Y.R. Su:** None.

Poster

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Title: Transient gamma power peaks upon monocular deprivation during critical period plasticity

Authors: *R. K. REH[1,2], T. K. HENSCH[1,2];
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Abstract: The EEG gamma power band (30-80 Hz) reflects parvalbumin-positive (PV) interneuron function. Maturation of these particular GABA circuits also initiates the onset timing of cortical critical periods for experience-dependent brain development. Indeed, PV-cells act as "first responders" by rapidly reducing their firing rate and shifting eye preference within hours of monocular deprivation (MD). Here, we explored whether a transient alteration in gamma rhythm would serve as a biomarker of sensory deprivation during critical period plasticity. Electroencephalographic (EEG) recording over primary visual cortex of mice at postnatal day 25-30 directly upon MD revealed a significant peak in spontaneous gamma power at 40 Hz persisting for up to 2 hours in the majority (> 68%) of mice. The peak was triggered by imbalanced visual input, but not observed in non-deprived controls, under complete darkness or when EEG electrodes are placed over somatosensory cortex. Gamma power elevation was present not only in waking, but also during REM sleep bouts. Notably, gamma power induction by MD was drastically diminished (< 27%) in adult (> P60) mice beyond the critical period for plasticity. Instead, in 60% of Lynx1-/- mice, which maintain an open-ended ocular dominance plasticity, the peak in gamma power remained inducible by MD in adulthood. When present, peak gamma frequency in adult mice was slightly but significantly higher (at 44 Hz) than in youth. To establish whether the gamma peak following MD was dependent upon inhibitory circuit function, we tested GAD65-/- mice, bearing constitutively low inhibitory tone and a delayed critical period. These animals failed to show a change in gamma upon MD, which could be rescued (> 54% of mice) with diazepam treatment for 3 days to restore a critical period prior to MD. In several of these mice, the gamma peak persisted significantly longer than in juvenile controls (> 4 hours), perhaps due to the constitutive loss of GAD65. Taken together, our data reveal EEG gamma power to be a robust indicator of the early stages of critical period plasticity. Further investigation will focus on whether the gamma increase contributes to the ocular dominance shift per se.

Disclosures: **R.K. Reh:** None. **T.K. Hensch:** None.

Poster

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Topic: D.04. Vision

Title: Neurons in visual cortex retain a memory of their inputs after monocular deprivation

Authors: *T. ROSE, J. JÄPEL-SCHAEL, M. HÜBENER, T. BONHOEFFER;
Max-Planck-Institute of Neurobio., Martinsried, Germany

Abstract: A classic example of experience-dependent cortical plasticity is the shift in ocular dominance (OD) after monocular deprivation (MD). Up to now, however, changes in cortical responsiveness after MD have largely been studied on the population level, and it therefore has remained open how alterations in tuning and response strength of individual neurons give rise to global OD changes. In particular, it is unclear if population OD shifts are realized by changes in the tuning of individual neurons, or by selective recruitment or silencing of distinct populations of cells. Moreover, it is unknown if and how single cells recover from an OD shift, given that MD is accompanied by the formation of stable spine synapses. We perform chronic two photon imaging of cellular structure and function after viral cotransduction with a genetically encoded Ca2+ indicator together with a bright structural marker (AAV1/2-mRuby2-P2A-GCaMP6s). In adult mice, the OD of the same excitatory L2/3 neurons imaged repeatedly over months is largely stable under baseline conditions (session-to-session Pearson's correlation coefficient r=0.51). The change in OD after MD is achieved by single-cell tuning changes as a result of a variable combination of increased open eye and decreased deprived eye responses. Whereas 34% of the originally deprived eye responsive neurons are silenced after MD, the number of neurons responding to the non-deprived eye remains stable. Even though cellular OD-shifts after MD can be pronounced, the majority of cells faithfully return to their pre-deprivation OD after ~3 weeks of recovery (pre-MD to full recovery correlation r=0.51). Interestingly, when challenged with a second MD episode, predominantly the same cells undergo repeated OD plasticity (1st to 2nd MD correlation r=0.57). Therefore, individual L2/3 pyramidal neurons retain a memory of both their baseline OD and their capacity for experience-dependent plasticity. We currently assess how these reversible, cellular OD shifts relate to functional and structural synaptic changes by chronic dual color imaging of dendritic spines of sparsely transfected neurons.

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Poster

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Title: Role of specific interneuron subtypes in ocular dominance plasticity during the critical period

Authors: *R. RAJENDRAN, M. SAIEPOUR, A. OMRANI, J. HEIMEL, C. LEVELT;
Mol. Visual Plasticity, Netherlands Inst. For Neurosci., Amsterdam, Netherlands

Abstract: To ensure that neuronal networks function in a stable fashion, neurons receive inhibitory inputs that are well-matched to their excitatory inputs. A temporary change in this balance of excitation and inhibition is an important component of plasticity, and recent evidence indicates that a reduction in inhibition-excitation ratio may be required for plasticity. This is also true for Ocular Dominance (OD) plasticity in the visual cortex, a well-studied model of cortical plasticity, where in closure of one eye shifts cortical responsiveness towards the open eye. While studies have shown that both excitatory as well as inhibitory network alterations are involved in the OD shift, controversy exists on whether altered inhibition drives OD plasticity by directly affecting eye-specific responses or through increasing the plasticity potential of excitatory connections. This is further complicated by the functional and network diversity of interneurons in the cortex. Here we have used optogenetic tools to acutely suppress inhibition from specific interneuron subtypes to address the effect on the OD shift. Our results suggest that in undeprived mice, eye specific excitation and inhibition are matched. In monocularly deprived mice, removal of inhibition from Parvalbumin (PV), Somatostatin (SST) or Vasoactive intestinal polypeptide (VIP) interneurons did not alter the OD shift when compared to control conditions. Time series experiments revealed that removal of inhibition did not alter the expression of OD during any stage of the OD shift. Our results indicate that the expression of the OD shift is not mediated by alterations in cortical inhibition.

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Topic: D.04. Vision

Support: CRSNG

FRQ-NT

Title: Comparative effects of adaptation on supra & infragranular layers in cat's visual cortex

Authors: ***N. CHANAURIA**[1], V. BHARMAURIA[1], L. BACHATENE[1], S. CATTAN[1], J. ROUAT[2], S. MOLOTCHNIKOFF[1];
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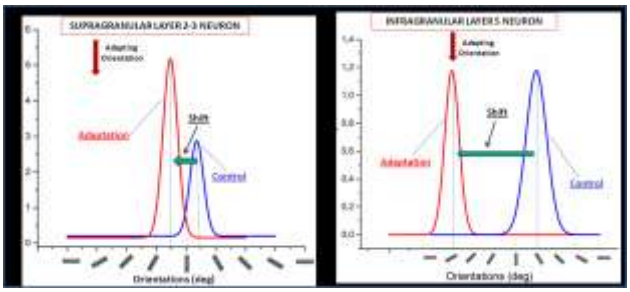
Abstract: Neurons have a fascinating tendency to change their properties in response to the environment that confers the brain plasticity. Now-a-days, it is widely known that forceful application of a stimulus for a certain duration of time could lead to changes in neuronal properties (Ghisovan et al., 2008) (Bachatene et al., 2013).Visual neurons are classical paradigm to study these adaptation effects. Reports demonstrate that layer2-3 primary visual neurons change their selectivity either towards adapter or away from it contingent upon stimulus duration. Neurons behave in a repulsive fashion in response to short duration (<3 min) whereas longer duration (upto 12 min) mostly reveals attractive shifts (Ghisovan et al., 2008). Layer 2-3 neurons, are the primary computational substrates that receive information from LGN through layer 4 and relay the information to layer 5-6 neurons. Because of their extensive dendritic trees L5 neurons are considered most important information processing units continuously involved in feed forward and feedback loops in response to an input, but how these neurons respond to adaptation, is a domain yet to be explored. Our preliminary data, through conventional simultaneous recordings in layer2-3&5 of anaesthetised cats, shows that infragranular layer 5 neurons also reprogram themselves in response to the imposed stimulus. L5 neurons shift their orientation tuning curves in a similar fashion as L2-3 suggesting that L2-3 neurons transmit their newly attained orientation tuning to L5 neurons. These preliminary results for the first time

indicate that in an adult brain not only the cells pertaining to specific layer learn to respond to an imposed stimulus, but the entire neuronal column changes its preferred orientation. **Support:** NSERC,FRQ-NT, University de Sherbrooke



Disclosures: **N. Chanauria:** None. **V. Bharmauria:** None. **L. Bachatene:** None. **S. Cattan:** None. **J. Rouat:** None. **S. Molotchnikoff:** None.

Poster

819. Striate Cortex: Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 819.20/GG6

Topic: D.04. Vision

Support: BBSRC grant BB/J002089/1

Title: Effects of dark exposure on visual cortex recovery from monocular deprivation in juvenile and adult mice

Authors: I. ERCHOVA, A. VASALAUSKAITE, *F. SENGPIEL;
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Abstract: Monocular deprivation (MD) disrupts normal visual input causing profound changes in binocular visual cortex and lasting vision deficits even when normal pattern vision is restored. The deficits are most severe when deprivation occurs during the critical period (CP) early in life and binocular vision is not restored until adulthood. However, a recent study on cats suggested that a period of dark exposure (DE) can trigger rapid visual recovery (Duffy & Mitchell, 2013). Using optical imaging of intrinsic signals and two-photon imaging we assessed changes in response properties of neurons in the binocular portion of the primary visual cortex of C57BL/6J mice following MD starting either during the CP or in adulthood. Short term (1 week) MD of the

contralateral eye in adults caused a shift in OD through an increased ipsilateral, non-deprived eye response, as well as a broadening of orientation tuning of responses through the deprived eye. These changes were reversible; 3 days of binocular vision was sufficient to restore a near-normal OD index (ODI) but deficits in deprived-eye orientation selectivity remained. Three days of DE immediately after MD accelerated recovery, with both ODI and orientation selectivity completely restored within 3 days. Long term (3 weeks) MD in adults had similar effects on neuronal response properties but recovery times were slightly longer at 6 days. When long term MD was initiated during the CP, it resulted in a pronounced OD shift predominantly caused by an increase of non-deprived eye responses combined with a smaller decrease in deprived-eye responses which also exhibited reduced orientation selectivity. Restoration of binocular visual experience in adulthood resulted in very limited recovery of deprived-eye responses within 3 or 6 days. However, 3 days of DE and subsequent binocular exposure for just 3 days resulted in nearly complete restoration of contralateral (previously deprived) eye dominance. These results suggest that DE activates homeostatic mechanisms and greatly enhances adult cortical plasticity.

Disclosures: I. Erchova: None. **A. Vasalauskaite:** None. **F. Sengpiel:** None.

Poster

819. Striate Cortex: Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: NIH/NIAAA AA13023

Title: Astrocyte implantation reinduces ocular dominance plasticity in adult ferret visual cortex

Authors: *W. A. FOXWORTHY, P. TRINDADE, A. MEDINA;
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Abstract: Muller and Best (1989) demonstrated that the critical period of ocular dominance plasticity (ODP) could be reopened in adult cats by implanting immature astrocytes in the visual cortex. These results have never been replicated in another species. Here we test the hypothesis that implantation of cultured immature astrocytes into the visual cortex of adult ferrets will reopen the critical period of ODP. The replication of these findings will pave the way for using modern genetic manipulation techniques to investigate the mechanisms underlying the role of astrocytes in the critical period. Astrocytes were cultured from the caudal pole of P7-P8 ferrets

and allowed to grow until reaching confluence (~14 days). These astrocytes were then implanted into the visual cortex of (n = 6) adult ferrets (>P70) who subsequently received a monocular deprivation (MD) on the eye contralateral to the implantation site. Control animals (n = 4) received a MD but no implantation. After a period of 5-14 days, the ocular dominance (OD) distribution was assessed using single unit electrophysiology in anesthetized animals. Number of spikes per stimulus above baseline spontaneous activity was quantitatively determined for each cell by presenting an optimally oriented, computer-controlled bar of light to each eye. To quantify cortical neuron OD, an index was calculated for each cell using the following equation: LE/(LE + RE), where LE stands for response to stimulation of left eye and RE for right eye. An OD index of 1.0 indicates that a neuron is responsive only to the left eye, and an OD index of 0.0 indicates responses only to the right eye. To quantify the changes in OD after monocular deprivation, we calculated a contralateral bias index (CBI) for each animal as ([P0.00-0.19 − P0.80-1.00] + [P0.20-0.39 − P0.60-0.79]/2 + 100)/200, where PA-B denotes the percentage of cells with OD indices between A and B. A CBI of 0 indicates that the ipsilateral eye dominated the responses in every neuron tested whereas a CBI of 1 indicates that the contralateral eye dominated every neuron. Significant differences in the OD distributions were found between the astrocyte implanted animals and the control animals ($F = 5.4$; d.f. = 1; $P = 0.048$). The control animals showed OD distributions that were biased towards the contralateral (deprived) eye (mean CBI: 0.63 ± 0.01), as is normal for adult ferrets. The animals which received astrocyte transplants showed distributions that were shifted towards the non-deprived eye (0.55 ± 0.03). These results are in agreement with those previously reported by Muller and Best (1989) in cats - implantation of immature astrocytes in adult ferrets can also reopen the critical period of ODP.

Disclosures: W.A. Foxworthy: None. **P. Trindade:** None. **A. Medina:** None.

Poster

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Topic: D.04. Vision

Support: NIMH Silvio Conte Center 1P50MH094271

Title: Parvalbumin network disinhibition prolongs critical period plasticity

Authors: *L. J. BOGART[1,2], H. H. C. LEE[1,2], T. K. HENSCH[1,2];
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Abstract: Parvalbumin (PV) circuits in mouse primary visual cortex (V1) mature in register with the critical period for ocular dominance plasticity. They densely innervate the perisomatic domain of target cells, where their inhibitory transmission triggers critical period onset and is sensitive to experience, such as dark-rearing from birth (DR) which delays plasticity into adulthood. To determine which PV-cell targets regulate critical period timing, we first examined anatomical labeling for Synaptotagmin-2 (Syt2), a high-fidelity presynaptic marker of PV+ boutons, upon both pyramidal cell (PC) subtypes and other PV-cells in V1. In normal, 2 month-old light-reared (LR) mice, Syt2+ boutons apposed to callosal-projecting Satb2+ PCs were found at lower density (0.079±0.005 boutons / um^2 Nissl-stained cell body surface area) than onto both sub-cortical projecting Ctip2+ PCs (0.128±0.005) and other PV-cells (0.134±0.007) (1way ANOVA $P<0.0001$; separately Ctip2+ and PV+ $P<0.001$ vs. Satb2). In DR mice at 2 months of age, Syt2+ bouton densities around both Satb2+ and Ctip2+ PCs remained unchanged (0.073±0.004 and 0.118±0.005, respectively; n.s., 2way ANOVA), while the density around PV-cells decreased dramatically (0.082±0.004; $P<0.001$ effect of rearing, 2way ANOVA). This level of Syt2+ inputs to PV-cells was similar to that of immature 15-day old mice (0.076±0.006; 1way ANOVA), and the effects of DR were rescued by re-exposing mice to light for several weeks (0.125±0.008, n.s. from LR in 1way ANOVA). We therefore tested the importance of the PV-PV connection itself for critical period regulation. As PV+ basket synapses on both PCs and PV-cells are enriched for alpha1 subunit-containing $GABA_A$ receptors, we crossed $alpha1^{f/f}$ mice with PV-Cre mice to produce cell-specific deletion (KO), leaving the PV-PC connection intact. Four-day monocular deprivation (MD) in adult (P60) wild-type mice failed to affect acuity (no MD: 0.47±0.04 cycles/degree, N=7; with MD: 0.46±0.02, N=3; P=0.82, t-test). However, MD even at P150 led to a significant loss of visual acuity in the PV-alpha1 KO mice (no MD: 0.50±0.03, N=3; with MD: 0.32±0.02, N=6; $P<0.01$, t-test). Two weeks of daily zolpidem injection, a $GABA_A$-alpha1-specific agonist, prevented adult plasticity in the KO (with MD: 0.43±0.02, N=5). Taken together, our results reveal that the PV-PV connection gates critical periods of cortical plasticity.

Disclosures: L.J. Bogart: None. **H.H.C. Lee:** None. **T.K. Hensch:** None.

Poster

819. Striate Cortex: Plasticity

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Title: Use-dependent cortical plasticity of structure and function in primary visual cortex following central vision loss

Authors: *W. BURGE, J. GRIFFIS, R. NENERT, A. ELKHETALI, D. DECARLO, R. H. CHEN, K. VISSCHER;
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Abstract: The influence of central vision loss on visual cortex is not fully understood. This study aimed to investigate structural and functional differences in primary visual cortex in participants with central vision loss due to macular degeneration vs. normally sighted controls. Ten participants with macular degeneration were paired with ten normally sighted controls matching for age, gender, and education. Structural and functional MRI data were collected at rest and while participants completed a visual task. Nine regions of interest were created that divided primary visual cortex (V1) into sections delineating centrally vs. peripherally responsive V1. We compared cortical thickness of these regions in participants with macular degeneration vs. controls. Functional connectivity (both at rest and background connectivity during performance of a visual task) was also assessed between these V1 regions and a set of pre-defined regions of interest. Compared to controls, participants with macular degeneration showed thinner cortex in the region of V1 corresponding to their retinal lesions; but they showed thicker cortex in peripherally responsive regions. This increase was specific to the part of V1 corresponding to the preferred retinal locus, the retinal location participants used for tasks such as reading. Compared to controls, participants with macular degeneration showed stronger modulations of functional connectivity (task vs. rest) between peripheral V1 and several regions thought to be involved in the 'default mode.' We hypothesize that this effect may relate to macular degeneration patients' enhanced ability to attend to peripheral locations. Our results suggest use-dependent changes in V1 cortical thickness following central vision loss, both increases associated with more use and decreases associated with less use. We also find use-dependent changes in the level of modulation of connectivity. Taken together, these data suggest

use-dependent structural and functional plasticity in primary visual cortex following central vision loss.

Disclosures: **W. Burge:** None. **J. Griffis:** None. **R. Nenert:** None. **A. Elkhetali:** None. **D. DeCarlo:** None. **R.H. Chen:** None. **K. Visscher:** None.

Poster

819. Striate Cortex: Plasticity

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Topic: D.04. Vision

Support: NIH Grant R01 EY022987

NIH Grant T32 EY015387

Title: Effects of early, pervasive exposure to stripes on visual acuity and visual response properties in the short-tailed opossum

Authors: ***J. C. DOOLEY**[1], L. A. KRUBITZER[2];
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Abstract: The short-tailed opossum (Monodelphis domestica) continues to be an important animal model for cortical sensory development due to the altricial age at which they give birth to young. Our laboratory has previously examined the organization, connectivity, and response properties of neurons in primary visual cortex (V1) in opossums, as well as their normal visual acuity as measured by the optokinetic response (Kahn et al., 2000; Dooley et al., 2012a; Dooley et al., 2012b). The goal of the present investigation was to characterize how pervasive alterations in early visual experience alter response properties of neurons within V1 as well as visually mediated behavior. We investigated how the neuronal response properties in V1 differed between animals exposed to vertical stripes from eye opening to adulthood and those reared in standard cages. Specifically, we examined orientation selectivity, temporal and spatial frequency, and contrast sensitivity. Additionally, we investigated differences in visual acuity between these groups using the optokinetic response. Once the animals reached adulthood, they were anesthetized and their eye was immobilized. At each recording site, the receptive field was isolated and sinusoidal bars were presented on a tangential screen. Stimuli varied across one of several parameters mentioned above. Neurons in V1 displayed the same temporal frequency

response curves between groups. However, among orientation selective neurons, stripe-reared animals had an over-representation of the cardinal axes. Neurons in V1 of stripe-reared animals also showed a stronger response when presented with low contrast stimuli compared to control opossums. Finally, on average the preferred spatial frequency of neurons was lower for stripe-reared animals when compared to controls. These electrophysiological data are consistent with behavioral results, which show that stripe-reared animals have a lower visual acuity when tested in the optokinetic drum. Our results are the first demonstration in a marsupial mammal that the visual environment in which an animal is reared has a large impact on both neural responses within V1 as well as visually mediated behavior, a phenomenon already demonstrated in several placental mammalian species. Given that marsupial and placental mammalian species diverged over 180 million years ago, and that Monodelphis is considered to reflect the brain organization and body morphology of early mammals, our results suggest experience-dependent plasticity was a feature present early in mammalian evolution.

Disclosures: J.C. Dooley: None. **L.A. Krubitzer:** None.

Poster

820. Extrastriate Cortex: Connections and Function

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Program#/Poster#: 820.01/GG11

Topic: D.04. Vision

Support: NIH Grant EY022090

NIH Grant EY020523

McDonnell Center for Systems Neuroscience

Title: Distinct inter-areal connection strengths in mouse visual cortex provide a pathway-specific drive of excitation and inhibition

Authors: *R. D'SOUZA, A. BURKHALTER;
Anat. and Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Mouse visual cortex is a hierarchical organization of distinct posterior brain areas that are interconnected via feedforward (FF) and feedback (FB) axonal projections (Wang and Burkhalter, 2007; Wang et al., 2012). The primary targets of these inter-areal excitatory connections are pyramidal (Pyr) cells and the parvalbumin (PV)-expressing GABAergic

interneurons, the latter of which provides strong local inhibition to Pyr cells. PV cells are therefore a major mediator of feedforward inhibition (FFI) between visual areas (Dong et al., 2004). FFI is a common motif in the brain, and controls the time window for the integration of convergent inputs in thalamocortical circuits (Gabernet et al., 2005). To understand how visual information is routed through the visual cortical hierarchy, we asked how the strength of FFI varies between primary visual cortex (V1), and the higher lateromedial (LM) and posteromedial (PM) areas, three areas with distinct spatiotemporal and receptive field properties (Wang and Burkhalter, 2007; Marshel et al, 2011; Andermann et al, 2011). To do this, we employed subcellular Channelrhodopsin-2 (ChR2)-assisted circuit mapping (sCRACM; Petreanu et al, 2009; Mao et al, 2011) in cortical slices from 30-45 day old PV-Cre X Ai9 mice to measure the relative strengths of inter-areal synaptic input to neighboring Pyr and PV cells. ChR2-expressing axon terminals were excited by a blue laser delivered in an 8 X 16 grid spaced 75 μm apart, and postsynaptic currents (EPSCs) were recorded from neighboring Pyr and PV cells, the latter identified by the expression of the red fluorescent protein tdTomato. We find that in layers 2/3, inputs to PV cells are, on average, stronger than inputs to Pyr cells in all pathways studied here (V1→PM, PM→V1, LM→PM, and PM→LM). The relative excitation of a PV cell normalized to its neighboring Pyr cell, however, is more than 3-fold stronger in the FF V1→PM than in the FB PM→V1 pathway. Relative excitation of PV cells is also significantly stronger in the FB PM→LM than in the FB PM→V1 pathway in layers 2/3, implying that FFI is stronger between hierarchically closer areas. In all four layer 5 pathways, inputs to PV and Pyr cells are equally strong. These results, along with previous data studying the V1→LM and LM→V1 pathways (Yang et al, 2013), indicate that the balance of excitation and inhibition is pathway-specific, suggesting that different pathways play distinct roles in the detection and top-down control of visual information. We hypothesize that FF circuits are more sensitive to convergent, synchronous inputs, while weaker FFI in the FB pathway may more broadly select for salient visual input.

Disclosures: **R. D'Souza:** None. **A. Burkhalter:** None.

Poster

820. Extrastriate Cortex: Connections and Function

Location: Halls A-C

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Program#/Poster#: 820.02/GG12

Topic: D.04. Vision

Title: Visual detection of signal in noise by mice

Authors: *L. WANG, R. KRAUZLIS;
NIH, Natl. Eye Inst., Bethesda, MD

Abstract: Mice are increasingly used as an animal model to study the visual system, largely because they provide an opportunity to target specific populations of neurons using advanced genetic methods. Although mice can perform many visually guided tasks, a concern is that they may not be able to perform the same types of challenging tasks that have been used to characterize the properties of the primate visual system. To address this issue, we studied the performance of mice trained to detect the presence of an oriented Gabor patch embedded in masking visual noise. Ten mice were trained to perform the visual detection task. Mice were implanted with a titanium head-holder that allowed us to fix their position within a sound-attenuated chamber during presentation of the visual stimuli, displayed on two flat panels forming the anterior walls of the chamber. Each display was centered on a visual axis 45 degrees to the left or right of straight ahead and subtended about 90 deg visual angle. The visual stimuli had two components - visual noise and an oriented Gabor patch. The visual noise filled the displays and consisted of individual luminance checks (1.6 deg/check) with values drawn from a Gaussian distribution (12.5% contrast). The Gabor patch contained a vertical grating (0.06 cycles/degree) of variable contrast (3-38%) added to the background visual noise. During individual trials, mice were presented with visual noise, followed randomly by newly drawn visual noise that either did or did not include an oriented Gabor patch on one side of the display. The task of the mice was to lick if a Gabor patch was present in the updated noise, and withhold from licking if absent. Correct responses were rewarded by a drop of water; false alarms prompted a brief airpuff to the snout and a timeout delay. Because the Gabor appeared in newly updated noise, mice could not respond based on local luminance changes but had to detect the presence of the Gabor pattern. Nonetheless, mice performed the task reliably, completing ~250 trials each day. We analyzed the data by counting the number of licks immediately following the updated noise, and performed an ROC-style analysis to measure perceptual discrimination and construct psychometric curves for each mouse. Performance varied with the relative contrast of the Gabor patch; asymptotic performance approached ~70% correct when the Gabor contrast exceeded the noise contrast. For most mice, reaction times were independent of relative contrast; time to first lick after Gabor onset was typically 300-400 ms. These data illustrate that mice can be trained to perform challenging visual tasks similar to those that have been used to study visual-motor function in primates.

Disclosures: L. Wang: None. **R. Krauzlis:** None.

Poster

820. Extrastriate Cortex: Connections and Function

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Topic: D.04. Vision

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Title: Amblyopia reduces neuronal and perceptual sensitivity to naturalistic image structure

Authors: *C. M. ZIEMBA, N. J. MAJAJ, R. D. KUMBHANI, C. SHOONER, L. E. HALLUM, A. VOYLES, V. GARCIA-MARIN, J. G. KELLY, J. A. MOVSHON, L. KIORPES; Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: V2 neurons, but not V1 neurons, respond more vigorously to naturalistic stimuli matched for the statistical dependencies found in natural images of visual texture than to "noise" images matched only for orientation and spatial frequency content (Freeman, Ziemba et al, 2013, Nat Neuro). V2 circuits therefore transform the information they receive from V1 to create an explicit representation of the statistics of the natural environment. Evidence from evoked potential recordings in infant monkeys suggests that this representation emerges relatively late in development. We therefore wondered whether the enhanced representation of naturalistic structure would be degraded in individuals in whom abnormal early visual experience had produced a form of amblyopia. Behavioral experiments in a few amblyopic monkeys revealed that detection of naturalistic structure was impaired in the amblyopic eyes relative to the fellow eyes. To investigate the corresponding neuronal deficit, we used 96-channel multielectrode arrays to record from single neurons and multiunit clusters in V1 and V2 of paralyzed, anesthetized monkeys in which amblyopia had been induced either by surgical alteration of eye alignment (strabismus) or by optical defocus in one eye (anisometropia) early in life. We measured responses evoked by 4 deg patches of naturalistic and noise texture, presented alternately to the amblyopic and fellow eyes. When driven through the fellow eye, responses were like those we observed previously - V2 showed reliably enhanced responses to naturalistic stimuli, while V1 responded similarly to both naturalistic and noise stimuli. When driven through the amblyopic eye, however, neurons in both areas responded similarly to the two kinds of texture, only weakly differentiating naturalistic textures. Amblyopia often reduces the responsiveness of neurons driven by the amblyopic eye relative to the fellow eye, and this pattern was evident at most of our V2 recording sites. Even for sites with roughly equal responsiveness to the two eyes, naturalistic sensitivity was greater in the fellow eye. Our results suggest that amblyopia not only weakens the influence of the amblyopic eye in cortex, but also disrupts V2's

ability to respond selectively to information captured by the statistical structure of natural images. Amblyopia thus modifies cortical computations downstream of V1.

Disclosures: **C.M. Ziemba:** None. **N.J. Majaj:** None. **R.D. Kumbhani:** None. **C. Shooner:** None. **L.E. Hallum:** None. **A. Voyles:** None. **V. Garcia-Marin:** None. **J.G. Kelly:** None. **J.A. Movshon:** None. **L. Kiorpes:** None.

Poster

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Topic: D.04. Vision

Support: NIH Grant EY021894

Title: Development of texture perception in infant monkeys: Physiology and behavior

Authors: ***A. VOYLES**, C. M. ZIEMBA, N. J. MAJAJ, J. A. MOVSHON, L. KIORPES; New York Univ., New York, NY

Abstract: Form perception in neonates is immature, and develops over a time course that is longer for more complex form tasks. Recent studies have used naturalistic texture stimuli synthesized to include statistical features of natural images to explore how the visual system processes complex visual patterns. Extrastriate visual area V2 is the earliest area in which neurons are sensitive to the naturalistic features of these textures, and we wondered whether the perceptual and electrophysiological correlates of this sensitivity would develop with a time course that perhaps reflected the development of this neural computation in area V2. We recorded visually evoked potentials (VEPs) from four infant monkeys longitudinally between the ages of 8 and 36 weeks, and compared VEP thresholds to those measured behaviorally. A custom-designed 27-channel EEG cap was used to record VEP signals as the infants passively viewed a naturalistic texture stimulus alternating with a spectrally-matched noise stimulus (3Hz). The “naturalness” of the texture (the magnitude of the naturalistic visual features) decreased throughout the trial in logarithmic steps. In a control condition, the subjects viewed a texture alternating with a homogeneous background while contrast decreased. A subset of the infants and three adults monkeys were behaviorally tested with the same stimuli to evaluate their ability to discriminate naturalistic texture stimuli from spectrally-matched noise control stimuli in a 2-alternative forced choice task. To quantify the selectivity of the VEP response to the stimulus

manipulation, we computed the amplitude of the first harmonic of the evoked potential. Contrast modulation evoked significant and reliable responses in the occipital channels from the earliest age. Naturalness did not evoke a strong response on any channels in the young animals, but showed reliable responses in the older animals. These responses were monotonically related to the naturalness of the stimulus, allowing us to estimate a VEP naturalness threshold. Behavioral ability to discriminate textures mirrored the measured VEP thresholds. We conclude that the VEP response to naturalistic textures increases with age, contains components selective to the "naturalness" of the image, and parallels behavior. Taken together with previous findings of selective V2 responsiveness to naturalistic textures, this suggests that the change in VEP selectivity to textures with age may reflect developmental changes in V2 or downstream areas that support improved form perception with age.

Disclosures: A. Voyles: None. **C.M. Ziemba:** None. **N.J. Majaj:** None. **J.A. Movshon:** None. **L. Kiorpes:** None.

Poster

820. Extrastriate Cortex: Connections and Function

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Program#/Poster#: 820.05/GG15

Topic: D.04. Vision

Support: CIHR to CC

Title: Morphology of area 21a terminals in the LP-pulvinar complex of the cat

Authors: *R. ABBAS FARISHTA[1], M. VILLENEUVE[1], F. HUPPÉ-GOURGUES[1], D. BOIRE[1,2], C. CASANOVA[1];
[1]Sch. of optometry, Univ. De Montréal, Montréal, QC, Canada; [2]Dept. d'anatomie, Univ. du Québec à Trois Rivières, Trois Rivières, QC, Canada

Abstract: The lateral posterior pulvinar (LP-pulvinar) is a higher-order (HO) thalamic nucleus with reciprocal connections with virtually all visual areas of the neocortex and is likely involved in the transmission of information between them. Two types of axon terminals have been identified in cortico-thalamic (CT) pathways: the class I (modulators) terminals, characterised by thin axons with small terminals on distal dendrites; class II (drivers) terminals characterised by thick axons and large terminals on proximal dendrites. In recent years, studies have shown that the projection from the primary visual cortex to the LP comprises mainly type II terminals

whereas the projection originating from the extra-striate (XC) area PMLS (Postero Medial lateral Suprasylvian cortex) comprises mainly type I terminals. It is hypothesised that, in HO thalamic nuclei, the proportion of type I versus type II axon terminals of CT projections would increase with the hierarchical level of cortical visual areas. To test this hypothesis, we charted the distribution of class I and class II terminals from area 21a, an XC area considered to be at a higher hierarchical level than the PMLS cortex. Given its hierarchical order, the proportion of class I/class II terminals was expected to be higher in area 21a than in the PMLS and area 17. Methods : Biotinylated dextran amine (10kDA) was injected by iontophoreris in area 21a of adult cats. After 10-15 days survival, brains were harvested and frozen; coronal sections were collected for the visualisation of BDA. Results: Labeled CT terminals were found in the lateral and medial part of the LP. Projections were classified according their size, preterminal axon morphology, and complexity of bouton groupings. The vast majority of labelled terminals in the LP nucleus were type I single small boutons located on short stalks of thin axons. Some terminals were slightly larger than type I terminals but formed simple single terminal swellings at the end of long axonal side branches and were classified as singletons. None of the labelled projections exhibited large terminals with complex rosette-like structures previously classified as class II. In conclusion, the majority of the projections from the high order cortical area 21a in the LP exhibit a class I like morphology, suggesting that area 21a exerts mainly a modulatory influence on the pulvinar complex. This also supports the hypothesis that the proportion of type I/type II axon terminals in higher order thalamic nuclei increases with the hierarchical order of cortical visual areas.

Disclosures: R. Abbas farishta: None. **M. Villeneuve:** None. **F. Huppé-Gourgues:** None. **D. Boire:** None. **C. Casanova:** None.

Poster

820. Extrastriate Cortex: Connections and Function

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Topic: D.04. Vision

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Royal Society

Title: Predicting the topographic organisation of visual area V5/MT from probabilistic cortico-cortical connections to V1

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Abstract: The primate extrastriate visual area (V5/MT) receives projections from primary visual cortex (V1), but a complete topographic map from each point of V5/MT to V1 in one individual cannot be established by histological or electrophysiological methods. Probabilistic tractography, using diffusion MRI (dMRI), offers the potential of non-invasive estimation of connectivity between identified cortical areas across the whole brain. We tested the visual-field representation across V5/MT in the rhesus macaque, based on estimates of its connections to different parts of V1 and the known retinotopic organisation of V5/MT and V1. dMRI was obtained from six perfusion-fixed *Macaca mulatta* brains, scanned *ex vivo* with a 4.7T preclinical scanner using a single shell of 61 gradient directions (b=4300s/mm^2, isotropic 0.5x0.5x0.5mm voxels; Dyrby *et al.* 2011); data were processed and analysed using FSL (Jenkinson *et al.* 2012) and MATLAB. A ball-and-stick model was used to estimate the distribution of axon fibre orientations in each voxel (allowing two crossing fibres; Behrens *et al.* 2007), which the tractography algorithm sampled to estimate the axonal trajectories and connectivity between cortical areas. The algorithm was impaired by ambiguity in the diffusion direction of the cortical white matter neighbouring our targets (mean fractional anisotropy: V5/MT = 0.41; central V1 = 0.47; peripheral V1 = 0.57; vs corpus callosum = 0.85, SD 0.08), which we overcame by extending the cortical target masks into well-connected, immediately-adjacent white matter. Exclusion masks were applied to sulci and ventricles. Based on the relative strength of probabilistic connection to central or peripheral V1, each V5/MT voxel was classified as either central (<10° from fixation) or peripheral (>12°) visual field. Using this simple classification, the resultant V5/MT map distinguished consistent peripheral and central representations. On average, V5/MT voxels assigned as central were 2.92mm lateral, 1.11mm anterior, and 2.46mm ventral relative to voxels assigned as peripheral (mean distance = 4.67mm; one-sided permutation test $p < 0.01$, all hemispheres), consistent with neurophysiological mapping from other studies. In one hemisphere scanned *in-vivo* (at 3T, 61 directions, b=1000s/mm^2, 1mm^3), the same classification technique yielded qualitatively similar topography for V5/MT. We conclude that dMRI can reveal topographic order in the projections between V1 and V5/MT – and that probabilistic tractography can correctly identify such connections to a precision that permits assessment of within-area topography. Supported by BBSRC, Wellcome Trust, Royal Society.

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Poster

820. Extrastriate Cortex: Connections and Function

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Topic: D.04. Vision

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ANR-11-LABX-0042

Title: The role of the Exponential Distance Rule in establishing the functional dorsal and ventral pathways

Authors: *L. MAGROU[1], A. FALCHIER[2], M. ERCSEY-RAVASZ[3], C. LAMY[1], P. MISERY[1], P. GIROUD[1], D. C. VAN ESSEN[4], Z. TOROCZKAI[5], K. KNOBLAUCH[1], H. KENNEDY[1]; [1]INSERM U846, SBRI, Bron Cedex, France; [2]The Nathan S. Kline Inst. for psychiatric research, Orangeburg, NY; [3]Physics, Babes-Bolyai Univ., Cluj-Napoca, Romania; [4]Anat. and Neurobio., Washington Univ. Sch. of Medecine, St Louis, MO; [5]Physics, Univ. of Notre-Dame, South Bend, IN

Abstract: There is a large body of evidence showing that foveal and peripheral representations of the early visual areas possess major differences in their connectivity (Falchier et al., 2002, Ungerleider et al., 1986). High frequency sampling of connectivity following retrograde tracer injections in cortical areas reveals high fidelity connectivity profiles (Markov 2011). The Exponential Distance Rule (EDR), discovered in the macaque brain (Markov et al., Science 2013 ; Ercsey-Ravasz et al., Neuron 2013), describes a predictive relationship between distances and connexion strength (FLN).. Random networks, constrained by EDR exhibit a number of local and global statistical properties observed in the cortico-cortical inter-areal connectome (Markov et al., 2013, 2014) including the motif distribution, wire minimization, conduction efficiencies; and a core/periphery Bowtie structure within the network. These successful predictions argue for the EDR constituting an important organization principle of the cortex. We here explore whether the EDR can also predict the differences in the organization of visual functional streams. Taking their roots in early visual areas, the dorso-parietal stream is associated with movement perception, and the ventro-temporal stream in identification of objects (Mishkin et al., 1983). Inspection of a 2D map of visual cortex shows that peripheral parts of V1 and V2 are localised in proximity with the dorsal stream areas, while foveal regions of these areas are near the ventral stream. Here we investigate if EDR sets up the two networks. We investigate the EDR between (i) peripheral representations and dorsal areas; (ii) foveal representations and ventral areas. We

performed 8 injections of retrograde tracers (Fast Blue and Diamidino Yellow) in cynomolgus macaques at different visual eccentricities in V1, V2, V4 and MT. We observe that, while injections performed in territories corresponding to the central (i.e. foveal) representation of the visual hemifield preferentially receive inputs from areas belonging to the ventral pathway, injections placed in peripheral representations preferentially labels neurons in areas known to be part of the dorsal pathway. Analysis of the relationship between FLN values and inter-areal distances from injections at different eccentricities return an exponential decay of FLN with increasing distances similar to that reported in our previous work. A generative model employing the EDR is needed to confirm the role of the EDR in differential connectivity patterns.

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Poster

820. Extrastriate Cortex: Connections and Function

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Title: Cytoarchitecture and distance predict connectivity of the primate cerebral cortex

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Abstract: Information processing in the brain is strongly constrained by anatomical connectivity. However, the principles governing the organization of corticocortical connections remain elusive. Here, we tested three models of relationships between the organization of cortical structure and features of connections in the cortex of a non-human primate, the macaque monkey. Factors taken into account were relative cytoarchitectonic differentiation (structural model), relative spatial position (distance model), and relative hierarchical position (hierarchical model) of potentially connected cortical areas. Quantitative measures of corticocortical connections were obtained from an extensive report of anatomical tract-tracing experiments in the macaque monkey (Markov et al. 2014, Cerebral Cortex 24:17). The data set comprises results

of retrograde tracer injections in 29 cortical areas, with a total of 2639 reported projections from 91 areas of the macaque cortex. Relative cytoarchitectonic differentiation and spatial distance (themselves only weakly correlated) correlated strongly with the presence or absence of inter-areal connections, whereas no correlation was found with relative hierarchical position. Moreover, cytoarchitectonic differentiation correlated with the absolute number of corticocortical connections formed by the areas. The two factors of structural type difference and spatial distance were integrated into a model of corticocortical connectivity which allowed us to predict the existence of connections in the data with more than 80% accuracy. Thus, anatomical connectivity of the primate cerebral cortex can, to a large part, be explained by the two factors of relative cytoarchitectonic differentiation and spatial distance of brain regions. Notably, the structural model provides a compelling framework for explaining the occurrence of long-distance connections. Hierarchical area rankings, by contrast, did not add explanatory value. These results are in excellent correspondence with findings reported for the cat cortical connectome (Beul et al. 2014). As both the structural and distance model were originally formulated specifically for the primate prefrontal cortex, their applicability across a wide range of cortices in different species suggests a general principle of cortical organization. These principles may be further extended to the human brain, to complement currently available information on structural cortical connectivity. Acknowledgements: We thank Helen Barbas for cytoarchitecture data.

Disclosures: C.C. Hilgetag: None. **S. Beul:** None.

Poster

820. Extrastriate Cortex: Connections and Function

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Topic: D.04. Vision

Title: A new theory for dorsal and ventral streams in the visual system

Authors: *B. R. SHETH[1], R. YOUNG[2];
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Abstract: The idea of a dissociation of the visual pathway into two distinct streams_ventral and dorsal_that each processes distinct kinds of information is a powerful one. Two proposals along those lines state that the ventral stream processes information about object identity (“what”), whereas the dorsal stream processes information about either object location (“where”;

Ungerleider & Mishkin, 1982;) or to perform motor acts ("how"; Goodale & Milner, 1992). Both proposals are influential but contradicted by recent data (e.g. ventral stream is involved in where/how computations; the dorsal stream is involved in what computations). We suggest a robust dichotomy breaking down into 1. a ventral stream sampling high-resolution/*focal* spaces, and therefore, macularly-biased, and 2. dorsal *ambient* sampling, and therefore less spatially biased streams. This dichotomy may derive from pressures exerted during evolution by dense receptive surfaces. The idea further hews more closely to the theme of embodied cognition: Function arises as a consequence of an extant sensory underpinning. A continuous, rather than sharp, segregation based on function emerges, and carries with it an undercurrent of an exploitation-exploration dichotomy. Under this interpretation, cells of the dorsal stream, which individually have large receptive fields and poor spatial localization, do not provide precise information about location but rather of the presence/absence of salient objects for novel exploration and subsequent exploitation; cells of the ventral stream, which individually have more punctate receptive fields that generally include the fovea or parafovea, provide detailed information about object shapes and features and lead to the systematic exploitation of said information. We leverage our dichotomy (focused/ambient or exploitation/exploration) to unify neuropsychological cases (e.g. visual agnosia, optic ataxia) under a common umbrella, account for the increased prevalence of multisensory integration in the dorsal stream under a Bayesian framework, offer a basis for spatial illusions, predict conditions under which object recognition will utilize both streams, help understand why cells of the dorsal stream respond to spots of light and generally have lower selectivity for objects, and provide a dynamic component to the ventral-dorsal dichotomy that underscores a unified, seamless perception. Existing theories for ventral-dorsal processing are subsumed under our proposal. Our model of the primate/hominid visual system is extendable to the bat auditory system and suggests an evolutionary link with the development of the fovea and mechanisms for eye tracking.

Disclosures: B.R. Sheth: None. **R. Young:** None.

Poster

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Searle Foundation

Title: Brain-wide imaging of neural activity during a visual discrimination task

Authors: *J. B. WEKSELBLATT, E. D. FLISTER, D. M. PISCOPO, C. M. NIELL;
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Abstract: Visually-driven behaviors engage multiple brain regions in order to convert sensory information into motor output. This includes primary visual cortex and extrastriate regions to extract cues from the visual scene, higher-order association areas involved in decision-making, and motor regions to generate output. Determining the flow of information between these regions is a key goal in understanding how vision is translated into action. In order to analyze brain activity at this scale, we have developed a system that allows functional imaging across large areas of cortex during a visual discrimination task. To facilitate imaging, we have generated a transgenic mouse expressing the fluorescent calcium indicator GCaMP6s in excitatory neurons throughout cortex. Using a widefield macroscope, we can thereby image patterns of neural activity across nearly the entire the dorsal surface of cortex simultaneously. Although this imaging modality does not provide cellular resolution, it allows us to map activity in regions of cortex on ~50um spatial scale with ~100msec temporal resolution. Using this method, we have delineated cortical areas that are selectively engaged by visual stimulation, whisker stimulation, or locomotion. Furthermore, we have integrated this imaging modality with a visual task, by training head-fixed mice on a spherical treadmill to report their response in an orientation discrimination by running either left or right. Widefield imaging while animals perform this task demonstrates a progression of activity in different cortical regions, including selective engagement of extrastriate and other posterior areas, which are associated with different phases of the task such as stimulus presentation and behavioral response. Importantly, once the cortex-wide patterns of activity are determined, we can “zoom in” on key regions using two-photon microscopy to measure responses during the task at the level of ensembles of individual neurons to study the circuit mechanisms underlying distinct aspects of the task. We expect that this paradigm will also be useful in probing brain-wide networks involved in other sensory or cognitive processes. This work is supported by the University of Oregon Developmental Biology Training Program (5 T32 HD007348-23), the Sloan Foundation, the Searle Foundation, and NIH (DP2 EY023190-01).

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Poster

820. Extrastriate Cortex: Connections and Function

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SFB889

Title: The disorganized visual cortex in reelin-deficient mice is highly functional and allows for enhanced plasticity

Authors: *J. PIELECKA-FORTUNA[1], R. J. WAGENER[3], A.-K. MARTENS[2], B. GOETZE[2], K. F. SCHMIDT[2], J. F. STAIGER[3], S. LÖWEL[2];
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Abstract: A hallmark of neocortical circuits is the segregation of processing streams into six distinct layers. The importance of this layered organization for cortical processing and plasticity is - however - little understood. One of the molecules crucial for the proper establishment of laminar organization is reelin, a large extracellular matrix glycoprotein. In reelin-deficient mice (rl-/-), an inversion of neocortical layers was initially described. However, recent studies examining the whisker-to-barrel pathway in rl-/- mice showed that the lamination of the primary somatosensory cortex was not just inverted, but also highly disturbed. To check whether there are similar changes in the primary visual cortex (V1), we investigated the structure, function and plasticity of V1 of adult rl-/- mice and their wild-type littermates. Using molecular methods of histology we found that in V1 of rl-/- mice, cells with different laminar fates were present at all cortical depths but afferents from the lateral geniculate nucleus contacted their appropriate layer IV fated cells irrespective of their ectopic position. In addition, optical imaging of intrinsic signals revealed that the (vertically) disorganized rl-/- cortex is capable of maintaining normal (horizontal) organization of visual cortex maps, which are not different from WT mice. Utilizing behavioral techniques we found that basic visual capabilities (measured via optomotry) of rl-/- mice were similar to WT mice, but more elaborate functions (measured via visual water task) like orientation discrimination were severely compromised. In addition, despite a massive defect

in hippocampal organization, rl-/- animals were able to learn and maintain the memory of an acquired visual task as well as their WT littermates. Most surprisingly, we found that reelin deficiency enhanced visual cortical plasticity: juvenile-like ocular dominance plasticity after monocular deprivation was preserved into adulthood. Altogether, the present data offer an astonishing insight into the capabilities of a disorganized cortical system to maintain basic functional properties. In addition, the significantly increased cortical plasticity of reelin-deficient mice opens new avenues to study the role of reelin for developmental and adult cortical plasticity.

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Poster

820. Extrastriate Cortex: Connections and Function

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Topic: D.04. Vision

Support: NEI EY020516

Title: Projecting V1 retinotopy to extrastriate cortex using connective field modeling and freely viewed naturalistic stimuli

Authors: *A. S. BOCK, G. K. AGUIRRE;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Dumoulin & Wandell (2008) introduced the population receptive field (pRF) approach for the analysis of traditional retinotopic mapping fMRI studies. Haak et al. (2013) extended the pRF method to describe the 'connective field' response of a voxel in terms of activity in another region of cortex. However, these approaches still require traditional retinotopic mapping stimuli (e.g., bars, rings, wedges). Here, we extend these methods and project V1 retinotopy to extrastriate cortex using a retinotopic template of V1, connective field modeling, and freely viewed naturalistic stimuli. fMRI data were acquired for two subjects at 7T (Siemens Magnetom, 1.5mm3, TR=2s) while they viewed without defined fixation the movie 'The Artist' (Hazanavicius, 2011) for 50 minutes, split over 5 runs, using an HD LCD monitor (1920x1200 resolution, 16 degrees visual angle). V1 location and retinotopy were defined using a V1 template (Benson et al., 2012; 2014). The connective field for each extrastriate voxel was

determined using connective field modeling (Haak et al., 2013) with reference to V1 time courses; polar angle and eccentricity assignment of voxels in extrastriate cortex were based on their connective field center in V1. The retinotopic arrangement of V2 and V3 was readily recovered, and found to closely match the organization defined using a cortical surface template (Benson 2014). Further, a large extent of occipital and parietal cortex beyond V3 was found to have signals that could be well modeled by reference to V1 signals. We find that extra-striate retinotopy can be conducted using complex and continuous 'naturalistic' visual input, without the need for fixation. The ability to saccade to various points on the screen not only increases the size of the stimulated visual field, but allows for retinotopic mapping in subjects with difficultly fixating on a central target due to behavioral or ophthalmologic limitations. Further, our approach allows for the presentation of different naturalistic stimuli better suited to activate and map visual regions along the dorsal, ventral, and subcortical pathways.

Disclosures: A.S. Bock: None. **G.K. Aguirre:** None.

Poster

820. Extrastriate Cortex: Connections and Function

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Topic: D.04. Vision

Support: NEI EY11001

Title: Hebbian plasticity leads to biased representations in parietal cortex

Authors: *W. ZHANG[1,2], K. D. MILLER[2];
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Abstract: When monkeys learn to group visual stimuli into arbitrary categories, neurons in the lateral intraparietal area (LIP) become category-selective. Surprisingly, the representations of learned categories are overwhelmingly biased: while different categories are behaviorally equivalent, nearly all LIP neurons in a given animal prefer the same category (Fitzgerald et al., 2013). Inspired by models of ocular dominance segregation (Miller, 1990), we propose that Hebbian plasticity, at the synapses to LIP from prefrontal cortex and from lower sensory areas, could lead to the development of biased representations. In our model, LIP category selectivity arises due to competition between inputs encoding different categories, and bias develops due to

excitatory lateral interactions among LIP neurons. Our model reproduces the different levels of category selectivity and bias observed for visual and persistent activity, as well as the redevelopment of bias after monkeys learn redefined categories. Our model predicts that when LIP neurons develop different preferences, neurons with similar preferences would have receptive fields that cluster in space; this prediction is confirmed in two animals during a visual discrimination task where weak response biases were observed in LIP (Oristaglio et al., 2006). Furthermore, our model predicts that biased representations would develop in LIP not only for discrete categories, but continuous stimulus variables as well. References Fitzgerald JK, Freedman DJ, Fanini A, Bennur S, Gold JI, Assad JA. (2013) Neuron. Biased associative representations in parietal cortex. Miller KD. (1990) Correlation-based models of neural development, in Neuroscience and Connectionist Theory, M.A. Gluck and D.E. Rumelhart, Eds. (Lawrence Erlbaum Associates, Hillsdale NJ), pp. 267-353. Oristaglio J, Schneider DM, Balan PF, Gottlieb J. (2006) J Neurosci. Integration of visuospatial and effector information during symbolically cued limb movements in monkey lateral intraparietal area.

Disclosures: W. Zhang: None. **K.D. Miller:** None.

Poster

821. Visual Motion: Heading and Orientation

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NEI Grant EY016178

Title: Joint encoding of observer translation and rotation in the ventral intraparietal area

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Abstract: As we navigate through the world, it is often necessary for the visual system to decompose self-motion into its translational and rotational components. For instance, walking down a sidewalk (translation) while turning one's head to look at a passing car (rotation) requires extracting the translation direction to maintain a straight trajectory. On the other hand, a

representation of rotations is necessary for motor tasks and to estimate curvilinear trajectories. Optic flow projected onto the retina is the sum of translations and rotations, such that the two components are confounded. We have previously shown that both retinal and extra-retinal (efference copy) signals in the ventral intraparietal area (VIP) are used to create a rotation-invariant representation of translation. While previous studies have focused on discounting rotations to encode translations, little attention has been paid to estimating the rotational component from neural responses. We examine if neurons in area VIP jointly encode both the translation and rotation components of self-motion. The experimental protocol consists of adding three rotation velocities (5, 10, 15°/s) to translational optic flow stimuli. Rotations are added in two ways: 1) Real pursuit - monkey makes smooth pursuit eye movements while translational optic flow is presented. 2) Simulated pursuit - monkey fixates at the center of the screen and rotation is added to the translational optic flow. Tuning curves for pure translations in the horizontal plane are compared to tuning curves during real and simulated pursuit. We find that the representation of translation is invariant across rotation velocities, validating our previous results. Using singular value decomposition, we further show that neurons in VIP use both retinal and extra-retinal cues to encode translations independent of the rotation velocity. More interestingly, a subset of cells (42%) show gain modulation based on the rotation rate, thus jointly encoding rotation velocity and translation direction. We also show that separable encoding of translation and rotation can be achieved using purely retinal cues. Since optic flow represents the rotation of the eye relative to the world, it is ambiguous to the source of the rotation (eye, head or body rotations). We thus propose that an 'eye-in-world' rotation signal derived from optic flow contributes to the rotation estimate in area VIP. Such a separable joint representation of translation and rotation provides a flexible mechanism for estimating self-motion trajectories using the translation component (for straight paths) or for estimating a curvilinear trajectory by adding translations and rotations.

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Poster

821. Visual Motion: Heading and Orientation

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Topic: D.04. Vision

Support: NIH R01EY019317

Title: Visual and non-visual contributions to the perception of object motion during locomotion

Authors: *B. R. FAJEN[1], M. S. PARADE[2];
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Abstract: To guide locomotion in the presence of independently moving objects, moving observers must perceive object motion in world coordinates (Fajen, Parade, & Matthis, 2013). However, the local optical motion of moving objects is influenced by both observer motion and object motion, and reflects object motion in observer coordinates. According to the "flow parsing" hypothesis (Warren & Rushton, 2009), observers recover object motion in world coordinates by using global optic flow to factor out the influence of self-motion. However, judgments of object motion in world coordinates during simulated self-motion are biased, as if the visual system does not completely compensate for the influence of self-motion (Matsumiya & Ando, 2009). Perceived object motion is less biased when both visual and vestibular self-motion information is available, but is still not completely veridical (Dokka, Macneilage, Deangelis, & Angelaki, 2013). The aim of this study was to investigate the accuracy of object motion perception when self-motion is real and actively generated by walking over a ground surface. The experiment was conducted in a virtual environment viewed through a stereoscopic head-mounted display. Subjects observed an object move along a textured ground surface across their path and judged whether the object was approaching or retreating. They performed this task while remaining stationary and viewing optic flow simulating self-motion and while actually walking. We found a bias to perceive objects as approaching when self-motion was simulated, consistent with previous studies. However, judgments were unbiased when self-motion was real, demonstrating that observers are capable of accurately perceiving object motion in world coordinates when self-motion is actively generated by walking over a ground surface. We replicated these findings in a second experiment using a different task. Taken together, the findings suggest that non-visual self-motion information (and, in particular, proprioceptive and/or motor information) plays a critical role in the accurate perception of object motion during self-motion. We introduce a new model to account for these and previous findings. The model proposes that non-visual information generated during locomotion is used not to improve the self-motion estimate but rather to allow object motion to be perceived relative to the physical ground surface, so that locomotion and object motion perception are in the same reference frame.

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Poster

821. Visual Motion: Heading and Orientation

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Support: NEI R01 EY10287

T30 EY01319

ONR N000141110525

Title: Steering transforms cortical self-movement representation from direction to destination

Authors: *M. S. JACOB[1], C. J. DUFFY[2];
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Abstract: Steering demands the rapid detection of deviations in self-movement heading direction. This signal must drive the manual control of heading back to the intended destination. Extrastriate visual cortical neurons in the dorsal medial superior temporal area (MSTd) respond to the visual motion in optic flow that guides self-movement. We trained monkeys in an active steering task and recorded the activity of MSTd neurons to assess their role in real-time heading correction. We used a delayed match by steering task in which the monkeys first viewed a randomly selected Sample optic flow stimulus. After a brief delay, a second Steer optic flow stimulus appeared in which the heading location was offset from that in the Sample. The monkeys then used a joystick to steer the heading location back to Match the heading location in the Sample. Our two monkeys performed similarly in the steering task, achieving >80% correct performance with all trials being completed in 2-3 s. Neuronal responses to the Sample optic flow show strong radial pattern selectivity. However, most neurons change the magnitude and stimulus preferences of their optic flow responses across the stages of the task. Neuronal population responses revealed a decrease in optic flow selectivity that is attributable to systematic changes in neuronal heading preferences. The net effect is a transformation of the neuronal population response from representing a heading direction in the Sample optic flow, to representing the task specified heading location in the Match optic flow. We find that the optic flow selectivities of cortical neurons reflect interactions between bottom-up sensory cues and top-down task goals: in this task, from sensing a heading direction, to matching a spatial location. These findings support the view that MSTd neurons integrate bottom-up visual processing and top-down task demands.

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Poster

821. Visual Motion: Heading and Orientation

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NSF Grant 0845749

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Title: Neural control of steering by self-movement stimuli: A closed-loop system identification analysis

Authors: *M. S. MADHAV[1], W. K. PAGE[2], N. J. COWAN[1], C. J. DUFFY[2];
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Abstract: Steering relies on the real-time analysis of self-movement stimuli integrated with vehicular motor control. This sensorimotor integration is a closed-loop system that supports real-time responses to irregular changes in vehicular motion dynamics. We have now studied these responses, and the underlying neural mechanisms, in monkeys trained to drive a motorized sled. We trained monkeys to steer between nine room locations in a random sequence specified on a tablet video display that presented intermittent navigational cues. The monkeys moved on a large-scale, two-dimensional sled system during open viewing of the room. Self-movement visual cues were enhanced by the presence of a luminous patterned background light array creating an array of optic flow and object motion cues. The monkeys deflected a joystick and its position was translated into a force input applied to the programmed inertial dynamics of the sled. The monkeys were trained to drive towards the specified goal to earn liquid reward. We recorded behavioral responses as two-axis joystick deflection, sled position, velocity, and acceleration, and goal location. Neurophysiological responses were recorded as the activity of single neurons in dorsal medial superior temporal cortex (MSTd) that are known to integrate visual and vestibular signals about self-movement. In some driving trials, we superimposed systematic changes on the sled's dynamics to assess the impact of unexpected external forces on behavioral and neuronal responses. These perturbations were composed of the sum of five sinusoidal signals with randomized phases designed to be spectrally separate from frequencies elicited by sled movement. Joystick steering resulted in epochs of ballistic and corrective excursions, approximating the goal position with a ballistic movement and refining position with

smaller, corrective movements. These epochs were reflected in the monkeys' joystick deflections. Imposed perturbations also elicited joystick responses, reflecting externally induced corrective motor responses that reflected the frequency compositions of the perturbation. We find that the cortical processing of complex stimulus arrays is directly transformed into motor commands that diminish the effects of unexpected changes in motion dynamics. These studies establish a foundation for a system identification analysis of real-time steering.

Disclosures: **M.S. Madhav:** None. **W.K. Page:** None. **N.J. Cowan:** None. **C.J. Duffy:** None.

Poster

821. Visual Motion: Heading and Orientation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

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Office of Naval Research Grant N000141110525

Title: Receptive field dynamics shape optic flow responses of MSTd neurons

Authors: ***W. K. PAGE**, W. VAUGHN, C. J. DUFFY;
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Abstract: The panoramic radial patterns of optic flow inform us about our direction of self-movement. Optic flow is analyzed by the visual motion processing areas of dorsal extrastriate cortex, particularly neurons in the dorsal segment of medial superior temporal cortex (MSTd). We have previously described interactions between segments of the large receptive fields of MSTd neurons, finding evidence that spatial interactions promote optic flow selectivity. We now test that hypothesis by comparing conventional receptive field mapping to maps derived from reverse correlation analyses of large-field arrays of segmental local motion. We recorded MSTd neuronal activity during the presentation of visual stimuli on the central 90o circular area of a large-field video display. Monkeys were trained to maintain centered visual fixation during the interleaved presentation of three types of stimuli: 1) Full-field optic flow moving dot fields that simulate observer self-movement in 20 directions distributed throughout monkey-centered 3D space. 2) Twelve directions of planar local motion presented in 1 of 13 M-scaled segments of the stimulus area, the other segments being filled with stationary dots. 3) The randomized sequential

presentation of 12 directions of local motion simultaneously occupying all of the 13 stimulus segments as specified by a Kautz spatio-temporal sequence. Each stimulus presentation consisted of a 5s period of sequential presentation of 500 ms visual motion stimuli while the monkey maintained centered fixation to earn a liquid reward. We recorded 45 MSTd neurons in all three stimulus conditions. The single segment stimuli showed clear direction selectivity in idiosyncratic distributions, consistent with the range of receptive field architectures seen in MSTd. The Kautz sequences revealed response dependence on the combination of motion stimuli across segments, in a manner consistent with excitatory and inhibitory spatio-temporal interactions across neuronal receptive fields. The optic flow responses showed evidence of both local motion and global pattern response effects, suggesting the impact of the Kautz interactions in shaping optic flow response properties. We find evidence of robust spatio-temporal interactions between stimulus segments. Our current efforts focus on characterizing the non-linear interactions across the spatial extent and temporal sequence of stimulus presentation. Our goal is to test the hypothesis that these spatio-temporal interactions contribute to MSTd neuronal optic flow selectivity.

Disclosures: W.K. Page: None. **W. Vaughn:** None. **C.J. Duffy:** A. Employment/Salary (full or part-time):; Cerebral Assessment Systems.

Poster

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Title: Visual evoked potentials in behaving monkeys: Accessing selective attention and spatial orientation

Authors: *C. J. DUFFY[1], C. T. LOCKWOOD[2], W. VAUGHN[2], W. K. PAGE[2];
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Abstract: Self-movement surrounds us with radial patterns of visual motion that indicate our heading direction. These optic flow fields are accompanied by various objects, visually

stationary as they move with us (e.g., texting while driving), that can critically distract us from self-movement processing. We previously described optic flow and object evoked responses from scalp recordings in human subjects. Those responses show systematic changes with aging and Alzheimer's disease reflecting bottom-up/top-down interactions related to their navigational impairments. We have now recorded optic flow and object shape evoked potentials in monkeys performing stimulus-related tasks. Our goal is to understand the neural mechanisms of bottom-up/top-down interactions evident in human ERPs. Monkeys maintained centered visual fixation during the presentation of optic flow and three-shape objects. The optic flow alternates between 100% and 5% random motion coherence at 1.04 Hz; the objects alternate between three-shapes and dot clouds at 0.76 Hz. The monkeys perform a continuous left-right detection-discrimination task during sustained periods of visual stimulus presentation. When viewing optic flow, 80% of the stimuli have a centered radial centers-of-motion (COM), with 20% having COMs shifted to the left or right to require the monkeys to press the corresponding button. When viewing objects, 80% of the stimuli have three identical shapes, with 20% differing at the left or right shape to require pressing the corresponding button. Optic flow and object evoked potentials were recorded from 32 intracranial electrodes. Optic flow evoked posterior-lateral positive-negative responses peaking 95 and 158 ms after stimulus transition from random to patterned motion. The transition from patterned to random motion evoked a single negative response peaking 89 ms later. Shape objects evoked posterior-lateral positive responses peaking 121 ms after stimulus transition from dot clouds to shapes. The transition from shapes to dot clouds evoked a negative response peaking 79 ms later. We find that optic flow and object shape stimuli evoke discriminable evoked potentials in behaving monkeys. Both optic flow and object shapes yield distinct responses to the target and noise phases of alternating stimuli. These findings suggest that the visual processing of optic flow and object shapes can be accessed in monkey ERPs to analyze neural mechanisms of selective attention and spatial orientation.

Disclosures: **C.J. Duffy:** A. Employment/Salary (full or part-time):; Cerebral Assessment Systems, Inc.. **C.T. Lockwood:** None. **W. Vaughn:** None. **W.K. Page:** None.

Poster

821. Visual Motion: Heading and Orientation

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Topic: D.04. Vision

Support: DFG SFB/TRR 135 (A2)

Title: Visual selectivity for heading in the macaque ventral intraparietal area

Authors: *F. BREMMER[1], A. SCHLACK[3], A. KAMINIARZ[2], K.-P. HOFFMANN[3], M. LAPPE[4];
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Abstract: The patterns of optic flow seen during self-motion can be used to determine the direction of one's own heading. Tracking eye movements which typically occur during everyday life complicate this task since they add further retinal image motion and distort the retinal flow pattern. Humans employ both visual and non-visual (extraretinal) information to solve a heading task in such case. We have previously shown, that neurons in the monkey medial superior temporal area (area MST) use both signals during the processing of self-motion information. Here we report that also neurons in the macaque ventral intraparietal area (area VIP) use visual information derived from the distorted flow patterns to encode heading during (simulated) eye movements. We recorded responses of VIP neurons from two macaque monkeys to simple radial flow fields and to distorted flow fields that simulated self-motion plus eye movements. Stimuli were back projected onto a tangent screen, 48 cm in front of the monkey, covering the central 90° x 90° of the visual field. Optic flow sequences simulated self-motion over an extended horizontal plane, located 37cm below eye-level. In the simulated eye movement condition, monkeys had to fixate a central target. Real (natural) eye movements were induced in blocks of trials by removing the fixation target and allowing the animal to freely move his eyes. In almost two thirds of the cases cell responses kept the same heading selectivity irrespective of the simulated eye movement. As a consequence, responses of the population of neurons allowed to decode heading direction across all simulated eye movement conditions. Remarkably, response modulations, i.e., differences between the strongest and the weakest heading response for a given eye-movement condition, were smaller during real as compared to simulated eye movements, being indicative of predictive processing of the visual consequences of eye movements in area VIP. We conclude that the motion selectivities found in area VIP, like those in area MST, provide a way to successfully analyze and use flow fields during self-motion and simultaneous tracking movements.

Disclosures: F. Bremmer: None. **A. Kaminiarz:** None. **K. Hoffmann:** None. **A. Schlack:** None. **M. Lappe:** None.

Poster

821. Visual Motion: Heading and Orientation

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Support: National Eye Institute grants R01-EY10287

Office of Naval Research Grant N000141110525

Title: The neurophysiology of visual processing during simulated self-movement in an active driving task

Authors: *K. C. MUNGER[1], W. VAUGHN[2], C. J. DUFFY[2];
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Abstract: Driving is a survival skill integrating visual self-movement processing with motor control for steering. Visual cues about self-movement derive from the radial patterns of visual motion in optic flow, and the visual motion of discrete objects relative to a moving observer. Our goal is to understand how naturalistically superimposed optic flow and object motion interact to guide steering. We recorded behavioral responses and visual evoked potentials to optic flow and object motion in human subjects who were actively engaged in a steering task. Optic flow and object motion stimuli were projected onto a screen during centered visual fixation. The focus-of-expansion in outward radial optic flow, or the object stimulus location, was moved from the center of the screen to the left or right along the horizontal meridian. The subjects then turned a steering wheel to return the cue to the center. The optic flow alternated between 100% and 50% random motion in coherence. The object motion alternated between filled and dashed outlines. These stimuli alternated at different frequencies (1.04 and 0.76 Hz) to allow the separate analysis of the responses evoked by each. In a block-wise series of stimulus-task conditions, we presented optic flow or object motion alone or as superimposed, combined stimuli. In alone conditions, subjects steered by the presented cue. In combined conditions, subjects were told which cue to use in each stimulus block. We randomly alternated whether the superimposed cues moved congruently, where steering by either cue led to the successful re-centering of both, or incongruently, where only steering by the designated cue led to correct re-centering. We find that adding a second stimulus increases the time required to successfully complete steering trials when subjects were steering by optic flow. There was no comparable effect on trial completion time when subjects were steering by object motion. These effects were paralleled by second stimulus reduction in the N200 amplitude, both to optic flow when steering by flow, and to object motion when steering by the object. When steering by object motion, the flow N200 lost its later negative component. When steering by optic flow, the object N200 lost its later negative component. We conclude that optic flow and object motion interact in guiding steering behavior,

and that those interactions are reflected in stimulus and task effects on the ERPs. Task effects on the cortical processing of naturalistically combined optic flow and object motion are similar to those seen in earlier studies of task effects on single neuron responses in monkey extrastriate visual cortex.

Disclosures: K.C. Munger: None. **W. Vaughn:** None. **C.J. Duffy:** A. Employment/Salary (full or part-time):; Cerebral Assessment Systems Inc.

Poster

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Topic: D.04. Vision

Support: NEI Grant EY12576

NEI Grant EY022087

Title: The role of MST in visual-motor processing for navigation

Authors: *X. LI[1], S. W. EGGER[2], K. H. BRITTEN[1];
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Abstract: For most animals, it is an essential ability to guide themselves to goals in the surrounding environment. This navigation must rely on efficient visual-motor processing based on changing environmental cues while traveling. However, the neural mechanisms underlying such navigation are still unclear. The medial superior temporal area (MST) of the extrastriate visual cortex is known for responding to optic flow from self-motion. In this study, we used a monkey model of steering to study the role of MST neurons in processing visual cues for navigation behavior in a virtual environment. During physiological recording of MST neurons, the macaque monkey used a joystick to steer over a ground plane to pursue a target on the virtual horizon. The monkey fixated on an eccentric point, such that both target and ground plane were in the cell's receptive field. In this task, behavior depends on two cues: the angle to the steering target and optic flow rotation that results from the steering itself, which provides visual feedback about ongoing steering. Our results showed that MST responses were correlated with both cues, with the rotation response typically being of higher amplitude and shorter latency. We also compared responses during active steering with those during passive replay of retinally identical

stimuli. We found that during active steering, responses were more highly correlated with the target cue and showed shorter time-lags. These data show unexpectedly large responses to small, behaviorally relevant stimuli in MST, and help to reveal how multiple visual stimuli interact during naturalistic behavior.

Disclosures: X. Li: None. **S.W. Egger:** None. **K.H. Britten:** None.

Poster

821. Visual Motion: Heading and Orientation

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Support: NIMH IRP

JSPS 241108

Title: Differential contribution of external versus self-generated visual motion during natural viewing: An fMRI study in the macaque

Authors: *T. KANEKO[1], B. E. RUSS[2], D. A. LEOPOLD[2];
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Abstract: In natural vision, a substantial amount of retinal image motion is derived from one's own actions, and from movement of the eyes in particular. Accurate visual perception of the world thus hinges on being readily able to distinguish between the true movement of external objects and the visual signals consequent to one's own actions, sometimes termed reafference. The relative contribution of external movement, visual reafference, and internal signals generated in concert with action (e.g. efference copy) has been previously investigated in certain visual areas of monkeys, most notably cortical areas V1 and MT. In this study, we asked whether it is possible to map the relative contribution of external versus self-generated visual motion across the entire cerebral cortex. We performed fMRI in two monkeys while they viewed various types of natural movies. The subjects freely scanned the movies, and eye position was monitored throughout. Two different types of activity maps were generated, each based on correlating the voxel response time courses with regressors based on "retinal movies". The first analysis, which focused on the effects of eye movements, created such a movie by moving the position of a still image over a virtual retina on each frame according to the eye position. Applying motion

algorithms to this spatiotemporal sequence provided a family of functions allowing us to investigate the impact of eye movements, and the associated reafference, on visual processing. The second analysis, which focused on external motion, created a retinal movie from the real stimulus movie aligned on the center of visual field, as if monkey had continuously maintained fixation at the center of the movie. We applied the same algorithms to extract motion as in the first analysis. We found that external and self-generated motion exhibited striking difference in their patterns of activation throughout the visual system, even when the mathematical methods for extracting motion were the same between two analyses. Self-generated motion showed strong activation on the occipital cortex in areas V1, V2 and V3, as well as mid-level visual areas, such as ventral V4 and TEO. By contrast, external object motion dominated responses throughout the superior temporal sulcus (STS), including area MT, as well as in area V3a, lateral intraparietal sulcus and the fundus to posterior bank of the arcuate sulcus. Our results suggest that, while reafferent signals impact processing in several regions of the visual cortex, the brain is readily able to discount this input in its analysis of complex visual motion in several key areas of the dorsal visual pathway and throughout the STS.

Disclosures: **T. Kaneko:** None. **B.E. Russ:** None. **D.A. Leopold:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Title: Decoding depth: Representations of 3D versus 2D spatial information in human visual cortex

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[1]Psychology, [2]Ctr. for Cognitive and Behavioral Sci., The Ohio State Univ., Columbus, OH

Abstract: We live in a 3D world, and yet the majority of vision research is restricted to 2D phenomena. Previous research has shown that neural representations of 2D visual space are retinotopically mapped in the visual cortex. Certain visual areas are also known to be sensitive to depth information (including V3, V3v, V3A, V3B/KO, V7, LO, and MT). Here we investigate the precise nature of 3D spatial representations in the brain in comparison to 2D spatial information. We used fMRI and multi-voxel pattern analysis to explore how information about horizontal (x), vertical (y), and depth (z) location is represented in the brain. Participants viewed

random dot stereograms with red/green anaglyph glasses. Eight different locations were stimulated in a blocked design: each location was defined by x, y, and z location (left or right, above or below, and in front of or behind the fixation cross). The responses to each of the x, y, and z location conditions were compared across the brain with a searchlight analysis and within functionally localized ROIs. As expected, both x and y location information was present all along the visual pathways, with x information outperforming y information in higher visual areas. Importantly, depth location information could be decoded in several higher visual cortex regions, including V3a, IPS, and LOC. Interestingly, we found opposite trends for y and z location information, with y information decreasing from early to later visual areas and z information increasing in both dorsal and ventral streams. Our design allows us to go further and explore whether these depth representations are sensitive to or tolerant of changes in x and y location. These results suggest that despite the 2D retinotopic nature of visual input on the retina, 3D location information is present in visual cortex. We conclude that neural representations for visual objects are spatially specific in 3D space, with the relationship between x, y, and z location representations varying across different visual areas.

Disclosures: **N.J. Finlayson:** None. **J.D. Golomb:** None. **C.N. Kupitz:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

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Title: Perception of planar surface orientation relative to earth vertical

Authors: ***L. C. ELMORE**[1], R. M. CASSIDY[1], A. ROSENBERG[1], G. C. DEANGELIS[2], D. E. ANGELAKI[1];
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Abstract: Interacting with objects requires combining visual signals with information about one's head/body orientation to establish a representation of the visual environment relative to gravity. The role of the vestibular system in visual orientation perception is often studied using a

subjective visual vertical task in which subjects at different roll-tilts align a bar stimulus with perceived earth vertical (EV) in the 2D frontoparallel plane. However, 3D visual perception also requires a representation of object orientation relative to gravity about the pitch/slant axis. We investigated visual perception in this dimension by training 2 rhesus monkeys to judge the slant of a plane in a gravity-centered (G-C) reference frame, requiring the monkeys to integrate visual and self-orientation information. The monkeys sat in an apparatus that pitched the animal and an LCD monitor which displayed visual stimuli (random dot stereograms). Monkeys viewed identical planar slants (-40°:5°:40° in eye coordinates) at a range of pitches (-30°:5°:30°). Experiments took place in darkness with no visual cues other than the stimulus (a mask around the monitor blocked view of the room). Monkeys judged whether the planes were slanted forward or backward relative to EV. This decision was based on the computation of planar slant in world coordinates, which required the combination of a visual estimate of stimulus slant in eye coordinates with an estimate of self-orientation relative to gravity. We plotted responses as a function of stimulus slant in eye coordinates, such that accurate judgment relative to EV should manifest as a horizontal shift in the psychometric function equal to the magnitude of body pitch. For example, when pitched 30°, the monkeys' psychometric function shifted roughly 30°. We define percent compensation as the ratio of psychometric shift to body pitch (x 100). 100% compensation indicates a G-C reference frame, whereas 0% compensation reflects an eye centered reference frame. After training, monkeys Z and P had 94 and 96% mean compensation for the range of pitches tested. We performed a bilateral labyrinthectomy on one monkey to determine the role of vestibular signals in slant perception relative to EV. Without vestibular information, this monkey's ability to judge slant in a G-C reference frame was markedly impaired. Overall percent compensation was reduced to 34%, showing that vestibular information is critical to perceive slant in a G-C reference frame. The remaining compensation is likely due to other cues, such as proprioception. Our findings indicate that vestibular signals play a critical role in computing planar surface orientation in world coordinates.

Disclosures: L.C. Elmore: None. **R.M. Cassidy:** None. **A. Rosenberg:** None. **G.C. DeAngelis:** None. **D.E. Angelaki:** None.

Poster

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Support: NIMH IRP

Title: Dorsal stream contribution to shape perception

Authors: *V. ZACHARIOU, C. V. NIKAS, L. G. UNGERLEIDER;
LBC:Section on Neurocircuitry, NIH/NIMH, Bethesda, MD

Abstract: Recent evidence suggests that location-processing mechanisms within the dorsal visual pathway may contribute to some aspects of shape perception (Zachariou et al, 2013). The common resource for shape and location perception could arise because changes in an object's shape alter locations as well, namely, with respect to the edge-based parts or features that constitute an object (rather than with respect to other objects within a scene). By such an account, the spatial arrangement of the shape-based parts that constitute an object is a location process and the analysis of location is intrinsic to shape perception. Here, we explored this hypothesis in human adults performing a same-different object task on two categories of objects (faces and chairs) while undergoing functional magnetic resonance imaging (fMRI) at 3T. In each category, two exemplars presented simultaneously on a screen could differ in terms of the shape (featural differences) or the spatial configuration of their shape features (configural differences). For both the face and chair categories, configural differences led to significantly stronger activation within the dorsal visual pathway compared to featural differences and the magnitude of this activation correlated with behavioral performance. The dorsal visual pathway was localized using an independent distance-estimation localizer task. We conclude that location-processing mechanisms within the dorsal visual pathway process the spatial arrangement of the shape or edge-based features that constitute whole objects and through this mechanism contribute to shape perception.

Disclosures: V. Zachariou: None. **C.V. Nikas:** None. **L.G. Ungerleider:** None.

Poster

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Topic: D.04. Vision

Support: NWO Vici

NWO MaGW

Title: Figure-ground modulation of Gestalt objects in the visual cortex of the macaque monkey

Authors: *D. JEURISSEN[1], M. W. SELF[1], A. VAN HAM[1], P. R. ROELFSEMA[1,2,3];
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Abstract: The segregation of the visual scene into figure and ground is thought to require a combination of local analysis of features and boundaries combined with global analysis of scene layout in accordance with the Gestalt rules of visual perception. Neurons in primary visual cortex of macaque monkeys show enhanced responses on figures compared to background, an effect known as figure-ground modulation (FGM). This has been taken as evidence that global scene analysis can influence activity at the earliest cortical processing stages. However, FGM has typically been measured using relatively small, texture-defined squares on a uniform background and the possibility therefore remains that FGM arises through local computations. In the current study, we examined the neural responses in V1 and V4 to stimuli in which figure-ground relationships were defined by more global Gestalt rules. We recorded multi-unit activity from areas V1 and V4 of two macaque monkeys. On every trial, two horizontal strips of texture divided into segments were presented: one strip without Gestalt cues functioned as the baseline; the other strip contained figure-ground organization based on the Gestalt rules enclosure, convexity, symmetry, or the combination of these three cues. We also investigated whether these Gestalt figures lead to figure-ground perception in monkeys by measuring the perceived contrast of a Gabor patch that was presented on each strip. Specifically, the monkeys were trained to make a saccade to the Gabor patch of higher contrast and we compared perceived contrast of Gabor patches presented on a figure, background, and on ambiguous regions. The behavioral results showed that the monkeys perceived Gabors on figures as higher in contrast than Gabors on the background. This change in perceived contrast suggests that the monkeys indeed segregated the textured-strips into figures and a background according to the Gestalt criteria. We next examined neural responses in both V1 and V4 and observed that enhanced neuronal activity was elicited by texture elements that belonged to figures compared to elements that belonged to the background. The strength of this modulation correlated with the strength of perceptual segregation as judged by the perceived contrast differences. We conclude that neuronal responses in early visual areas are sensitive to the global structure of the visual scene. This result implies that figure-ground modulation in early visual cortex is established through feedback from higher visual areas, so that the global scene structure can influence the activity of neurons with small receptive fields.

Disclosures: **D. Jeurissen:** None. **M.W. Self:** None. **A. van Ham:** None. **P.R. Roelfsema:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Title: Coarse to fine-grained representation of object spatial layout in the Lateral Occipital Cortex (LOC)

Authors: *E. FREUD, T. GANEL, G. AVIDAN;
Ben Gurion Univ. of the Negev, Beer-Sheva, Israel

Abstract: Processing spatial configuration is a fundamental requirement for object recognition. Yet, little is known about the processes underlying the representation of object spatial layout in the human brain. Recent theories imply that visual perception operates in a multi-level fashion, such that a rapid, coarse representation is first generated and only later, a fine-grained description is formed. Here, we utilized fMRI adaptation to provide novel evidence that this processing scheme may support the representation of object spatial layout in the LOC. Participants preformed same / different classifications on pairs of 3D line-drawings of objects, half of which were spatially possible while the other half were spatially impossible. These latter stimuli could not be created in 3D space due to their incoherent spatial layout. Importantly, the impossible objects still possess valid Gestalt cues that may support a coarse representation; nevertheless, their spatial ambiguity may distort finer-level representation. In Experiment 1, the non-repeated trials were of possible and impossible objects which differed by a few features that defined object impossibility. Hence, this task required fine detailed representation of object layout. An adaptation effect was found only for possible, but not for impossible objects suggesting that LOC failed to form a fine-grained representation of impossible objects. In Experiment 2, the stimuli in the non-repeated trials were of fundamentally different possible and impossible objects, thus, this task could be performed based on a coarse object representation. Accordingly, similar adaptation levels were observed for the two object categories, implying that coarse object representations were generated in a similar fashion for the two object categories. Taken together, these findings suggest that a coarse-to-fine detailed processing scheme plays an important role in the representation of object spatial layout in LOC and that spatial ambiguity impairs fine-grained object representation.

Disclosures: **E. Freud:** None. **T. Ganel:** None. **G. Avidan:** None.

Poster

822. Visual Processing: Objects and Scenes

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NSF GRFP DGE-1256082

Title: Behavioral relevance changes feature selectivity in area V4

Authors: *D. V. POPOVKINA, A. K. PASUPATHY;
Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Our eyes receive an enormous amount of information which we are able to effortlessly use in a variety of time-demanding tasks. The visual system must be able to distinguish behaviorally relevant information, like a particular location or feature, from that which is less useful. How might this process affect the representation of objects in sensory visual cortex? For example, visual neurons often carry information about multiple features of a stimulus: in visual cortical area V4, neurons are selective for the shape and color of objects. When only one of these features is relevant for a behavioral task, how is a V4 neuron's feature tuning curve affected? Are there any changes in selectivity for the relevant or irrelevant feature?

To investigate this question, we recorded well-isolated single neurons in area V4 of 1 awake nonhuman primate (*Macaca mulatta*) as it performed delayed match-to-sample tasks with 2-D colored stimuli. The feature relevant for correct discrimination (shape or color) was indicated by a cue at fixation. Stimuli in the behavioral task were chosen based on detailed characterization of shape and color tuning during passive fixation, and included shapes/colors which elicited high, low, and intermediate levels of activity. Behavioral task effects were examined relative to responses during passive fixation. Preliminary results from 20 neurons suggest behavioral task conditions modulate shape and color tuning of individual V4 neurons. In 6/20 neurons, response to the most preferred value of a relevant feature was enhanced with respect to the others (a sharpening of the tuning curve); in 2/20 neurons, response to an intermediate value of the irrelevant feature was selectively enhanced (a broadening of the tuning curve). In 7/20 neurons, sharpening or broadening of tuning was observed in both behavioral contexts, suggesting this modulation was unrelated to the specific behavioral relevance of shape or color. Overall, these results demonstrate that behavioral relevance of a feature can modulate visual responses in some V4 neurons by changing tuning for specific features. V4 neuron responses are known to be affected by several types of attention; studies exploring this have typically reported increases in neuronal responses, reflecting a gain change for attended features/locations. Our findings suggest that beyond the expected response increase, behavioral relevance of features can change the feature selectivity of a V4 neuron. At this level of sensory processing, neuronal responses to a visual object are dependent on the behavioral context within which the object is viewed.

Disclosures: D.V. Popovkina: None. **A.K. Pasupathy:** None.

Poster

822. Visual Processing: Objects and Scenes

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 822.07/HH4

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Title: Advancing models of shape selectivity in V4

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Abstract: Our goal is to develop a biologically realistic model of processing in the ventral visual pathway that accurately predicts responses of V4 neurons to a wide variety of stimuli. Past studies have shown that V4 neurons are tuned to contour features at specific positions along the boundary of simple shapes (Pasupathy and Connor, 2001, J Neurophys 86:2505-19). This data has been successfully fit by the hierarchical model of Cadieu et al. (2007, J Neurophys 98:1733-50), which was designed to capture shape tuning and position invariance in V4 responses to isolated shape stimuli. Since the publication of this model, new physiological data has been collected in V4 using stimuli consisting of multiple shapes and involving partial occlusions. We seek to determine whether the Cadieu et al. model can account for responses to these more complex stimuli, thus providing deeper insight into mid-level visual representation, or whether significant modification of the model is required. The model follows a feed-forward hierarchy in which responses of many adjacent S1 units (Gabor filters modeling simple cells) are pooled by C1 units (complex cells) that compute the maximum over their inputs to generate modest scale and position invariance to boundary elements. A weighted sum of C1 units produces S2 units that are selective for particular spatial distributions of orientations. Finally, C2 units compute a max over similarly tuned but offset S2 units to amplify invariance while maintaining shape specificity. We fit the model to V4 responses for a battery of isolated shape stimuli, reproducing previous results on position invariance and boundary conformation tuning, and then tested it using stimulus perturbations that included rescalings, partial occlusions, and faux-shapes composed of discontinuous boundary elements. For partial occlusions, we found that the model responses were consistent with the initial transients of V4 neurons but not with later phases of the stereotypical response dynamics observed *in vivo*. This suggests that the initial shape responses may be captured by feedforward processing, but over time, more complex processing may transform the cortical representation to separate signals encoding the occluders from those encoding the occluded stimulus. The model also predicted strong responses to particular patterns of discontinuous oriented elements that optimally activated the C1 subunits, but that did not appear anything like the preferred shapes in our standard shape screen. This makes a strong prediction that we intend to test in V4 by closing the loop between model and experiment.

Disclosures: E. Nicholas: None. **A. Pasupathy:** None. **W. Bair:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: NI H R01-EY002966

NI H 11 R01EY016281-02

ONR N000141010278

Title: Behavioral importance of border ownership modulation in the visual cortex

Authors: *A. B. MARTIN[1], R. VON DER HEYDT[2,3];
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Abstract: We see a world filled with objects, but neurons in visual cortex signal elemental features such as pieces of contour. In cluttered scenes where one object occludes the other (which is the rule in ordinary visual environments) object recognition and searching for a specific object depend strongly on the correct assignment of contours to objects. Previous studies have shown that this assignment is reflected in differential responses of border ownership selective neurons in visual cortex which respond to a figure border with higher firing rate when the figure is on a neuron's preferred side compared to the opposite side. A model explains this by postulating feedback from "grouping cells" that enhance the responses of the widely distributed feature signals corresponding to an object. Here we show that these firing rate modulations are behaviorally important. We recorded single neuron activity from areas V1 and V2 of monkeys (macaca mulatta) while they performed a selective attention task. They had to discriminate between deformation and translation which required attending to the target as a whole. Neural activity preceding the critical change was analyzed. We found that stronger border ownership modulation during the waiting period was correlated with faster reaction times. The correlations were similar for target and distracter figures, but stronger for the target. Response measures unrelated to contour assignment, such as response onset latency and amplitude, were not correlated with reaction times. Our findings indicate that the border ownership processing in these neurons is a critical stage in object perception.

Disclosures: A.B. Martin: None. **R. von der Heydt:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: Mozaiek grant 017.005.107 from the Netherlands Organization for Scientific Research (NWO)

Title: Recurrent processing contributes to natural scene categorization only for scenes of high complexity

Authors: *I. I. GROEN, S. JAHFARI, H. SCHOLTE;
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Abstract: Numerous experiments show that object recognition in natural scenes occurs extremely rapidly, suggesting that it requires only feed-forward visual processing. On the other hand, recurrent processing is believed to be involved in many operations relevant for object recognition, such as grouping and figure-ground segmentation. Here, we examined whether recurrent activity during object recognition depends on scene complexity. We systematically manipulated scene complexity by selecting natural scenes on two low-level, biologically plausible image statistics: contrast energy (CE) and spatial coherence (SC). These statistics summarize local edge intensity and higher-order correlations between edges in scenes. Together, they are diagnostic of scene complexity because they index the degree of sparseness versus clutter in the scene (e.g. whether it contains a bird against the sky versus a deer in the woods). Subjects performed an animal vs. non-animal categorization task in the fMRI scanner for scenes with low, medium or high CE/SC values. Slowed reaction times and increased error rates indicated that animal categorization was especially difficult for scenes from the high complexity condition, i.e., scenes with high CE/SC values (low sparseness, high clutter). Accordingly, in early visual cortex (V1), animal scenes gave rise to increased fMRI activity for high complexity scenes only. High complexity scenes further decreased activity in the parahippocampal place area (PPA; more active for non-animal scenes), but no modulations by condition were found in the lateral occipital cortex (LOC; more active for scenes with animals). Separate ERP recordings showed that the increased V1 activity for animal scenes with high complexity is not due to low-level differences between animal and non-animal scenes, but that it instead reflects visual feedback activity present from ~200 ms after scene onset. Thus, upon presentation of a complex scene, a lack of differential activity for animal vs. non-animal scenes in high-level scene-selective cortex is accompanied by increased recurrent activity in early visual areas. These results suggest that the degree of recurrent activity during object detection in natural scenes depends on the complexity of the scene as described by CE and SC. For sparse scenes with clear

figure-ground segmentation, feed-forward activity may be sufficient, whereas for cluttered scenes, object recognition involves feedback.

Disclosures: I.I. Groen: None. **S. Jahfari:** None. **H. Scholte:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: NIMH Intramural Research Program

Title: The time course of scene processing: A multi-faceted EEG investigation

Authors: *A. HAREL[1], I. I. A. GROEN[2,3], D. J. KRAVITZ[4], L. Y. DEOUELL[5,6], C. I. BAKER[1];
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Abstract: Humans are extremely adept at recognizing complex visual scenes, an ability supported by a network of scene-selective cortical regions. In spite of growing knowledge about the functional properties of these regions, much less is known about the temporal dynamics underlying scene processing. We measured electroencephalography (EEG) activity and used three distinct approaches to identify electrophysiological markers of scene processing. First, we adopted an event-related potential (ERP) approach and contrasted responses to faces, objects and scenes. The first ERP component to evidence scene selectivity was the posterior-lateral P2, peaking around 200 ms post-stimulus onset, with highest amplitude to scenes relative to both objects and faces. In comparison, the sensory-driven P1 component was equal in amplitude to all categories, and the N170 showed the expected face selectivity with highest amplitude to faces relative to both objects and scenes. Second, to investigate what scene properties are represented by the scene-selective P2, we presented the same participants with a set of 96 diverse real-world scenes spanning three stimulus dimensions: spatial layout (open/closed), relative distance

(near/far), and semantic content (manmade/natural). Consistent with its putative role in scene processing, P2 was sensitive to the spatial layout of the scene (higher amplitude to closed than open scenes). P2 amplitude was also diagnostic of the distance and the semantic content of the scene, but each of these dimensions separately interacted with P2's sensitivity to spatial layout. Finally, we evaluated the extent to which these scene properties map onto low-level summary statistics previously shown to be diagnostic of global scene recognition (Groen et al., 2013). Focusing on individual scenes, we found that the spatial coherence of the scene can be used to differentiate manmade from natural scenes, and closed from open scenes. Single trial analysis revealed that spatial coherence modulates EEG activity as early as 100 ms post stimulus onset but persists through the P2 time window as well. In sum, using a multi-faceted approach, our results demonstrate how multiple scene properties influence the processing of scenes. We further suggest that P2 can be used as an index of scene-related processing.

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Poster

822. Visual Processing: Objects and Scenes

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Program#/Poster#: 822.11/HH8

Topic: D.04. Vision

Title: Visual material perception in the human brain: Glossiness versus roughness

Authors: *H.-C. SUN[1], A. WELCHMAN[2], M. DI LUCA[1];
[1]Psychology, Univ. of Birmingham, Birmingham, United Kingdom; [2]Psychology, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Identifying what objects are made of from their appearance is crucial in our daily life since material properties determine how we should interact and how we can manipulate such objects. In previous studies, information about the appearance of materials was found to be represented in ventral visual areas such as fusiform gyrus (FG), inferior occipital gyrus (IOG) and collateral sulcus (CoS) (Cant, Arnott, & Goodale, 2009; Cant & Goodale, 2007; Hiramatsu, et al., 2011). However, the specific network of areas for the representation of materials and of material properties remains unclear. Here we compared the network involved in processing glossiness with the network involved in processing visual roughness. Our rationale is that if glossiness and roughness provide similar information about object material, then they should

share neural resources. Alternatively, the two cues could inform about complementary aspects of objects and then they should be analysed separately. We used Blender to render three abstract objects in four conditions: 1. Glossy, specular components were present, 2. Glossy control, these specular components of the image were rotated by 45 degree making the objects look not glossy while keeping most information of the in Glossy condition. 3. Rough, wave textures were applied to the displacement mapping, creating bumps in the object geometry. 4. Rough control, the same wave textures were applied to the reflectance of the surface, creating similar texture patterns while leaving a smooth object geometry. The images in Glossy condition were rated significantly glossier than in Glossy control condition and the images in Rough condition were rated significantly rougher than in Rough control condition by five naïve participants. Another 12 participants took part in a block-designed fMRI session and performed 1-back matching task on the images. Functional MRI activations were measured over the whole brain with echo-planar imaging (EPI) sequence (32 slices, TR 2000 ms, TE 35 ms, voxel size 2.5 × 2.5 × 3 mm) and an 8-channel head coil. We analyzed the data using a general linear model (GLM) and found that glossiness and roughness activated different areas in the brain: fusiform gyrus (FG) and collateral sulcus (CoS) regions were more active in Glossy condition than its control condition (see Cant, et al., 2009; Cant & Goodale, 2007; Hiramatsu, et al., 2011) while primary somatosensory area (S1) showed stronger activation in Rough condition compared with its control. Thus, results indicate that different networks are involved in the processing of surface glossiness and roughness, suggesting a different informational contribution of the two visual signals.

Disclosures: H. Sun: None. **A. Welchman:** None. **M. Di Luca:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: National Science Foundation of China 61071003

China Postdoctoral Science Foundation 2012M510398

Title: Hierarchical processing underlying object representation in human visual cortex

Authors: *R. XU, B. HONG;
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Abstract: The human brain is hierarchically architected, which arguably enables high-level abstractions. Here we used electrocorticography (ECoG) to reveal the rapid dynamics of object processing within and across multiple stages in human visual cortex. We evaluated millisecond-resolution neural responses to 120 objects from six object categories, by high gamma (broadband) ECoG activty from 141 visually responsive electrodes (50-300 ms poststimulus, $p<0.0001$) in 15 epilepsy patients. After the object category was first decodable (~100 ms), the structure of between-category neural distance continued to evolve in extrastriate visual areas along a converging direction. Meanwhile, nontrivial invariance to object categories emerged, and its dynamics were quantified by linear classification analysis: the invariant representation was initially observed in lower-level areas, but then rapidly build up in higher-level areas to a much larger extent. Although the early neural codes appeared majorly due to feedforward mechanisms, build-up of more sophisticated representation was accompanied by top-down dominated interareal connectivity. These results suggest that recurrent and feedback computations may be crucial in hierarchical visual processing of objects.

Disclosures: R. Xu: None. **B. Hong:** None.

Poster

822. Visual Processing: Objects and Scenes

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Program#/Poster#: 822.13/HH10

Topic: D.04. Vision

Title: Functional networks mediating convergence of object- and space-centered scene processing pathways in the PPA: Information-based connectivity analysis

Authors: *D. LINSLEY, S. MACEVOY;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Behavioral data suggest that scene recognition draws heavily upon analysis of scenes' spatial properties, particularly their layout (e.g. Greene & Oliva, 2009). At the same time, scene recognition is strongly influenced by the kinds of objects scenes contain (e.g. Davenport & Potter, 2004). We recently used multivariate analysis (MVPA) of fMRI data (Linsley and MacEvoy, Cerebral Cortex 2014) to demonstrate that these two routes to scene recognition

converge, at least partially, in the parahippocampal place area (PPA), an area of ventral-temporal cortex sensitive to scenes' spatial properties (e.g. Kravitz et al., 2011). Along PPA pattern dimensions that encoded scenes' spatial properties, extremely small and large scenes were encoded as spatially more similar to their category average when category-informative objects were visible versus masked. We hypothesized that this object-triggered bias aids scene categorization by harmonizing scenes' encoded spatial properties with expectations derived from their object contents. In the present study, we applied information-based functional connectivity analysis (iFCA) to identify brain regions contributing to PPA the object information needed to instantiate this bias. Brain activity patterns were recorded while participants viewed images of bathrooms varying in size and with their informative objects (e.g., toilets and bathtubs) either visible or masked. Next, we used whole-brain searchlight MVPA to identify voxel clusters containing significant information about scenes' object-masking state. For each such cluster, we used mediation analysis to measure the extent to which single-trial scores along the pattern dimension capturing object-masking state explained the trial-wise variability of PPA spatial codes. This analysis revealed contributions to PPA spatial codes by regions of the visual system and the default-mode network (DMN). Consistent with PPA resting-state connectivity (e.g. Baldassano et al., 2013), regions of the DMN were linked preferentially to anterior PPA (APPA) while visual areas connected primarily to posterior PPA (PPPA). The greatest contributor of object information to PPPA spatial codes was the lateral occipital complex, a region previously implicated in object-based scene recognition (e.g. MacEvoy et al., 2011). Taken together, these results provide further evidence for a view of PPA as a point of functional convergence between object- and spatial property-based routes to scene categorization.

Disclosures: D. Linsley: None. **S. MacEvoy:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: McKnight Foundation

Sloan Foundation

Title: Rapid plasticity of visual object selectivity in inferior temporal cortex

Authors: *D. J. FREEDMAN[1], J. L. MCKEE[2];
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Abstract: Our ability to recognize complex visual stimuli depends critically on our past visual experience. For example, we easily and seemingly automatically recognize familiar visual stimuli such as a friend's face, our bicycle, or the characters on a written page. Visual form recognition depends on neuronal processing along a hierarchy of visual cortical areas (e.g. areas V2, V4, TEO) which culminates in the ITC (area TE), which contains neurons which show exquisite selectivity for complex visual stimuli. Although both passive experience and explicit training can modify or enhance visual selectivity in ITC, the mechanisms by which experience and learning establish new mnemonic representations for visual recognition are not understood. In order to investigate how neuronal responses to visual stimuli in inferior temporal cortex (ITC) change with increasing familiarity due to stimulus repetition, we recorded from ITC neurons while monkeys viewed a group of initially novel stimuli approximately 50 times each. Across the population of all ITC neurons, we observed an increase in stimulus selectivity with increasing repetitions, measured by the sparseness of encoding. In examining how stimulus representations change with increasing viewings, we observed a subset neurons that while initially weakly or non-selective, developed strong stimulus selectivity over the course of 10-40 image repetitions. The neurons showing this strong emergent selectivity were over-represented in putative inhibitory (narrow-spiking) compared to putative excitatory (broad-spiking) neurons. When separating these two populations, we observed an average decrease in activity with increasing repetitions in putative excitatory neurons and an increase among putative inhibitory neurons. While neurons showing this emergent selectivity were relatively rare (about 10% of all recorded cells) and have not been reported before, their discovery helps to explain how the strong stimulus selectivity often observed in ITC neurons may develop through learning.

Disclosures: D.J. Freedman: None. **J.L. McKee:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: Office of Naval Research – MURI contract number N000141010934

Title: High-dimensional encoding of scenes in the PPA, RSC, and OPA

Authors: *E. M. AMINOFF[1], M. TONEVA[1,4], A. GUPTA[2], M. TARR[1,3];
[1]Ctr. for Neural Basis of Cognition, [2]The Robotics Inst., [3]Dept. of Psychology, Carnegie Mellon, Pittsburgh, PA; [4]Dept. of Computer Sci., Yale Univ., New Haven, CT

Abstract: Three regions of the cortex have been associated with scene perception: the parahippocampal cortex (PPA), the retrosplenial complex (RSC), and the occipital place area (OPA). However, little is known about how these regions relate to one another, and what aspects of scenes are processed in these regions. We examined the neural patterns - as measured by fMRI - associated with individual scenes within these three regions. In a slow event-related design, participants viewed 100 different scenes - 50 scene categories, two exemplars from each. To assess the differences in how the three regions encode scene information we used a representational similarity analysis in which pairwise correlations between the voxel-by-voxel patterns associated with each scene were analyzed to create a similarity matrix for a given region. We found that the PPA's pattern of fMRI activity was strongly correlated with both the RSC and the OPA, whereas the RSC and the OPA were correlated the least. These data suggest that the PPA may be an intermediate encoding phase between the OPA and the RSC. To examine the specific aspects of scenes that are encoded in these regions we employed a machine vision system that relies on large-scale data methods to measure mid- and high-level scene statistics (Never Ending Image Learner, NEIL, http://neil-kb.com/). NEIL has analyzed over one million images to learn commonsense relationships between scene categories and visual attributes. These relationships and attributes form a pool of candidate mid-level features that may be encoded within the human brain. NEIL defines a high-dimensional scene space for characterizing scenes based on such features, for example a baseball field has an open area, or the inside of a tent can have a diamond shape. In our study we tested how well NEIL attributes account for the measured variance in the neural encoding of scenes. This computationally-derived candidate model of scene attributes was able to account for the encoding variation in all three brain regions. Moreover, increasing the number of attributes used to define the space increased the correlation between attribute space and the PPA and the OPA, but not the RSC. These results indicate that scene encoding within the OPA and the PPA may be based more on visual information than within the RSC. To further address this observation, we also examined how much high-level semantic information explained neural variation over and above that of the visual attributes. Together our results explicate a network of brain regions supporting scene processing. We propose that information may flow through the OPA to the PPA to the RSC, with the first two regions emphasizing the visual analysis of scenes.

Disclosures: E.M. Aminoff: None. **M. Toneva:** None. **A. Gupta:** None. **M. Tarr:** None.

Poster

822. Visual Processing: Objects and Scenes

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Athinoula A. Martinos Imaging Center at the McGovern Institute for Brain Research, MIT

Title: Convolutional neural networks predict the representational geometry embodied in the human visual processing hierarchy

Authors: *R. M. CICHY[1], A. KHOSLA[1], D. PANTAZIS[2], A. TORRALBA[1,3], A. OLIVA[1]; [1]CSAIL, [2]BCS, [3]Eecs, MIT, Cambridge, MA

Abstract: Humans recognize objects in real world settings with ease and efficiency, subserved by cascades of hierarchically organized cortical areas. Here, we combined human functional magnetic resonance imaging (fMRI) and state of the art computational modeling by representational analysis. In particular, we tested the hypothesis that ascending layers in deep convolutional neural networks correspond to ascending cortical regions in the visual hierarchy. For this, we used an 8-layer deep convolutional neural network (CNN) trained to categorize real-world images into 1000 different categories. We presented the same set of 118 images of different categories to the CNN, and human participants while recording fMRI data. We then used representational similarity analysis and searchlight analysis to determine where in the brain representational geometry was similar to layers of the neural network. Importantly, the CNN was in no way constrained by the acquired fMRI data. We used permutation tests and family-wise error correction for multiple comparisons ($p<0.05$) across voxels to establish significance. Our findings show that early layers of the CNN corresponded to posterior early visual regions, and later layers of the CNN corresponded to higher cortical regions (Figure 1) reaching deeply both in the ventral and dorsal visual stream. Our results indicate that convolutional neural networks are promising models of the visual processing hierarchy embodied by the human visual brain.

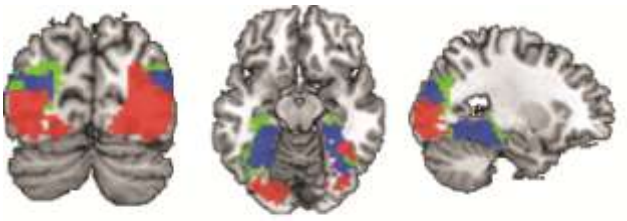

Figure 1. Correlation in representational geometry between convolutional neural network layers 1 (red), 5 (blue) and 8 (green) and fMRI patterns in the brain.

Disclosures: **R.M. Cichy:** None. **A. Khosla:** None. **D. Pantazis:** None. **A. Torralba:** None. **A. Oliva:** None.

Poster

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BPENDURE

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Title: Non-human primates behaviorally demonstrate object invariance and categorization

Authors: ***M. RABADI**, N. MAJAJ, L. KIORPES;
Ctr. for Neural Sci., New York, NY

Abstract: Object recognition and categorization are essential for our ability to function in the world. Human adults effortlessly recognize objects while tolerating changes in their relative size, pose, place in the visual field, or other identity preserving transformations, a property called 'invariance.' Furthermore, humans are able to organize similarly shaped objects into categories. Many studies suggest that the neurons of inferior temporal cortex give rise to the perceptual experience of objects. These neurons are invariant to identity preserving transformations and respond similarly to pictures of objects that are in the same category. However, these neurophysiological studies are done in monkey cortex while the monkeys are fixating rather than

while discriminating objects, so it remains unclear whether monkeys perceive and categorize objects the same way that humans do. To test for invariance, we trained two adolescent pigtailed macaques (Macaca nemestrina) to recognize objects despite certain identity-preserving transformations. Utilizing an object oddity task, we trained the subjects to touch the odd one of three stimuli despite transformations of size, changes in relative rotation, and the presence of a background. Like humans, the monkeys learned to ignore changes in rotation, place in the visual field, and the presence of a background. The monkeys also demonstrated size invariance over a restricted range, but their performance dropped with larger size differences. To address the issue of categorization we trained the monkeys on eight human-defined categories. In this case the two distractors were exemplars from one category and the target was an exemplar from a second category. We used multidimensional scaling to compare monkey and human performance. While the monkeys performed similarly to each other, there were some differences in their category definitions. The monkeys' performance on the task also differed from a human tested under the same conditions, suggesting that even explicit training on human-defined categories may be limited by inherent visual similarities. These results are a first step toward establishing a stronger link between neuronal responses and invariant object recognition ability.

Disclosures: **M. Rabadi:** None. **N. Majaj:** None. **L. Kiorpes:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: Wellcome Trust - DBT India Alliance

Title: How do object parts determine dissimilarity relations?

Authors: ***P. R. TARANATH**[1], S. P. ARUN[2];
[1]Ctr. for Neurosci., Dr. S.P.Arun's Lab., Bangalore, India; [2]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: It has long been thought that reducing objects into their component elemental parts would elucidate object perception. This is possible only if the object parts have a systematic relationship with the whole object. If this is true, it suggests that dissimilarity between objects can be explained in terms of dissimilarities between parts. This relationship may be linear or

nonlinear. Here we set out to investigate this problem by studying dissimilarity relations in a set of abstract objects. Each object contained two parts joined together by a stem. Seven parts were used at each location in a combinatorial fashion to produce 49 objects. We estimated perceived dissimilarity using a visual search paradigm in which human subjects had to find the oddball item in an array. The reciprocal of the time taken by humans to find one object among multiple instances of another object was taken as a measure of dissimilarity between the two objects. The main finding of our study is that the dissimilarity between a pair of objects is a linear sum of the dissimilarities between every pair of their constituent parts ($r = 0.75$, $p < 0.005$). Thus, for two objects AB and CD, their overall dissimilarity is a linear sum of the dissimilarity between AC, AD, BC and BD. Our analyses revealed several interesting insights about how object parts contribute to the overall dissimilarity: (1) Dissimilarity relations between parts is independent of their spatial location in the object; (2) Parts at corresponding locations in a pair of objects (i.e. AC & BD) contribute more to the overall dissimilarity than parts at opposite locations (i.e. AD & BC). This indicates that dissimilarity computations involve some degree of part registration; (3) the contribution of opposite parts was significantly weaker for objects in a vertical orientation compared to the same objects in a horizontal orientation. This indicates that opposite part dissimilarities might be modulated by mirror confusion; (4) the contribution of opposite parts became significantly weaker on elongating the stem connecting the two parts. Thus, the part registration process is specific to spatial location; (5) Symmetric objects tended be more dissimilar than expected in the model; (6) Objects that were mirror images of each other were more similar than expected in the model – this effect was stronger for horizontally oriented objects than for the same objects in a vertical orientation. Taken together our findings suggest a surprising and systematic linear rule by which objects are related to their parts.

Disclosures: **P.R. Taranath:** None. **S.P. Arun:** None.

Poster

822. Visual Processing: Objects and Scenes

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: Wellcome-Trust DBT India Alliance

Council for Scientific and Industrial Research

Title: Effect of silhouetting and inversion on object representations in monkey inferotemporal cortex

Authors: *N. R. MURTY, S. P. ARUN;
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Abstract: Our visual system has a remarkable tolerance for objects across changes in the viewing angle. We have previously shown that, for natural upright objects, neuronal responses in the inferior temporal cortex exhibit a transition from view dependence early in the response to view invariance later in the response. Is this transition a hallmark of a specialized 3-d object representation? Specifically, does it still occur upon reducing objects to their silhouettes? Does it occur for inverted objects? To address these questions, we recorded the responses of neurons in the monkey inferotemporal cortex to upright, inverted and silhouette versions of a set of 24 natural objects in their profile & oblique views. The main findings are as follows: (1) IT neurons show widespread view invariance for upright, silhouette and inverted objects; (2) However, there was a weaker transition from view dependence to invariance for inverted and silhouette versions; (3) silhouetting and inversion affected view invariance differently for animate and inanimate objects: silhouetting influenced inanimates more compared to animates, whereas inversion affected animates more than inanimates. (4) We obtained similar results in an analysis restricted to single views, suggesting that the impact of silhouetting and inversion on view invariance was due to changes in the object representation itself rather than in view invariance per se. That animate representations are less affected by silhouetting can be explained by the fact that animates contain less internal features. Likewise, the greater impact of inversion on animates might be because animate representations are specialized for an upright orientation. Our results suggest that view invariance in IT neurons is intimately linked to three-dimensional structure and possibly specialized for orientation.

Disclosures: N.R. Murty: None. **S.P. Arun:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Support: NIH Grant EY022538

Title: Reliability-dependent convergence of 3D visual cues in parietal cortex

Authors: *A. ROSENBERG, D. E. ANGELAKI;
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Abstract: Creating accurate three-dimensional (3D) representations of the environment from 2D retinal images is a fundamental task for the visual system. However, the reliability of 3D spatial information conveyed by different visual signals depends inherently on viewing geometry, such as how much an object is slanted in depth. Human psychophysical studies correspondingly show that qualitatively different visual signals including texture and binocular disparity are combined according to their slant-dependent reliabilities to create robust 3D object representations, but where and how this occurs in the brain is unknown. Here we examine how the convergence of these cues depends on their relative reliability in the caudal intraparietal area (CIP) of the macaque monkey by recording slant tuning curves using mixed-cue (texture + disparity) and cue-isolated (texture or disparity) planar stimuli. Most slant tuning curves measured with the disparity stimuli were significantly tuned (58/59 cells, 98%, N = 59 cells). A smaller percentage of tuning curves measured with the texture stimuli viewed either binocularly (32/49, 65%, N = 49 cells) or monocularly (15/22, 68%, N = 14 cells) were also significantly tuned. Interestingly, the accounted variance between texture and mixed-cue responses was found to increase on average by 14% when the texture stimuli were presented monocularly rather than binocularly. Using Z-scored partial correlations between the mixed-cue and cue-isolated tuning curves (71 comparisons), we classified the mixed-cue responses as 'disparity dominated' (56/71, 79%) or 'texture dominated' (8/71, 11%). The remaining 7/71 mixed-cue responses (10%) were not classifiable since the texture and disparity tuning curves were highly similar, and can thus be considered 'cue-invariant.' We then took the difference between Z-scored partial correlations (texture minus disparity) as an index of the relative contributions of the two cues, such that larger positive values indicate a greater contribution of texture cues. This index was positively correlated with the preferred slant measured with the mixed-cue stimuli ($r = 0.41$, $p < 0.005$). Consistent with theoretical and psychophysical results showing that the reliability of texture relative to disparity cues increases with slant angle, this indicates that texture cues contribute more to the mixed-cue responses of CIP neurons that prefer larger slants. Together, the present results demonstrate the reliability-dependent convergence of 3D visual signals in parietal cortex, suggesting that area CIP may play an important role in creating a robust, multimodal 3D representation of the environment.

Disclosures: A. Rosenberg: None. **D.E. Angelaki:** None.

Poster

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Topic: D.04. Vision

Title: Action affordances among semantically related objects mediates competition in V4

Authors: *E. WAGER[1], G. HUMPHREYS[2], P. E. SCALF[1];
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Abstract: When multiple items fall within the receptive field of a common V4 cell population, their representations compete in a mutually suppressive manner (Scalf, Basak and Beck, 2011). This competition can be reduced when stimulus factors such a color (Beck & Kastner, 2007) or shape (McMains and Kastner, 2011) cause them to form a unified percept. Here, we ask whether higher-level grouping factors also reduce competition for representation amongst cells in V4. Parietal lobe patients are less likely to extinguish an object grouped appropriately for action (i.e. a wine bottle pointing towards a glass) than an object positioned inappropriately for action (a wine bottle pointing away from a glass), possibly because they form a single perceptual unit whose members are less likely to compete for representation (Riddoch et al., 2003). Here, we use fMRI to directly test this hypothesis. We presented subjects with pairs of action objects (e.g. pizza and pizza cutter) in the upper left visual field (center to center separation = 2.2 degrees). Objects were either positioned with correct (pizza cutter above and right of pizza) or incorrect (pizza cutter below and left of pizza) action affordances. We manipulated the items potential to compete for representation by presenting them either simultaneously (likely to compete) or sequentially (unlikely to compete). Items compete less for representation in V4 when presented in the correct action affordance (Sensory Suppression Index (SSI) = .097 for correct and SSI=.246 for incorrect affordance, $p < .05$). In experiment two we explore whether competition in V4 can be mediated by action affordance amongst non-semantically related action pairs, as suggested by Roberts & Humphreys, 2010. Participants viewed only non-semantically paired action objects. Under these conditions, competition in V4 was insensitive to action affordance (SSI = .120 and .159 for correct and incorrect action affordance respectively, $p > .5$). We find that action affordances reduce competition among V4 representations of semantically related items.

Disclosures: E. Wager: None. **G. Humphreys:** None. **P.E. Scalf:** None.

Poster

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Topic: D.04. Vision

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NSF Science of Learning Center SBE0542013

Title: Neural coding of point-light dynamic objects

Authors: *J. A. PYLES[1], M. J. TARR[2];
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Abstract: Dynamic objects are ubiquitous in our visual environment, however the neural mechanisms for encoding moving objects are not well understood. In previous work we showed that a large areas of visual cortex subsuming LOC and hMT+ are involved in perception of dynamic objects (Pyles & Tarr, 2013). We also used multi-voxel pattern analysis (MVPA) to show that almost all visual regions active during dynamic object perception also encode information about these objects that is invariant to changes in viewpoint, articulation, and size. Here we attempt to focus our investigation on the kinematics of dynamic objects by using point-light animations that have sparse form information. We conducted an MVPA fMRI experiment across two scanning sessions. In one session, subjects viewed point-light animations of three novel, articulating, dynamic objects where only tokens on the object's joints were visible. In another session subjects viewed short animations of the same three objects, but with the structure clearly visible. In both sessions, subjects were presented with 80 different example animations (never repeated) of the dynamic objects that varied in viewpoint, size, and motion path. We then conducted both whole-brain searchlight and ROI analyses from independent localizers using a support vector machine (SVM) pattern classifier. Our results show that multiple regions of visual cortex have significantly above chance classification accuracy in decoding dynamic object identify from point-light animations. A second MVPA analyses sought to investigate if common patterns of neural activity were present when viewing point-light and structure visible counterparts of dynamic objects. To do so, the classifier was trained on point-light data only, and then tested on structure visible data, and vice versa. This training and testing across point-light and full structure presentations also yielded significantly above chance accuracy in multiple visual areas. Results indicated some shifting of accuracy to motion selective cortical areas, suggesting that the kinematic information present in both the point-light and full structure versions of the animations is similarly encoded. Finally, we present an analysis of patterns of information similarity in the network of identified ROIs derived from classifier performance in

each area. In sum, our results show the ability to classify novel dynamic object identify from point-light animations alone, and also to cross-classify dynamic object identity between point-light and full structure stimuli. This suggests that invariant kinematic information about dynamic object identity is encoded in multiple regions of visual cortex.

Disclosures: J.A. Pyles: None. **M.J. Tarr:** None.

Poster

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Eugene McDermott Foundation

Title: Invariant representations for action recognition in the human visual system

Authors: *L. ISIK, A. TACCHETTI, T. POGGIO;
MIT, Cambridge, MA

Abstract: The human brain can rapidly parse a constant stream of visual input. The majority of visual neuroscience studies, however, focus on responses to static, still-frame images. Here we use dynamic video stimuli to study invariant action recognition with MEG decoding analysis (also known as MVPA or readout). MEG provides an optimal tool for studying this problem, due to its high temporal resolution. In this study, subjects viewed a series of videos of different actors performing different actions (e.g. run, walk, jump) under different transformations (e.g. viewpoint, speed of execution) while in the MEG. In order to assess the amount of transformation invariant stimulus information in the neural signals, we decoded which action the subject was viewing based on their MEG data at each time point. Action identity can be decoded

with above chance accuracy during the period of 400-700 ms after stimulus onset. Both decoding accuracy and human behavioral performance are significantly lower when motion coherence is disrupted. These results show that even with form information preserved, a lack of motion coherence negatively impacts action recognition, and this impairment can be detected in the decoded MEG signals. Finally, we developed a computational model that agrees with these MEG data. Based on these results, we propose a biologically plausible computational framework to recognize actions across complex transformations.

Disclosures: L. Isik: None. **A. Tacchetti:** None. **T. Poggio:** None.

Poster

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Topic: D.04. Vision

Support: NIH 1 RO1 EY 02231801A1

Title: Differential temporal capacity across category-selective regions in human high-level visual cortex

Authors: *A. STIGLIANI, K. S. WEINER, K. GRILL-SPECTOR;
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Abstract: Human high-level visual cortex contains category-selective regions that respond more strongly to faces, bodies, words, and places compared to other stimuli, but the temporal processing capacity of these regions is unknown. Previous fMRI studies suggest that face and place-selective regions can process 6-9 items/sec (Hz). However, the results of these studies are difficult to interpret because they examined the temporal processing capacity of just one or two stimulus categories and confounded presentation rate with the number of stimuli in an experimental block. Using a novel paradigm that corrects these confounds, we determined the temporal processing capacity of multiple regions in ventral temporal (VTC) and lateral occipitotemporal cortex while maintaining the number of items in a block constant. Subjects were scanned with fMRI while viewing images of characters, bodies, faces, and places presented at 1, 2, 4, or 8 Hz. Critically, stimuli were presented in 8-image blocks at all rates to maintain a constant number of stimuli per block. We found that different category-selective regions have different temporal processing capacities, with character-selective regions manifesting the lowest

temporal capacity, face- and place-selective regions showing intermediate capacity, and body-selective regions illustrating the highest capacity. This pattern of results was evident in three ways. First, the proportion of VTC exhibiting selectivity for a particular category peaked at a specific rate: 2 Hz for characters, faces, and places, and 4 Hz for bodies. Second, the selectivity for the preferred category was highest at the optimal presentation rate. Third, the response magnitude was dependent on both the presentation rate and category, where responses (a) declined at 4 Hz and above for characters, (b) declined at 8 Hz for faces and places, and (c) remained sustained for bodies even at 8Hz. These results suggest that neural processing of different visual categories occurs at differential rates. These capacities may be related to the amount of time required to process information associated with specific categories and the natural time-varying properties of stimuli of various categories in the real world.

Disclosures: **A. Stigliani:** None. **K.S. Weiner:** None. **K. Grill-Spector:** None.

Poster

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Topic: D.04. Vision

Support: KAKENHI (21670004, 25242058, and 26560325)

Title: Neural correlates of pattern randomness judgment

Authors: *H. KADOTA[1], Y. YAMADA[2], T. DOTE[3], M. IWATA[1], T. KOCHIYAMA[4], M. MIYAZAKI[3,1];
[1]Kochi Univ. of Technol., Kochi, Japan; [2]Kyushu Univ., Fukuoka, Japan; [3]Yamaguchi Univ., Yamaguchi, Japan; [4]ATR, Kyoto, Japan

Abstract: Randomness is a feature that characterizes objects, environments or events in the world. A recent psychophysical study reported that human observers can adapt to randomness in two-dimensional visual patterns; that is, perceived randomness decreased/increased after prolonged exposure to high/low levels of physical randomness ("pattern randomness aftereffect," Yamada et al. 2013). This perceptual aftereffect suggests the existence of a process component to compute visual pattern randomness in the brain. Yamada et al. further found that the pattern randomness aftereffect was selective to the orientation of the pattern but not selective to the contrast polarity. Based on these psychophysical and previous neurophysiological findings,

Yamada et al. hypothesized that the lateral occipital complex (LOC) is involved in the perception of pattern randomness. In the present study, we tested this hypothesis using fMRI. During the fMRI scan, participants (n = 18) performed randomness judgment (RJ) and contrast judgment (CJ) of identical visual patterns. Comparing the behavioral results between the tasks revealed similar accuracy rates [0.91 ± 0.02 (mean ± SEM) for RJ, 0.91 ± 0.02 for CJ, P = 0.98] and reaction times (952.6 ± 45.7 ms for RJ, 930.8 ± 36.4 ms for CJ, P = 0.27). Brain imaging results revealed multiple activation regions in the RJ > CJ contrast, although there was no significant activation in the CJ > RJ contrast [extent threshold: P = 0.05 (FWE corrected), height threshold: P = 0.001 (uncorrected)]. RJ-specific activity was observed in the right occipitotemporal region, extending from the fusiform gyrus to the inferior occipital and temporal lobes, which supports the LOC hypothesis. Similar activity was observed in the left hemisphere, although the activation cluster was smaller. In addition, greater activity by RJ was observed in the right premotor cortex and bilateral inferior parietal lobes extending to the primary somatosensory cortices (S1, BA 2/3). Thus, as predicted, the LOC was found to be a neural correlate of pattern randomness judgment. In addition, neural correlates were also identified in S1, along with the premotor and posterior parietal areas. An fMRI study of object recognition proposed that the LOC constitutes a multimodal network across visual and somatosensory modalities (Amedi et al. 2001). The S1 activation observed herein may indicate that the brain relies on the somatosensory area to process visual pattern randomness via the LOC.

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Poster

822. Visual Processing: Objects and Scenes

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Title: How inferotemporal cortex cells use different cortical inputs for selectivity and tolerance

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Abstract: Background. Our ability to recognize visual objects, such as faces or places, is carried out by neurons in the inferotemporal cortex (IT). These cells show preferences for individual images or image categories (and are thus selective), and are able to maintain these preferences even under irrelevant contextual changes (they are tolerant to changes in retinal size, position or viewpoint). Posterior IT neurons (pIT) receive feedforward projections from over a dozen cortical regions, predominantly from area V4, but also from V3 and V2. In this study, we probe the contributions of areas V2, V3 and V4 towards selectivity and tolerance in pIT neurons. Image preferences are similar in V2, V3 and V4 but receptive field diameters, at any given eccentricity, are larger in V4. Thus our working hypothesis is that V4 inputs will be most important for size and position tolerance than V2/V3. Methods. We used reversible inactivation (cooling) to probe the differential contributions of V2/V3 and V4 to size and position tolerance. Two floating microelectrode arrays were implanted in posterior IT cortex of one macaque, along with three cryoloops onto areas V2/V3 and V4. We presented images from seven object categories (line contours, faces, body parts, places, plants, gadgets and animals), at different sizes and positions within the RFs, while the monkey performed a fixation task. We collected image preference curves from 64 pIT locations during, before and after inactivation of V2/V3 or V4. Results. Inactivating both V2/V3 or V4 did not fully silence pIT neurons, but rather reduced firing rates by a median of ~30-40%. The latency of the peaked tuned responses increased by 20-40 ms. We looked at the differential contributions of V2/V3 vs V4 by measuring preservation of image preferences of each IT site: specifically, we computed the correlation coefficients between the control (warm) tuning curves and either the V4-cooled or V2/V3-cooled tuning curves. IT neurons became less selective to their preferred stimuli during V4 inactivation compared to V2/V3 inactivation. Some IT sites maintained strong image selectivity throughout all cooling conditions: these loci showed a significant decrease in size tolerance during V4 cooling but not V2/V3 cooling. In summary, our results so far show that posterior IT neurons depend more on V4 inputs for both selectivity and size tolerance; we are now exploring how V2/V3 inputs contribute to the response properties of IT neurons.

Disclosures: C.R. Ponce: None. **M.S. Livingstone:** None. **S.G. Lomber:** None.

Poster

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Title: Estimating dimensionality of object representations in monkey inferotemporal cortex

Authors: *S. R. LEHKY[1,2], R. KIANI[3], H. ESTEKY[4,5], K. TANAKA[2];
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Abstract: We have calculated the intrinsic dimensionality of visual object representations in anterior inferotemporal (AIT) cortex, based on responses of a large sample of cells stimulated with photographs of diverse objects. As dimensionality was dependent on data set size, we determined asymptotic dimensionality as both the number of neurons and number of stimulus image approached infinity. Our final dimensionality estimate was 93 (SD: ± 11), indicating that there is basis set of approximately a hundred independent features that characterize the dimensions of neural object space. We believe this is the first estimate of the dimensionality of neural visual representations based on single-cell neurophysiological data. The dimensionality of AIT object representations was much lower than the dimensionality of the stimuli. We suggest that there may be a gradual reduction in the dimensionality of object representations in neural populations going from retina to inferotemporal cortex, as receptive fields become increasingly complex.

Disclosures: S.R. Lehky: None. **R. Kiani:** None. **H. Esteky:** None. **K. Tanaka:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Title: Orientation representation in object-selective cortex

Authors: *M. HATFIELD, M. MCCLOSKEY, S. PARK;
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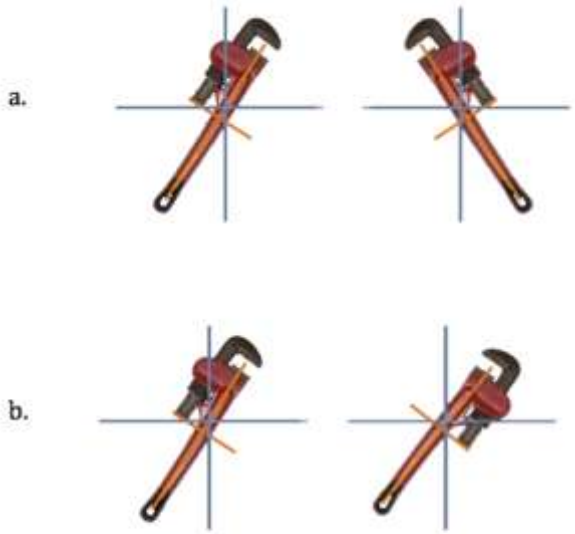
Abstract: b. How does the brain represent the orientation of objects? A clue comes from behavioral experiments showing confusions between mirror-reflected orientations (Corballis & Beale, 1976; Davidoff & Warrington, 2001). Crucially, object-axis reflections are more confusable than external-axis reflections (see figure) (Gregory & McCloskey, 2010). Does the neural representation preserve this similarity structure, coding object-axis reflections as more similar than external-axis reflections? Using an event-related fMRI-adaptation paradigm (Expt 1), we investigated the representation of orientation in two object-selective cortical regions [lateral occipital (LO) and posterior fusiform (pFs)]. Participants (N=19) viewed an object stimulus, followed by the same view repeated (Ident condition), an object-axis reflection (OA), an external-axis reflection (EA), or a different object (Diff). LO only adapted to identical views (Ident v. OA; EA; Diff [pairwise]: all $F's > 20$, p's <.05) and did not differentiate between object-axis, external-axis, and different object conditions (OA v. EA v. Diff [all pairwise]: all $F's < 6$, *n.s.*). pFs, in contrast, adapted to object- (Ident v. OA, $F(1,45) = 7.1$, *n.s.*) but not external-axis reflections (Ident v. EA, $F(1,45) = 18.7$, $p < .05$). These results suggest that the response to mirror images in pFs depends on the kind of reflection, modifying previous claims that pFs is mirror image invariant (Dilks et al., 2011). Additionally, these results suggest that pFs may preserve the behavioral similarity structure; however, comparison of more orientation changes is required. In Experiment 2 (in progress), we employ a continuous carry-over design (Aguirre, 2007) to compare 16 orientations at both within-voxel (fMRI-adaptation) and across-voxel (multi-voxel pattern analysis) scales. We model similarity of neural response in terms of the behavioral confusability between orientations as well as in terms of pixel-overlap. The results will provide a more complete profile of the neural representation of orientation in object-selective cortical regions.



An object with object-axes (orange) and external axes (blue) shown. **a)** An external-axis reflection (EA). **b)** An object-axis reflection (OA).

Disclosures: **M. Hatfield:** None. **M. McCloskey:** None. **S. Park:** None.

Poster

822. Visual Processing: Objects and Scenes

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Topic: D.04. Vision

Title: Are face representations depth-cue invariant?

Authors: ***R. FARIVAR-MOHSENI**, A. DEHMOOBADSHARIFABADI;
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Abstract: The human visual system can represent complex 3-D surfaces from multiple depth cues, but individual depth cues are processed by different cortical networks. For example, different cortical areas process depth from motion vs. shading cues to extract 3-D surface information that may ultimately be used for object recognition. It is not known whether there is only one underlying mechanism for object recognition which combines multiple depth cues or

whether there are multiple cue-specific representations of objects. Building on the proposed framework of O'Toole et al. (2002) for motion-defined facial surfaces, we asked whether mechanisms underlying the processing facial identification and object categorization are depth cue invariant. First, we utilized the face-identity aftereffect: the transitory distortion in perception of facial identity following extended periods of adaptation to a configurally distorted face called an anti-face (Leopold et al., 2001). Stimuli consisted of facial surfaces and their anti-face surfaces defined purely by shading, texture, motion, or stereo disparity. We measured face identification thresholds for each cue across four subjects using a four-alternate forced choice task with 3 conditions: matched anti-face adapter, non-matched anti-face adapter, as well as without adaptation. We found robust face-identity aftereffect for faces defined by each depth cue. Even when the adapter was defined by a different depth cue (cross-cue adaptation), the same effect was observed. These psychophysical results suggest that we can recognize objects defined purely by a depth cue, implying that complex object recognition mechanisms, such as face recognition, are depth cue invariant. We next tested whether adaptation effects of the MEG M170 component would be observed for faces vs. chairs (object categorization). Fourteen subjects viewed two interval trials, with the second interval always a shaded face. The first interval (the adapter) was either a face, chair, or an oval in the coarse shape of a face, and stimuli from all three categories were defined by shading, texture, structure-from-motion or disparity. We measured the amplitude and latency of the M170 response to the second stimulus (i.e., always a shaded face) and found robust adaptation of the M170 signal but only when the adapter was defined by shading or texture. The results are discussed in the context of depth-cue integration for object recognition and the cortical models that would support this integration.

Disclosures: R. Farivar-Mohseni: None. **A. Dehmoobadsharifabadi:** None.

Poster

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NSFC 31271115

Title: What is visual and what is not? Mapping the connectional and functional fingerprints of the visual cortex in congenitally blind individuals

Authors: *X. WANG[1,2], M. PEELEN[3], Z. HAN[2], C. HE[2], A. CARAMAZZA[4,3], Y. BI[2];
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Abstract: A large number of studies have shown that the function and wiring pattern of parts of visual cortex are shaped by visual experience. However, recent studies have also identified regions in higher-order parts of the ventral occipital temporal cortex (VOTC) that exhibited functional response patterns and connectional patterns that were largely independent of visual experience. In the present study we mapped out, across the whole visual cortex, the degree to which function is determined by visual experience by examining both the intrinsic functional connectivity and the responses to various object categories for each voxel, motivated by the insight that a cortical region's functional fingerprints - its pattern of functional responses -- is determined by its connectional fingerprints - its pattern of connections (Passingham et al., 2002; Mahon and Caramazza 2009; Saygin et al. 2012). We compared the functional and connectional fingerprints of 13 congenitally blind and 16 sighted subjects across the whole VOTC to map out vision-dependent and vision-independent regions. Connectional fingerprint was measured as each VOTC voxel's resting-state functional connectivity pattern with 180 regions covering the whole cerebrum; functional fingerprint was measured by each voxel's activation strength for 16 common object categories using an auditory object size judgment task. Sighted subjects additionally took part in a visual task involving the same object categories. Results of functional and connectional fingerprint analyses were highly convergent: Regions in anterior medial and posterior lateral parts of the VOTC showed similar connectional and functional fingerprints across blind and sighted, as well as across the functional fingerprints revealed by the sighted groups' auditory and visual experiments, suggesting that these regions were "polymodal" instead of strictly visual. Other regions, mainly in the early visual cortex and posterior fusiform gyrus, showed divergent connectional and functional fingerprints between blind and sighted, suggesting that bottom-up visual input significantly shapes the connectivity and function of these regions. These findings provide the first large-scale mapping of the degree to which connectional and functional fingerprints of the "visual" cortex depend on visual experience. In addition to revealing regions that are strongly dependent on visual experience, they point to regions in which connectional and functional patterns are surprisingly similar in blind and sighted individuals.

Disclosures: X. Wang: None. **M. Peelen:** None. **Z. Han:** None. **C. He:** None. **A. Caramazza:** None. **Y. Bi:** None.

Poster

823. Visual Processing: Faces

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 823.01/HH28

Topic: D.04. Vision

Support: NIMH-IRP

Title: Thetaburst TMS over human V5/MT reduces fMRI responses to dynamic but not static faces in the right posterior STS

Authors: *K. HOLIDAY, D. PITCHER, S. JAPEE, L. G. UNGERLEIDER;
NIMH/NIH, Bethesda, MD

Abstract: Neuroimaging studies reveal brain regions that exhibit preferential responses to dynamic or static images of faces. In humans, the functional magnetic resonance imaging (fMRI) response to dynamic faces is three times greater than to static faces in the right posterior superior temporal sulcus (rpSTS), while the right fusiform face area (rFFA) and right occipital face area (rOFA) exhibit equal responses to dynamic and static faces (Pitcher et al., 2011). The differential pattern of these responses suggests that face-selective regions may have different cortical inputs. We addressed this question by causally disrupting two cortical regions with thetaburst transcranial magnetic stimulation (TBS) and measuring the effects of this disruption in local and remote face-selective regions with fMRI. Participants were scanned over two sessions while viewing dynamic or static faces and objects. During these sessions, TBS was delivered over the rOFA or the motion-selective region V5/MT. Preliminary analysis showed that TBS delivered over the rOFA reduced the fMRI response to both dynamic and static faces in this region and in the downstream rFFA. In contrast, the fMRI response to dynamic and static faces was doubly dissociated in the rpSTS: disruption of the rOFA reduced the response to static but not dynamic faces, while disruption of V5/MT reduced the response to dynamic but not static faces. These results suggest that dynamic and static facial aspects are processed via dissociable cortical pathways that begin in early visual cortex, a conclusion inconsistent with current models of face perception.

Disclosures: K. Holiday: None. **D. Pitcher:** None. **S. Japee:** None. **L.G. Ungerleider:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: Grants-in-Aid for Scientific Research on Innovative Areas "Sparse modeling" (26120535)

Title: Members of face-responsive neurons in monkey area TE that contribute to global categorization of faces and to upright-face versus inverted-face categorization are different

Authors: *N. MATSUMOTO[1], Y. SUGASE-MIYAMOTO[1], K. KAWANO[2];
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Abstract: There are neurons that respond to faces in monkey temporal cortex. We have reported that face-responsive neurons in area TE represent information about a global category of faces, namely human vs. monkey vs. simple shapes earlier than information about more detailed categories about the faces, i.e. facial expression and identity. Recently, we found that when faces were presented with inversion, information about the fine categories decreased, whereas information about the global category remained. To examine whether face-responsive neurons respond to inverted faces showing similar characteristics to upright faces on a face-by-face basis, we analyzed activities of 119 face-responsive neurons in area TE of two rhesus monkeys (Macaca mulatta), performing a fixation task. Test stimuli were colored pictures of monkey faces (4 models with 4 expressions), human faces (3 models with 4 expressions), geometric shapes, and inverted pictures of the faces. Population activity vectors consisting of responses to upright vs. inverted faces were computed in a window 115-165 ms after stimulus onset; this is the time that the global categorization is observed. The Euclidean distances between 28 upright and 28 inverted faces were smaller than those of the 192 combinations of 16 monkey and 12 human faces, showing that inverted monkey/human faces evoked population responses similar to those evoked by upright monkey/human faces, respectively. Principal component analysis was applied individually to the upright monkey vs. human faces, to the upright vs. inverted human faces, and to the upright vs. inverted monkey faces. The distribution of eigenvector values for the first principal component was different in the upright monkey vs. human faces from that in the upright vs. inverted human faces (Pearson's $r = -0.33$, $p < 0.001$) and from that in the upright vs. inverted monkey faces ($r = 0.13$, $p = 0.16$). The distribution of the eigenvector values for the upright vs. inverted human faces showed a significant positive correlation with that for the upright vs. inverted monkey faces ($r = 0.44$, $p < 0.0001$). The results suggest that different

members of the neuronal population contribute to the global categorization and to the upright vs. inverted categorization, but that the upright vs. inverted categorization of the monkey faces and the upright vs. inverted categorization of the human faces are likely to have contributing members in common.

Disclosures: **N. Matsumoto:** None. **Y. Sugase-Miyamoto:** None. **K. Kawano:** None.

Poster

823. Visual Processing: Faces

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Program#/Poster#: 823.03/HH30

Topic: D.04. Vision

Support: FWO

IUAP

FP

FNRS

Title: Sensitivity to different orientation ranges of face information in the monkey IT cortex

Authors: J. TAUBERT[1,2], G. VAN BELLE[2], V. GOFFAUX[2], *R. VOGELS[1];
[1]Dept Neurosci., KU Leuven, Leuven, Belgium; [2]Res. Inst. for Psychological Sci., Université Catholique de Louvain, Belgium

Abstract: Humans process face identity best based on the horizontal ranges of face information. Here we used orientation filtering to compare the functional characteristics of cells belonging to different face-selective patches. Also we examined whether such a manipulation could decouple the responses of face cells that cluster within face patches from the responses of face cells that occur outside this functionally defined system. We used a standard fMRI procedure to localize the face patches in two male rhesus monkeys, then targeted the middle-lateral face patch (ML) and the anterior-lateral face patch (AL) with microelectrodes. We also recorded the responses of face cells that were found between ML and AL, in a region of STS that was not differentially activated by faces. Stimuli were 24 upright and inverted human face pictures. For each recorded cell, we selected the face that resulted in the highest spike activity. Monkeys were then presented with the preselected faces in 5 different filter conditions: full spectrum, horizontal-pass, vertical-

pass, phase-scrambled horizontal-pass and phase-scrambled vertical-pass. Stimuli were viewed either upright, or inverted in the picture plane. During the test, monkeys passively fixated stimuli, which were shown for 300ms, at the center of the screen. Face cells in ML responded most strongly to full spectrum faces; they also responded more to horizontal- than the vertical-pass faces in the early transient response. Turning the faces upside-down reduced the response of cells in ML all filtering conditions. In contrast, face cells in AL responded equally to full spectrum and vertical-pass faces; stimulus inversion reduced the firing rate in these two conditions. Face cells outside the patch were insensitive to the filtering manipulation and there was no reliable inversion effect, indicating that responses to intact upright faces reflect a bias related to the processing of a face-like shape. Phase scrambling diminished the responses of face cells inside the face-selective patches, however, these cells responded equally to phase-scrambled horizontal-pass and phase-scrambled vertical-pass stimuli, suggesting a face structure was necessary for orientation tuning. The effects of picture-plane inversion were also absent among the phase-scrambled conditions providing further evidence that inversion effects were limited to face stimuli. Overall these findings suggest that face cells in monkey ML and AL have specialized responses to the internal structure of a face and are differentially tuned to different orientation bands.

Disclosures: **J. Taubert:** None. **R. Vogels:** None. **V. Goffaux:** None. **G. Van Belle:** None.

Poster

823. Visual Processing: Faces

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Support: The Attias Family Foundation NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (A.S.G.)

NIMH Grant K01 MH081191

NICHD ACE grant HD055648

Title: The relationship between face-specific and domain-general configural processing in individuals with and without autism

Authors: E. KESSLER[1], A. C. LYNN[2], *K. O'HEARN[2], A. S. GHUMAN[1];
[1]Neurolog. Surgery, Univ. of Pittsburgh, PITTSBURGH, PA; [2]Psychiatry, Univ. of Pittsburgh Sch. of Med., PITTSBURGH, PA

Abstract: One of the central questions of cognitive neuroscience is whether processing is organized by domain-general computation or whether it is domain-specific, and a focus of this debate has become the study of face processing. Specifically, it remains unknown whether the "specialness" of face perception is due to a difference in processing style, such as relying on configural processing mechanisms more heavily than part-based mechanisms, or whether there is a distinct system devoted to processing of faces. Here we examined the correlation between face processing and both face-specific and domain-general configural processing ability in 23 individuals with autism spectrum disorders and 28 neurotypical individuals. We administered the Cambridge Face Memory Test (CFMT) to measure overall face processing and the Parts/Whole Identity section of the *Let's Face It!* Skills Battery (LFI) to assess configural processing of faces. Additionally, we measured domain-general configural processing using Pomerantz's test of configural superiority (1977), involving discriminating among various line segments when presented in isolation or in configural contexts. In this task, irrelevant contextual information can either facilitate processing (configural superiority) or interfere with discrimination ability. In neurotypical individuals, we found a significant correlation between CFMT and holistic processing in LFI ($0.4 \leq r \leq 0.8$, with $r = 0.4$ corresponding to $p < 0.5$). However, both the correlation between the CFMT and parts-based face processing in LFI and between the CFMT and the domain-general configural processing in the Pomerantz task failed to reach significance. Configural superiority was normal in individuals with autism, based on their performance on the Pomerantz task. However, all three behavioral measures showed significant correlations with one another in individuals with autism spectrum disorders. These results suggest that there are distinct face and non-face configural processing systems in the typically developing brain. In contrast, the separation between face-specific and domain-general configural processing systems is absent in individuals with autism.

Disclosures: E. Kessler: None. **A.C. Lynn:** None. **K. O'Hearn:** None. **A.S. Ghuman:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: NIMH IRP

Title: Projections of the lateral and basal nuclei of the monkey amygdala delineated with electrical stimulation and fMRI

Authors: *A. MESSINGER[1], J. M. SEIDLITZ[1], R. B. H. TOOTELL[2], L. G. UNGERLEIDER[1]; [1]Lab. of Brain and Cognition, NIMH, Bethesda, MD; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachussets Gen. Hosp., Charlestown, MA

Abstract: In both humans and monkeys, the amygdala responds to emotionally laden stimuli such as fearful facial expressions and is necessary for mediating goal-directed decisions (Izquierdo and Murray 2004). To better understand how information about emotional valence is transmitted from the amygdala to sensory and decision-making areas of the brain, we conducted an *in vivo* study of amygdala connectivity in two rhesus monkeys. We used electrical stimulation (300 Hz trains of 250 μA pulses) to elicit neuronal activity in specific amygdala nuclei and concurrent fMRI to detect activations both locally and in functionally connected brain areas. The lateral nucleus is the primary recipient of amygdala input. Stimulation sites in this nucleus resulted in reliable but circumscribed activation of the ipsilateral rhinal cortex, temporal pole, rostral auditory areas, insula, orbitofrontal area 13, and the medial dorsal nucleus of the thalamus. Stimulation of the basal nucleus, the primary output structure of the amygdala, resulted in activations of those areas activated by lateral nucleus stimulation and additional areas. Both the extent and degree of the additional activation varied with the position of the stimulating electrode within the basal nucleus, with dorsal sites projecting more widely and strongly than ventral sites. Stimulation at the most ventral sites, located in the parvicelluar subdivision of the basal nucleus, produced additional activations in the gustatory cortex, cingulate cortex, hippocampus, striatum, and hypothalamus. Stimulation at the most dorsal sites, corresponding to the magnocellular subdivision of the basal nucleus, activated the targets of the more ventral sites plus the prefrontal cortex, rostral agranular insula, somatosensory cortex, caudal auditory cortex, ventral stream visual (including area TE, area TEO, and early visual areas), nucleus accumbens, and basal forebrain. Tracer studies have shown that the basal nucleus sends non-reciprocated output projections to many of these activated areas (Yukie 2002; Amaral et al. 2003). Thus presumably, electrical stimulation of the basal nucleus generates orthodromic conduction along these pathways, which transmit output signals from the amygdala to a diverse network of sensory, limbic, and decision-making areas.

Disclosures: A. Messinger: None. **J.M. Seidlitz:** None. **R.B.H. Tootell:** None. **L.G. Ungerleider:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: Nancy Lurie Marks Postdoctoral Fellowship

NIH Grant EY16187

NIH Grant T32 NS007484

Title: Development of category-selective domains in infant macaque inferotemporal cortex

Authors: *K. SRIHASAM, J. VINCENT, M. LIVINGSTONE;
Neurobio., Harvard Med. Sch., BOSTON, MA

Abstract: In humans and in monkeys inferotemporal cortex is divided up into domains specialized for processing specific object categories, such as faces, places, and body parts. To monitor the normal development of these category selective domains we did functional MRI on infant and juvenile macaques while they passively viewed movies and images of faces and places in the scanner. We have developed techniques for safely and non-invasively doing functional MRI in alert infant macaques. We have scanned monkeys as early as 2 weeks of age and thereafter as IT develops. We find a rostro-caudal gradient of development of visual responsiveness. Our youngest monkeys (2 weeks old) showed visually evoked activity only in early visual areas (V1 and V2). By the 10th month, all of occipito-temporal cortex showed visually evoked activity. We further find that up to 4 months of age IT did not show clear segregation into category-selective domains but did show retinotopic organization. Category-selective domains emerged around 5 to 6 months and were robust by the 9th month.

Disclosures: K. Srihasam: None. **J. Vincent:** None. **M. Livingstone:** None.

Poster

823. Visual Processing: Faces

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IUAP P7/11

IDO/10/003

Title: Representations of dynamic faces in the brain: A closer look at identity and emotional expression using multi-voxel pattern analyses

Authors: C. DILLEN, L. HERMANS, J. STEYAERT, B. BOETS, *H. P. OP DE BEECK; Univ. Leuven, Leuven, Belgium

Abstract: Human neuroimaging studies have demonstrated the existence of several face-specific cortical regions which are more strongly activated by faces than by other stimuli (e.g., Kanwisher et al., 1997). Recently, studies have applied multi-voxel analyses methods to investigate the properties of the neural representations in these face-specific regions (Goesaert & Op de Beeck, 2013; Kriegeskorte et al., 2007; Nestor et al., 2011). From these studies it is unclear to what degree these face-selective regions contain functional maps of specific face dimensions, whether maps for face identity and face expression are dissociated and present in different regions, and whether such maps are different or less prominent in non-face-selective regions. In the present study we used faces with dynamic facial expressions to investigate neural representations of face identity and expression in 16 healthy adults. Participants had to detect changes in identity or expression. Regions of interest were delineated at different spatial scales (scale 1: lobes; scale 2: gyri; scale 3: face-selective areas and nearby non-face-selective clusters). Multi-voxel pattern analyses (MVPA) revealed significant identity decoding at all spatial scales, while decoding analyses of expression revealed significant decoding preferentially at the larger spatial scale. When the identity decoding required invariance (decoding identity across different emotions), decoding performance decreased and was mostly found at the larger spatial scale. Interestingly, decoding performance was very similar in face-selective regions and in non-face-selective (but responsive) voxels. In sum, information about facial identity and emotion as revealed by MVPA is relatively weak in local regions, distributed across many weakly informative voxels/regions, and is not related to the preference for faces that some voxels and regions show in univariate analyses.

Disclosures: C. Dillen: None. **H.P. Op De Beeck:** None. **J. Steyaert:** None. **L. Hermans:** None. **B. Boets:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: Natural Scientific Foundation of China 61104143

Natural Scientific Foundation of China 61104224

Title: The temporal cortex face expression area, the precuneus and posterior cingulate cortex, and autism

Authors: *E. T. ROLLS[1,2], W. CHENG[3], J. ZHANG[3], J. FENG[2,3];
[1]Computer Sci., Oxford Ctr. For Computat. Neurosci., Coventry, United Kingdom; [2]Univ. of Warwick, Coventry, United Kingdom; [3]Ctr. for Computat. Systems Biol., Fudan Univ., Shanghai, China

Abstract: Whole-brain voxel-based unbiased resting-state functional connectivity was analyzed in 396 people with autism and 351 typically developing individuals. We identified a key system in the middle temporal gyrus / superior temporal sulcus (STS) region which has reduced cortical functional connectivity (and increased with the thalamus), which is implicated in face expression and motion processing involved in social behavior, and which has onward connections to the orbitofrontal cortex/ventromedial prefrontal cortex and amygdala. The same system is implicated in theory of mind processing, and in audio-visual integration for e.g. speech, and possibly in further aspects of communication using language. This system has reduced functional connectivity to face somatosensory and motor areas in the pre- and postcentral cortex, which may normally be used for outputs for such face expression and speech-related processing. This middle temporal gyrus / STS system also has reduced functional connectivity with the amygdala and orbitofrontal cortex, which may be especially involved in the rewarding aspects of face processing and mentalizing for social communication. We have identified a second key system in the posterior cingulate cortex / precuneus / cuneus with reduced functional connectivity which is implicated in spatial functions including of oneself, and of the spatial environment, and suggest that this provides an important contribution to theory of mind processing which is impaired in autism. The findings are consistent with the hypothesis that these two types of functionality, face expression-related, and of one's self and the environment, are important components of the computations involved in theory of mind, whether of oneself or of others, and that reduced connectivity within and between these regions may make a major contribution to the symptoms of autism. Hasselmo,M.E., Rolls,E.T. and Baylis,G.C. (1989) The role of expression and identity in the face selective responses of neurons in the temporal visual cortex of the monkey. Behavioural Brain Research 32: 203-218. Rolls,E.T. (2014) Emotion and Decision-Making Explained. Oxford University Press: Oxford.

Disclosures: **E.T. Rolls:** None. **W. Cheng:** None. **J. Zhang:** None. **J. Feng:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: French ANR Grant (Plasmody)

Title: Face processing in profoundly deaf adults: Hemispheric asymmetry study with chimeric faces

Authors: M. DOLE, D. MEARY, G. LEROY, *O. PASCALIS;
Lab. De Psychologie Et Neurocognition, Grenoble, France

Abstract: A substantial body of research suggests the presence of cross-modal plasticity in cases of early sensory deprivation. We were interested in the impact of early deafness on face processing. Previous studies indicate that early deafness results in the reorganization of visual abilities, such better peripheral visual attention and modifications of face processing. Face processing changes included modifications of the eye movement patterns during the observation of static faces (Watanabe et al., 2011) or modifications of hemispheric asymmetry (Letourneau et al., 2013). A right hemisphere advantage for face processing is indeed found in hearing participants. The present study was designed to determine if face hemispheric asymmetry in profoundly deaf participants differs from hearing using chimeric faces. Chimeric faces are faces divided in two different halves (for example, half male, half female), and are used to establish a left visual field bias (right hemisphere advantage) in hearing participants, that is, participants use more the left part of the face (from the viewer point of view) to discriminate gender or identity. In our first study we presented 40 faces (10 chimeric male/female, 10 chimeric female/male, 10 female, 10 male) during a gender categorization task in a group of profoundly deaf participants and hearing controls. Results confirmed the presence of a left visual field bias in hearing participants; this leftward bias was however significantly reduced in deaf participants, suggesting a reduction of their face hemispheric asymmetry. In a follow-up study we investigated whether this reduction of asymmetry was reflected in the participants' scanning patterns. Eye movements were recorded by an Eye-Tracking system while participants underwent the same gender categorization task with chimeric or entire faces. First fixation direction, number of left/right fixations, and left/right fixation duration were investigated. Our results showed no significant

difference in the left/right scanning patterns between the two groups, suggesting that the reduction of asymmetry observed in the behavioral study did not result from differences in the left/right scanning patterns of the participants. Our results are discussed in the light of differences in the peripheral vision.

Disclosures: **M. Dole:** None. **D. Meary:** None. **G. Leroy:** None. **O. Pascalis:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: DFG Grant KO 3918/1-2

DFG Grant KO 3918/2-1

Title: Repetition and expectation suppressions affect fusiform activity independently

Authors: ***G. KOVÁCS**, N. WANKE, M. GROTHEER;
Inst. Psychol, FSU JENA, Jena, Germany

Abstract: Several functional magnetic resonance imaging (fMRI) studies have pointed towards the role of perceptual expectations in determining the adaptation of the BOLD signal (fMRIa) in humans. These studies showed that the probability of face repetitions (and therefore their expectation) further increases the magnitude of fMRIa in face-related areas of the brain. However, most of the prior studies did not attempt to separate the reduction of the response due to stimulus repetition itself from the signal reduction due to confirmed expectations. In order to achieve this, here we used pairs of repeated or alternating female and male faces (50%, randomly) and measured fMRIa in the fusiform face area. Critically, orthogonal to the repetition of the stimuli, the gender of the first stimulus (S1) predicted repetition or alternation of the stimulus (for example female S1 predicted repetition while male S1 predicted alternation with 0.75 probability, counterbalanced across subjects). We found significant fMRIa in the fusiform face area for repeated as compared to alternating stimuli. More importantly, although expecting repetitions decreased the target related responses as well, it had no interaction with the observed fMRIa. This suggests that stimulus repetition and expectation suppressions are independent

processes that determine the magnitude of the BOLD signal of face-specific areas of the human brain together.

Disclosures: G. Kovács: None. **N. Wanke:** None. **M. Grotheer:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: JSPS KAKENHI GRANT 25330173

JSPS KAKENHI GRANT 26350995

Title: Effects of signals originating from large blood vessels on BOLD signals of the fusiform face area

Authors: *Y.-W. SUNG[1], J. CHUNG[2], S. OGAWA[1];
[1]Kansei Fukushi Res. Institute, Tohoku Fukushi Univ., Sendai, Japan; [2]Gachon Univ., Incheon, Korea, Republic of

Abstract: In BOLD imaging, on which most fMRI studies are based, the paramagnetic property of blood produces a bulk susceptibility difference between a blood vessel and the surrounding brain tissue, thus producing resonance frequency shifts in extravessel molecules. This is, in itself, is a short-range phenomenon located within a few tens of microns from an activated neuronal site. However, two factors make an undesirable contribution to BOLD-based fMRI signals, leading to an exaggeration of the spatial specificity and signal amplitude compared with those expected. One factor is the propagation of the bolus of oxygen-enriched blood by neuronal activation to the larger draining venous structures. MRI signals are inherently sensitive to inflowing fully magnetized spins; therefore, hemodynamic changes, particularly in larger blood vessels, can create signal fluctuations that are coincident with neuronal activation due to the inflow effect. Face recognition is important for social communication. The fusiform face area (FFA) is known to play a pivotal role in face processing. The measurement of responses of the FFA can provide crucial information regarding face recognition. The FFA is located in the ventral region, at the base of the brain, through which large vessels. The location of the FFA via fMRI varies across subjects, although the average location is almost invariant across groups of

subjects. If the variation in location is dependent on large blood vessels, then the signal of the FFA can be under- or overestimated. However, this issue has not been examined. In this study, we investigated whether the FFA includes large blood vessels and/or whether inflow signals contribute to fMRI signals of the FFA. For this purpose, we used susceptibility-weighted imaging (SWI) sequences to visualize large blood vessels and dual-echo gradient-echo echo-planar imaging (GE-EPI) sequences to measure inflow signals. The data demonstrated that fMRI responses of the FFA were not affected by inflow signals. The data also showed that no large blood vessels were present in the FFA. This means that the FFA was not affected directly by inflow signals, and that its location was not biased by large blood vessel signals. However, the presence of large blood vessels and the activation by inflow effects around the FFA represent a warning that the evaluation of FFA responses via intersubject averaging may lead to a wrong direction. Therefore, the consideration of the contribution of large blood vessels by the presented methods in parallel with the typical functional analysis would be needed for ensuring reliability.

Disclosures: **Y. Sung:** None. **J. Chung:** None. **S. Ogawa:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: JSPS KAKENHI Grant Number 24700421

Title: The roles of category-selective regions for identifying the deteriorated images

Authors: *Y. MORITO, T. MURATA;
Natl. Inst. of Information and Communication Technol., Suita, Osaka, Japan

Abstract: Visual information into eyes is sometimes not sufficient for the observer to perceive some objects, so that we need to use top-down information based on the internal knowledge of the objective world. Our hypothesis is that the category-selective regions in the ventral and temporal visual areas are not only engaged in the bottom-up process for perceiving the object from the clear-sighted visual features, but also involved in the top-down process for identifying the uncertain visual objects from internal knowledge. To investigate this hypothesis, we conducted a functional magnetic resonance imaging (fMRI) experiments with 14 normal volunteers. In MRI scanner, subjects were required to observe the deteriorated images which

were gradually getting clear and to push the button when they could identify the image category: Human, Scene and Tool. We provided the 90 sets of the gray-scale deteriorated images. Each image set consisted of 14 level degradation performed by the Fourier-component deformation, for the first level of which no subjects could perceive the meaningful objects and the last level of which all subjects could provide confident discrimination. In addition, we identified the category-selective regions of each subject using the canonical visual stimuli: the fusiform face area (FFA), the extrastriate body area (EBA), the parahippocampal place area (PPA) and the lateral occipital complex (LOC). The MRI results showed that all the category-selective regions were significantly activated regardless of stimulus category though the subjects were not perceiving meaningful objects yet. Once subjects identified the image category, the regions which were selective to that category showed increased activity while the other category-selective regions showed decreased activity. On the other hand, the activation in primary visual area consistently increased after perceiving the meaningful object regardless of category of the object. These results suggest that the category-selective regions were involved in the top-down process for identifying the deteriorated images. The observed increase and decrease of activation of catergory-selective regions after the identification may play a role in forming stable perception of objects in the deteriorated image.

Disclosures: Y. Morito: None. **T. Murata:** None.

Poster

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We thank Adrian Nestor for his help with the data acquisition

Title: Mapping the topology of the face processing network in congenital prosopagnosia

Authors: *G. ROSENTHAL[1], M. TANZER[2], M. BEHRMANN[3], G. AVIDAN[2];
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Abstract: Converging studies suggest that face processing is mediated not by a single, localized brain area, but rather by the contribution of multiple posterior ‘core’ and anterior ‘extended’ regions which form a coherent, distributed face network. One approach for understanding the properties of the normal face network is to explore its dysfunction in individuals who have congenital difficulties in face processing in the absence of any obvious brain abnormality and in the presence of intact sensory and intellectual functions (congenital prosopagnosia, CP). Intriguingly, individuals with CP exhibit a seemingly normal pattern of functional magnetic resonance imaging (fMRI) activation profile in the 'core' face system. However, both structural and functional MRI studies have documented impairments in the connectivity patterns between the 'core' and the 'extended' systems, and specifically, connectivity with the anterior temporal cortex. To further investigate the pattern of abnormal connectivity in CP, in the current study, we performed whole brain network exploration while adopting tools from the field of complex system analyses to quantitatively infer network topologies. Specifically, we compared a group of CP individuals and matched controls while all participants performed a one-back task during fMRI scanning. Interestingly, using modularity, a graph theory measure which captures the network decomposition into sub-networks or communities, we found a distinct, largely right-lateralized module comprised of regions of the 'core' face system, the anterior temporal cortex and the inferior frontal gyrus which exhibited significantly reduced connectivity in CP compared to matched controls. Importantly, the anterior temporal cortex, served as the hub in this network. While consistent with our previous functional and structural results, these results offer new insights by providing a computational, quantitative framework for assessing network structure and topology in cases of impaired face processing.

Disclosures: G. Rosenthal: None. **M. Tanzer:** None. **G. Avidan:** None. **M. Behrmann:** None.

Poster

823. Visual Processing: Faces

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Topic: D.04. Vision

Support: ISF grant 384/10 to GA

Title: Visual expertise in the absence of holistic processing in congenital prosopagnosia

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Abstract: Background: A major question in face perception is whether faces comprise a distinct visual category that is processed by specialized cognitive and neural mechanisms, or whether face processing merely represents an extreme case of visual expertise. Method: Here, we address this issue, by studying O.H, 22 years old woman with congenital prosopagnosia (CP), a lifelong impairment in face perception in the absence of an obvious brain damage. Interestingly, despite her deficit, O.H reported having superior recognition skills for horses, due to her work with horses since she was 7 years old. To examine her holistic perception with faces and horses, we used the inversion effect, i.e. better performance for upright compared to inverted faces, which is a well-established indication of holistic face processing. We conducted an identical task for horses, and used response time, accuracy and eye movement data as dependent measures. Additionally, we conducted fMRI scan in order to investigate the implications of expertise on neural responses to faces and horses. O.H performance was compared to data obtained from two age and gender-matched control groups that were either horse experts, having 7-23 years of experience with horses or non-experts. Results: As expected, both control groups exhibited the face inversion effect, while O.H did not show the effect. As for horses, O.H. exhibited superior performance for horses compared to faces, but this was not qualified by an inversion effect for horses. Similarly, neither of the control groups exhibited an inversion effect for horses. Interestingly, gaze behavior toward upright and inverted horses was indicative of horse expertise. Particularly, non-experts tended to focus their gaze towards the upper part of image of the horse, while in contrast, experts and O.H focused their gaze towards the head, regardless of image orientation. Surprisingly, O.H exhibited BOLD response for horse stimuli that was similar to that of faces, in her face selective brain regions. Importantly, this pattern was dissociated from the response in both expert and non-expert control groups in which faces elicited a greater response compared to horses within the same regions. Conclusions: These results suggest that visual expertise can be acquired independently from the mechanisms mediating face recognition and is not necessarily dependent on holistic processing. Nevertheless, given a face perception deficit, expertise may possibly be mediated through the face neural network.

Disclosures: N. Weiss: None. **E. Mardo:** None. **G. Avidan:** None.

Poster

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Topic: D.04. Vision

Support: NIH Intramural Research Program

Title: Understanding the topography of face and body selectivity in human ventral temporal cortex

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Abstract: Faces and body parts are amongst the most salient visual and biological stimuli in our environment. Recent research from both human and non-human primates has reported multiple clusters of activation for faces and body parts in the ventral visual pathway. Body-selective regions are often found adjacent to face-selective regions, suggesting some sort of organizational principle. However, the nature of this organizational principle is not well established. Further, given the limited power of many experimental designs, the extent to which there are multiple discrete face- and body-selective clusters remains unclear. Here, we investigated the topographical organization of body and face selectivity in ventral temporal cortex at high resolution (1.2 mm isotropic voxels) using a 7T MRI scanner. First, to identify regions selective for faces and body parts, participants viewed large numbers of runs containing blocks of each stimulus type. Given prior reports of body part topography in lateral occipital cortex, we separately tested both hands and feet, which might be expected to have the most distinct representations. Second, we collected detailed retinotopic mapping data in each participant to determine the extent to which the location of face- and body-selectivity reflect underlying retinotopic biases. Third, we tested the selectivity across face- and body-selective regions in a condition-rich event-related design with multiple categories. As expected, we found that faces elicited strong activations along the mid fusiform sulcus, in a region that has often been referred to as the Fusiform Face Area (FFA). This face selectivity coincided with a foveal representation of the visual field. In contrast, both hands and feet produced robust activations adjacent and lateral to the face selectivity. The location of this limb selectivity overlapped with the Fusiform Body Area (FBA), defined by the contrast of response to bodies and objects. We found little evidence for alternating patches of face and limb selectivity. Instead, we observed what appear to be two parallel streams of face and limb selectivity extending from posterior to anterior ventral regions. Our results suggest an organizational principle for face and body selectivity reflecting the underlying retinotopic biases in the ventral visual pathway.

Disclosures: A. Chan: None. **E.H. Silson:** None. **C.I. Baker:** None.

Poster

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Topic: D.04. Vision

Title: Responses in the anterior fundus (AF) face patch to face size, identity, and viewing angle

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Abstract: Humans and nonhuman primates are able to recognize individual faces despite their similarity and a range of transformations that strongly affect the presentation of faces onto the retina. Face recognition is likely the result of activity in cortical areas to be specialized for face processing. In the macaque, several "patches" have been identified in the inferotemporal cortex (IT) using fMRI, and their roles in face perception are an active area of investigation. Previous work has found that many single neurons in the face patches in the superior temporal sulcus (ML/MF and AL) were tuned to head orientation and viewing angle, whereas those in ventral IT face patch AM were more strongly tuned to identity (Perrett et al, 1992; Freiwald et al., 2010). We previously reported that neurons in face patch AF are highly sensitive to the scale of a face when monkeys are either presented with flashed images or naturally viewing videos (Jones et al. SFN 2013; Elnaiem et al. SFN 2013). Here we asked to what extent face identity and viewing angle shape single-unit responses in AF, and how changes in these attributes might interact with spatial scale. We made use of chronically implanted microwire electrodes, which allowed us to accumulate responses of single-units over multiple sessions. We recorded the activity of 27 neurons over 68,000 trials collected during 12 recording sessions spanning 3 weeks. On the first day of recording, we screened 200 human faces at a fixed scale and orientation in order to identify the neuron's effective stimuli for testing on subsequent days. We subsequently focused on the same neurons' preferred stimuli and tested how transformations of scale, viewing angle, and in-plane rotation affected the neurons' responses. Initial results suggest that the spatial scale of the face predominates the response variance across stimuli for most neurons in AF. Similar to previous observations in AL, some AF neurons were selective for multiple views of faces, with this view tuning largely independent of identity tuning. Across the population, there was a bias in neural response preference toward faces with their gaze directed upward and toward right, which

was the visual field contralateral to the recordings. The majority of neurons tested showed response modulation for changes in each of the dimensions tested. Together with previously reported neural tuning properties from other face patches, these results contribute to an emerging picture of functional specialization in macaque face-processing system.

Disclosures: **L.N. Vasileva:** None. **A.P. Jones:** None. **D.B.T. McMahon:** None. **I.V. Bondar:** None. **D.A. Leopold:** None.

Poster

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Topic: D.04. Vision

Support: NIMH DIRP

Title: Encoding 10,000 pictures

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Abstract: Using chronically implanted microwires capable of tracking neurons across days, we recently showed that neurons in inferotemporal (IT) cortex maintain consistent visual properties across many months (McMahon et al. PNAS 2014). Together with the empirical finding that IT response patterns are stationary, the ability to record from neurons longitudinally makes it possible to conduct large-scale screenings of neuronal feature space using massive stimulus sets. Here we realized this possibility by screening a library of 10,000 stimuli comprised of 10 different object categories. In 30 neurons in the AF (anterior fundus) face patch within the superior temporal sulcus, visual responses were significantly correlated when the same images were presented on different days. Robust discriminative information was present in the neurons' responses to face stimuli, and to a lesser extent in the neurons' responses to non-preferred stimulus categories. Scale tolerance was observed in the selectivity patterns for both face and non-face stimuli. In the same neurons, the magnitude of evoked responses varied monotonically with images scale. The majority of neurons showed a dissociation between the gain factor of

scale tuning and stimulus category, in that response magnitude was positively coupled with face image size but negatively coupled with all other stimulus categories. In a separate set of experiments, we focused a comparable number of trials on much smaller image libraries. For many neurons, some stimuli appeared to be completely ineffective at driving the neurons in trials collected on a single day, but were found to induce rare spikes when observed over hundreds of trials collected over weeks. When present these rare spikes were both stimulus specific and temporally precise, and presumably arose from subthreshold synaptic events riding on top of noise fluctuations in the cell's membrane potential. This finding indicates that IT neurons are sensitive to a much broader range of visual inputs than their conventionally measured spiking output suggests. Taken together, the results of these two experiments illustrate how the technical advance of longitudinal recording can open up new avenues of investigation that would not be feasible by other means. By spending the extra trials accrued over days broadly across many stimuli, we can obtain a picture of neuronal selectivity analogous to surveying a landscape via satellite photography rather than at ground level. Alternatively by spending the extra trials in depth across few stimuli, we can resolve the fine-grained structure of a neuron's response pattern in a manner analogous to examining objects under a microscope lens.

Disclosures: D.B. McMahon: None. **D.A. Leopold:** None.

Poster

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Topic: D.04. Vision

Support: NIMH IRP

Title: The amygdala shows a greater selectivity for dynamic faces than static faces

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Abstract: Prior fMRI studies have identified multiple face-selective regions in the human cortex but the functional division of labor between these regions is not yet clear. One hypothesis that has gained some empirical support is that face-selective regions in the superior temporal sulcus (STS) preferentially respond to the dynamic aspects of faces, whereas the fusiform face area (FFA) computes the static or invariant properties of faces (Pitcher et al., 2011). We further

tested this hypothesis by examining how face-selective regions in the occipitotemporal cortex and the amygdala respond to dynamic and static face stimuli. Preliminary analyses from 18 healthy adult subjects indicated that the right FFA and right occipital face area (OFA) responded equally to dynamic and static faces. In contrast, the amygdala showed a two-fold increase in response to dynamic faces, as compared to static faces. A high-field strength (7 Tesla) and high resolution (1.25 mm isotropic) scan allowed us to functionally define face-selective voxels in the amygdala in almost all participants. This two-fold increase in response to dynamic faces was also seen in the right posterior STS region, while the right FFA and right OFA responded equally to dynamic and static faces. This pattern of responses in the amygdala and right posterior STS suggests that the two regions may be preferentially involved in computing the changeable aspects of faces, compared to the FFA and OFA.

Disclosures: G.R. Ianni: None. **D. Pitcher:** None. **L.G. Ungerleider:** None.

Poster

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NIH NEI R01 EY019684 to J.L.G.

Title: Object silhouettes and semantic tuning in human lateral occipital cortex

Authors: *M. D. LESCROART[1], S. NISHIMOTO[2], J. L. GALLANT[1];
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Abstract: Human lateral occipital cortex is comprised of several visual areas: multiple subdivisions of LO, the occipital face area (OFA) and the extrastriate body area (EBA). It is generally thought that LO represents intermediate shape features, and studies suggest that OFA and EBA are tuned for parts of faces and bodies rather than “faces” and “bodies” as rigid semantic categories (Pitcher et al, 2011; Taylor et al, 2007). Thus it is possible that areas in lateral occipital cortex might represent more general shape features, such as object contours that aid in figure/ground segmentation (a crucial step in object recognition). We investigated this possibility in an fMRI voxel-wise modeling experiment. We used Blender, an open-source

graphics and animation program, to create movies containing realistic rendered objects (e.g., humans, animals, vehicles, and tools). The objects were placed together in random settings that varied in color, texture, lighting and camera motion. We then used fMRI to record brain activity from four subjects while they watched the movies. Finally, we used two different feature spaces to fit voxel-wise models to these data. The semantic feature space was constructed by labeling each item in the movies. The silhouette (i.e., figure/ground) contour feature space was constructed by passing silhouette images of each scene through a spatio-temporal Gabor pyramid. We then used the semantic and silhouette feature spaces to fit a separate model to each voxel in each subject's brain. Finally, we evaluated predictions of each model using a separate data set reserved for this purpose. We find that the semantic category model accurately predicts responses in EBA and FFA. This is consistent with previous work showing that EBA and FFA voxels tend to respond whenever animals or people are present in movies (Huth et al., 2012). In contrast, the silhouette contour model accurately predicts responses in V4 and ventral LO. Surprisingly, predictions of the silhouette contour model are more accurate than those of the semantic model in OFA, an area that is conventionally thought to be selective for faces. (Our stimuli did not contain large, full-field faces.) Our results suggest that OFA also represents the boundaries of objects other than faces. Thus, representation of the bounding contours of objects appears to be common across ventral lateral occipital and posterior fusiform cortex, suggesting that these regions play a role in figure-ground segmentation in natural scenes.

Disclosures: **M.D. Lescroart:** None. **J.L. Gallant:** None. **S. Nishimoto:** None.

Poster

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Support: NSF STC: CCF-1231216

Title: Invariant face recognition in the presence of clutter

Authors: Q. LIAO[1], *J. Z. LEIBO[2,1], T. POGGIO[1];
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Abstract: In accord with a recent theory of ventral stream (Anselmi et al. 2013), which conjectures that invariance is the crux of object recognition, we investigate an unconventional

problem of "clutter invariance" on a number of visual recognition tasks. Specifically, we simulate feedforward "cortical modules" that can recognize faces, dogs and cats in unconstrained environments (i.e., with arbitrary transformations and complex backgrounds). The models are trained in an unsupervised fashion using natural videos. Despite substantial visual clutter in both training and testing phases, we show that these biologically plausible models are able to learn remarkably invariant and selective representations.

Disclosures: Q. Liao: None. **T. Poggio:** None. **J.Z. Leibo:** None.

Poster

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Title: Human right parietal cortex shows early preferential responses to naturalistic emotional stimuli: An MEG study

Authors: *B. DE GELDER[1], H. K. M. MEEREN[2], S. P. AHLFORS[3], M. S. HÄMÄLÄINEN[3], N. HADJIKHANI[3,4];
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Abstract: For highly social species, many signals with direct behavioral relevance are provided by the behavior of the others around them. For example, when a person's face and body expression shows fear, bystanders already prepare to act to possible danger. It is however not clear how our visual system achieves the earliest differentiation of emotional content necessary for rapid activation of the emotion-action link. There is longstanding evidence that emotional stimuli elicit enhanced activation in temporal cortex, but recent functional magnetic resonance imaging (fMRI) research has also begun to highlight involvement of the dorsal route. However, crucial information about the timing of neural events necessary to substantiate this proposed emotion-action link is still missing.To answer this question we investigated the cortical dynamics mediating early differentiation of fearful body language using magnetoencephalography (MEG),

which combines temporal resolution at the millisecond scale with good cortical spatial resolution. Event-related fields (ERF) were recorded using a 306-channel MEG system while healthy human volunteers watched greyscale photographs of human bodies expressing fear (fear condition) and performing a neutral action (neutral condition). The results show that the right lateral parietal cortex responded preferentially to fearful as compared to neutral whole body expressions already between 80 and 110 ms after stimulus onset, whereas no such early differential activity could be found in the object recognition system of the occipitotemporal cortex. The present findings provide the first empirical evidence for the hypothesis that in addition to the subcortical tectopulvinar system, the parietal cortex has a functional role in the rapid detection/orienting to threatening stimuli, and mediates a fast link between affective vision and behavioral output before detailed analysis in the ventral stream is completed.

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Poster

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Support: NIMH IRP

Title: The human posterior STS is causally connected to the amygdala and the thalamus: A combined TMS / fMRI study

Authors: *D. PITCHER, L. G. UNGERLEDIER;
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Abstract: Neuroimaging studies have identified multiple face-selective regions in human cortex, but the functional division of labor between these regions is not yet clear. Prior evidence suggests that the face-selective region in the right posterior superior temporal sulcus (rpSTS) is part of a cortical network dedicated to processing the changeable aspects of faces, such facial expression and eye gaze direction. The cortico-cortical connections of the rpSTS in the human brain remain unknown. In the present study we investigated what cortical regions are causally engaged when viewing dynamic faces by delivering thetaburst TMS (TBS) over the rpSTS, and the vertex control site, while 20 participants were scanned at 3T while viewing short movies of

faces, objects and bodies. Preliminary analyses showed that TBS delivered over the rpSTS reduced the fMRI response to faces, bodies and objects within the rpSTS itself. By contrast TBS delivered over the rpSTS selectively reduced only the fMRI response to dynamic faces in both the amygdala and the thalamus. These results suggest that the rpSTS is causally connected to both the amygdala and the thalamus when viewing dynamic faces.

Disclosures: D. Pitcher: None. **L.G. Ungerledier:** None.

Poster

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Title: Electrophysiological recordings reveal early facilitated visual processing of nude human bodies

Authors: *J. ALHO[1], N. SALMINEN[1], M. SAMS[1], J. K. HIETANEN[3], L. NUMMENMAA[1,2,4,5,6];
[1]Dept. of Biomed. Engin. and Computat. Sci., [2]O.V. Lounasmaa Lab., Aalto Univ. Sch. of Sci., Espoo, Finland; [3]Sch. of Social Sci. and Humanities, Univ. of Tampere, Tampere, Finland; [4]Turku PET Ctr., Turku, Finland; [5]MEG Core, Aalto NeuroImaging, [6]AMI Ctr., Aalto Univ., Espoo, Finland

Abstract: Electrophysiological studies have shown that a brain response at ~170 ms post stimulus (N170) is sensitive to perception of human faces and bodies. Considering the short evolutionary history of clothing, it is plausible that the brain networks specialized in body perception have been tuned to respond specifically to nude bodies, yet cerebral processing of nude vs. clothed bodies has not been studied in detail. We recorded simultaneous magneto- and electroencephalography (MEG/EEG) while subjects viewed photographs of male and female

faces, clothed bodies, and nude bodies. The following questions were addressed: How does the spatiotemporal organization of cortical activity differ between the visual processing of faces and bodies? How does clothing affect the cerebral processing of bodies? We observed larger N170 amplitudes to faces compared to clothed bodies. However, supporting our earlier findings (Hietanen and Nummenmaa, 2011), the N170 response to nude bodies was greater than that to clothed bodies or faces. MEG global field power revealed a prominent response peak at ~145 ms to all stimulus categories, at ~180 ms specific to faces, and at ~210 ms specific to nude bodies. Source analysis based on minimum-norm estimates and spatiotemporal clustering localized face-sensitive (faces > clothed bodies) responses to the right fusiform gyrus (FG) between 140-200 ms. In contrast, responses to clothed bodies were stronger in the left posterior occipito-temporal cortex (OTC; 100-300 ms) and at longer latencies in the right FG (240-300 ms). Stronger responses to nude vs. clothed bodies were localized first to the posterior OTC (100-200 ms) and later to more anterior temporo-parietal cortex (TPC; 200-300 ms). Responses were stronger to clothed vs. nude bodies only in the left medial occipital cortex (OC) between 220-300 ms. Compared to faces, nude bodies elicited stronger responses first in the OC (100-200 ms) and later in the TPC (200-300 ms). We conclude that the human brain is tuned to detect sexual cues from human bodies rapidly, and that this categorization process is reflected in enhanced activity in a widespread occipito-temporo-parietal network, including the extrastriate body area (EBA) in the lateral OC and the fusiform body area (FBA) in the FG. Such a perceptual 'highway' for processing of sexual cues is beneficial for triggering sexual behavior, subsequently ensuring mating and reproduction. Furthermore, our results point to a spatiotemporal organization of body perception where the EBA is involved in early and the FBA in later perceptual processing of human bodies.

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Poster

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EFA189045

Title: A causal role of the fusiform face area in face perception

Authors: *C. KELLER[1], I. DAVIDESCO[3], P. MEGEVAND[4], D. GROPPE[4], F. LADO[5,2], A. MEHTA[4];
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Abstract: Neuronal activity in the fusiform face area (FFA) has been shown to increase during the processing of facial features. However, the causal nature of the FFA in face perception is largely unknown. Here, we investigated the causal role of the FFA in face perception by using computer-controlled, event-related, direct electrical stimulation in six patients with intractable epilepsy. We compared the effects of stimulation of FFA and PPA on the speed and accuracy of detecting distorted faces. FFA and PPA sites were selected in each patient based on high gamma band (70-150Hz) selectivity to faces and objects, respectively, in a visual screening task prior to the current experiment. The level of difficulty was determined for each patient based on a set of training sessions with varying levels of distortion at a goal of 75% accuracy. Faces with distorted features (0-30%) were presented (250ms presentation; 1s ISI), while subjects discriminated between distorted and non-distorted faces. Electrical stimulation was performed by applying single current pulses to adjacent electrodes (bipolar, biphasic pulses, 100us/pulse, 5mA). For each trial, the following conditions were randomized: face with no electrical stimulation; no face with electrical stimulation; and face with electrical stimulation at -200, 100, 150, 200, and 500ms following face presentation. For each condition, high gamma power responses as well as the cortical evoked response to electrical stimulation (CCEP) was compared at rest and during visual stimulation. We demonstrate a location- and timing-specific causal relationship of neuronal activity in the FFA and the perception of distorted faces. Specifically, electrical stimulation of the FFA caused increased response times of detecting distorted faces. Importantly, these changes were not evident when stimulation occurred 1) in face selective regions prior to or following face presentation or 2) in place selective regions during face presentation. On a group and single trial level, neuronal activity within face-selective sites predicted the slowing of face distortion perception. Furthermore, electrical stimulation of the FFA elicited a decrease in the strength of the CCEP during presentation of visual stimuli compared to a resting period in the N2 but not the N1 time period. This was accompanied by a decrease in the suppression of high gamma power due to electrical stimulation, suggesting a release from stimulation-associated inhibition of neural activity in the FFA. Together, these behavioral and electrophysiological findings support a temporal and spatially specific causal role of the FFA in the perception of faces.

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Poster

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Topic: D.04. Vision

Title: Norm-based responses to identity in the macaque face patch AF

Authors: *A. P. JONES[1,2], D. B. T. MCMAHON[2], D. A. LEOPOLD[2];
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Abstract: Face perception is a fundamental aspect of primate social behavior that is thought to rely on a network of specialized visual cortical regions containing neurons that respond more to faces than to other visual categories. How such response selectivity contributes to primate face recognition is a topic of long-standing interest. At a theoretical level, face recognition is a difficult problem. Different faces share the same structural components in the same basic configuration; hence a face recognition system must be sensitive to details that distinguish individuals. One popular notion, which has received considerable support from human psychophysical studies, is that this process involves the comparison of individual faces with an internal, stored reference face. Such a reference, or norm, is thought to develop gradually with experience, and to approximate the statistical average of previously viewed faces. In an earlier electrophysiological study, our group showed that neurons in ventral IT cortex (TEav, face patch AM) respond to the identity of morphed human faces in a manner that is consistent with norm-based models (Leopold et al, 2006). Here we asked whether similar tuning is evident in the anterior fundus (AF) face patch of the superior temporal sulcus. We recorded the responses of neurons in awake, fixating macaques to brief presentation of macaque and human face stimuli. Each stimulus set consisted of twelve individuals morphed along a radial trajectory of face space and thus ranging from 0% identity (average face) to 100% identity, for a total of 197 images. Across a population of 51 neurons in one monkey that were significantly responsive, we found systematic tuning to face identity in roughly half the neurons tested. Seventeen neurons (33%) exhibited tuning that supported norm-based models, where responses showed similar trends (e.g. response increases) to identity level for at least half the faces, and with no examples of the opposite trend (e.g. response decreases) for other faces. Ten neurons (20%) were nominally inconsistent with norm-based models, as they showed at least one case in which there was a non-matching response change for different identities. The remaining neurons did not exhibit systematic tuning for face identity. Comparing response patterns over time revealed no evidence

for either within-session or between-session adaptation that could give rise to the observed identity tuning. These results point to an important role of the average face in the responses of neurons in face patch AF and, together with previous findings, suggest that norm-based responses may be widespread throughout the face processing system.

Disclosures: A.P. Jones: None. **D.B.T. McMahon:** None. **D.A. Leopold:** None.

Poster

823. Visual Processing: Faces

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 823.26/II21

Topic: D.04. Vision

Support: NIMH IRP

Title: Facial expressions evoke differential neural coupling in macaques

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Abstract: In humans and monkeys, face perception activates a distributed cortical network that includes extrastriate, limbic and prefrontal regions. Within face-responsive regions, emotional faces evoke stronger responses than neutral faces. We used functional MRI with an iron-based contrast agent (MION) to increase the contrast-to-noise ratio, and Dynamic Causal Modeling (DCM) to test the hypothesis that emotional faces differentially alter the functional coupling between the amygdala and inferior temporal (IT) cortex. Three rhesus monkeys performed a fixation task while viewing conspecific faces with neutral, aggressive (open-mouthed threat), fearful (fear grin), and appeasing (lip smack) expressions, which were presented in 32 s blocks. Based on the main effect of faces, five face-responsive regions-of-interest were selected: the posterior portion (area TEO) and the anterior portion (area TE) of IT cortex, the amygdala, the orbitofrontal cortex (OFC) and the dorsolateral prefrontal cortex (DLPFC). Various models of neural interactions (feedforward, feedback or bidirectional) between the amygdala and IT regions were tested. Our preliminary results indicate that the perception of neutral faces and the valence effect for fearful and appeasing expressions are mediated by feedback projections from the amygdala to IT cortex, whereas the valence effect for aggressive facial expressions is mediated by feedforward projections from IT cortex to the amygdala. These results suggest dynamic

alterations in neural coupling during the perception of socially relevant facial expressions that are vital for communication.

Disclosures: **N. Liu:** None. **A. Ishai:** None. **F. Hadj-Bouziane:** None. **L.G. Ungerleider:** None.

Poster

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Title: Temporal dissociation of sub-category level face processing

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Abstract: Functional human brain imaging studies have revealed regions within the ventral temporal cortex (VTC) with category specific visual responses to faces, objects, and words. These regions have been localized reliably across subjects with a consistent spatial organization with respect to anatomical landmarks such as the Fusiform Gyrus (FG) and mid-Fusiform Sulcus. Though several studies have attempted to classify the magnitude of VTC responses at the category level (i.e., selectivity for faces versus places), few studies have explored sub-category level neural activity (i.e., human faces versus mammal faces). In the current study, we first identified face-selective neuronal populations within the VTC and determined if the onset of their activation was different when subjects viewed human versus non-human faces. Towards this aim, we used electrocorticography (ECoG) in four patients implanted with subdural electrodes in the VTC. The ECoG data was collected while subjects performed two different tasks: Task 1 was a localizer task which contained images of human faces, places, English words, Spanish words, numerals, foreign numerals, corporate logos, and false fonts. In Task 2, the following categories and subcategories of images were presented: Faces (human, mammal, bird, fish); Bodies without heads (human, mammal, bird, fish); Objects; Places; and Limbs. In both tasks, subjects were instructed to press 1 when a red hashtag sign appeared. We analyzed High Frequency Broadband (HFB: 70-150 Hz) power changes in the VTC. In concordance with our previous work (Parvizi et al., JNeuro 2012), several face-selective FG electrodes ($p<0.01$,

FDR corrected) were identified in all subjects (S1: 3, S2: 2, S3: 5, and S4: 6 electrodes) using the Task 1 ECoG data. Only these electrodes were included in the analysis of data obtained from Task 2. Our analysis revealed a significant temporal dissociation between sub-categories of faces. In face-selective sites, the HFB power was most increased by viewing human faces compared to other face categories (mammal, bird, and fish). Moreover, we found that the onset of HFB activity for human faces was consistently earlier than the onset of activity for all other face categories. For example, the average onset of HFB activity for human faces preceded mammal faces by 26ms (+/- 11 ms) across electrodes. Our findings provide strong evidence for a temporal dissociation for processing human face images compared to other face sub-categories in the human VTC and add a temporal layer to the extensive body of literature on face per

Disclosures: V. Rangarajan: None. **B.L. Foster:** None. **S. Gattas:** None. **J. Parvizi:** None.

Poster

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Topic: D.04. Vision

Support: IWT

FWO

IUAP

PF

GOA

Title: Tolerance of macaque middle superior temporal sulcus body patch neurons to shape-preserving stimulus transformations

Authors: *I. D. POPIVANOV[1], J. JASTORFF[1,2], W. VANDUFFEL[1,3,4], P. G. SCHYNS[5], R. VOGELS[1];
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Abstract: Previously we demonstrated that although neurons in the middle Superior Temporal Sulcus (midSTS) body patch responded greater to images of bodies than to faces and objects, the body patch neurons show profound within-class stimulus selectivity (Popivanov et al. JNS 2014). Applying Bubbles showed that those neurons respond to relatively small stimulus fragments (Popivanov et al. SFN 2013). Here we assess the tolerance of the midSTS body patch neurons to stimulus transformations that preserve shape: changes in retinal location, in size and silhouette and outline rendering of the images (maintaining only shape information, but lacking textural or shading features). Also, we measured the effect of in-plane image rotation. We recorded spiking activity of single neurons from the left midSTS body patch of 2 awake and fixating macaque monkeys. After a search test with 100 stimuli (monkey and human bodies, faces, man-made objects and fruits/vegetables), stimuli were selected for further tests. All stimuli were presented for 200 ms with an inter-stimulus interval of ~ 400 ms. Analyses were done on baseline subtracted firing rates. RF mapping (4º size; 35 locations; 3º steps) showed that the RF of most of the neurons included the fovea. The RFs varied in size (mean > 8º, SD > 3º) and peak location with a bias towards the lower contralateral visual field quadrant. Stimulus preference (5 stimuli; 4º size) was preserved across 2 tested positions inside the RF. Presentations of two stimuli at 2º, 4º or 8º sizes showed strong tolerance of stimulus preference to size changes. Moreover, applications of Bubbles (Popivanov et al. SFN 2013) using two stimulus sizes revealed image fragments at similar relative locations that were correlated across the two sizes, demonstrating size tolerance. To assess whether these neurons still respond selectively when only shape features are present, we showed 10 stimuli in 3 versions: original (shaded and textured), their silhouettes and outlines. The large majority of the neurons responded as well to the silhouette as to the original image and maintained their selectivity for the silhouette versions (median correlation between the responses to the original and the silhouette $r = 0.63$). Furthermore, the degree of tolerance to the silhouette transformation correlated positively with the body category selectivity ($r = 0.35$). These data demonstrate that shape features are sufficient to drive body selective neurons of the midSTS body patch. Responses to outlines were reduced and showed less preserved selectivity. In-plane rotation (step: 45º) of the effective stimulus showed that responses of midSTS body patch cells strongly depended on orientation.

Disclosures: I.D. Popivanov: None. **R. Vogels:** None. **J. Jastorff:** None. **W. Vanduffel:** None. **P.G. Schyns:** None.

Poster

823. Visual Processing: Faces

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Program#/Poster#: 823.29/II24

Topic: D.04. Vision

Title: Neurons in the posterior STS extract facial information for the guidance of gaze following and the establishment of joint attention

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Abstract: Eye and head gaze orientation are powerful directional cues defining the object of a conspecific´s attention and attracting the observer´s overt gaze or covert attention to this object. This "gaze following" is an automatic response that results in the establishment of "joint attention", a key step in developing a theory of mind. Noninvasive imaging as well as brain lesion studies have implicated parts of the superior temporal sulcus (STS) in gaze following, the same general region known to play a role in face processing. In order to unravel the neural underpinnings of gaze following and its relationship to face processing, we have started to record from the posterior superior temporal sulcus (pSTS) of rhesus monkeys engaged in "active" tasks requiring gaze following towards distinct spatial targets or, alternatively, the identification of the same targets based on learned associations with facial identities. Finally, we also collected neuronal responses to passively viewed faces and a variety of biological and non-biological non-face stimuli, presented while the monkey maintained fixation of a dot ("passive" task). In the active tasks we presented portraits of 4 individual conspecifics ("senders") looking at one out of four spatial targets. For each trial the observing monkey was cued to either follow the sender´s gaze to a target or to select the target based on the sender´s identity while ignoring gaze orientation. Our microelectrode penetrations targeted a region in the pSTS that we have previously shown to exhibit a selective BOLD activation by active head gaze following. This region is spatially close to but not congruent with patches of cortex activated by the passive vision of faces (Marciniak et al., SfN No. 197.07, 2011). The 50 single units recorded from the right pSTS of one monkey exhibit a bewildering variety of response preferences, reflecting fine grained topographical differences. We could identify units that respond to the passive viewing of faces or to non-face biological objects, but not to faces used as sources of information driving shifts of attention. A second group of units were modulated whenever facial information was used to shift attention, independent of whether the monkey relied on head gaze orientation or identity. Finally, we could also identify a small set of units that differentiated between the need to extract head gaze orientation or identity by showing a clear response in the gaze following condition only. These preliminary results suggest that the pSTS of rhesus monkeys encodes different aspects of facial information cues in order to initiate joint attention.

Disclosures: H. Ramezanpour: None. **K. Marciniak:** None. **P.W. Dicke:** None. **P. Thier:** None.

Poster

823. Visual Processing: Faces

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Title: Representation of multiple stimuli by face selective neurons in the macaque temporal lobe

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Abstract: Primates are capable of recognizing faces even in highly cluttered natural scenes. In order to understand how the primate brain achieves face recognition despite this clutter, it is crucial to study the representation of multiple objects in object selective cortex. However, contrary to the essence of natural scenes, most experiments use only one or a few visual stimuli simultaneously to study neural response properties. It thus remains unclear how face selective neurons respond to multiple stimuli, some of which might be encompassed by their receptive fields (RFs), others not. How is the neural representation of a face affected by the concurrent presence of other stimuli? Two lines of evidence lead to opposite predictions: first, given the importance of MAX-like operations for achieving selectivity and invariance, as suggested by feedforward circuitry for object recognition, face representations may not be compromised in the presence of clutter. On the other hand, the crowding effect - the reduced discriminability (but not detectability) of an object in clutter - suggests that a face representation may be impaired by additional stimuli. To address this question, we conducted electrophysiological recordings in the macaque temporal lobe, where bilateral face selective areas are tightly interconnected to form a hierarchical face processing stream. For each neuron, the most preferred face stimulus was determined, then presented at the center of the neuron's RF. In addition, multiple stimuli (preferred stimuli or non-preferred distracters) were presented in different numbers (1,2,4 or 8), from different categories (face or non-face object), or at different proximity (adjacent to or separated from the center stimulus). Our findings are fourfold: the majority of neurons reduced their responses more (1) with increasing numbers of distracters, (2) with face distracters rather

than with non-face object distracters, (3) at closer distracter proximity, and, additionally, (4) the response to multiple preferred faces depends on RF size. Although these findings in single neurons may indicate reduced discriminability, we found that each stimulus condition was well separated in a high-dimensional space spanned by the neural population. Only a few neurons showed MAX-like behavior. These findings could be explained using the framework of a divisive normalization model, with implications for the neural circuitry that underlies these representations.

Disclosures: A.F. Ebihara: None. **A. Hocevar:** None. **M.O. Magnasco:** None. **W.A. Freiwald:** None.

Poster

824. Visual Learning, Memory, and Categorization

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Support: NSFC

Title: Short-term and long-term temporal entrainment in primary visual cortex

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Abstract: Many living organisms have the ability to remember temporal information such as time interval. Previous studies from zebra fish, monkeys and rats demonstrate that neural circuits in the optic tectum, prefrontal cortex, and the thalamus have ramping activities that temporarily correlate with periods of external rhythmic stimuli (also named entrainment). However, it remains unknown what the origin of entrainment is and how it appears in visual cortex. Here we chose Thy1-ChR2-GFP mice to do whole cell recording *in vitro* in order to test whether entrainment phenomenon is derived from the retinal ganglion cells (RGCs). We also recorded the response of V1 to repetitive visual stimulation *in vivo* using microelectrodes. In the *in vitro* experiments, we did not observe entrainment phenomenon in any RGC. What's more, we did not observe the replay phenomenon, which has been observed in the *in vivo* experiments in V1. Thus, it seems that entrainment and replay may not originate from the retina, but from the visual pathway. In the *in vivo* experiments, periodic blue laser stimuli (period 10s, duration 1s), was presented to one eye of Thy1-ChR2 mice mildly anesthetized by isoflurane, A few cycles of

rhythmic activity in V1 retained the interval, duration and even amplitude of previous periodic stimulation after periodic stimulation. Interestingly, we noticed an accidental discovery that the neural circuits seem to be able to 'replay' the 10s interval during stimulation of 20s intervals, right after 10s stimulations finished. There may be common neural mechanisms between the entrainment and replay phenomena, and it is quite possible that entrainment is the internal dynamics of replay.

Disclosures: **Q. Yu:** None. **N. Qin:** None. **J. Zhang:** None.

Poster

824. Visual Learning, Memory, and Categorization

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Title: Enhanced effective connectivity among early visual areas during visual skill learning: A longitudinal FMRI study

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Abstract: Models of visual skill learning emphasize contributions of either local plasticity in low-level visual areas (Low-Level Theory, LLT, [1]), or enhanced readout efficiency from low-level areas by high-level areas (Readout [2] and Reverse Hierarchy Theories, RRHT [3]). In

LLT, feedback is thought to be especially important during acquisition, but less so after asymptotic learning, when low-level intra-cortical re-wiring is finalized and skill performance automated. In RRHT, as the skill becomes perfected, more efficient readout is hypothesized to take place involving increasingly lower areas in the visual hierarchy. Hence, according to LLT, effective connectivity among V1/V2/V3 would be comparable before and after learning (with perhaps a bias towards increased feedforward, FF). By contrast, according to RRHT, there might be more evidence for effective connectivity among areas after learning than before (with perhaps a bias towards increased feedback, FB). To test these ideas, we trained 7 human participants in one quadrant with square wave gratings in an easy orientation discrimination task at a range of contrasts (1-32%). Similar, ignored stimuli were shown in another quadrant, and a third quadrant served as unstimulated control. We performed 3-Tesla FMRI measurements at 4-6 sessions during learning. Behavioral performance increased strongly for lower-range but not high contrasts. GLM FMRI analysis did not show changed responses to stimuli as a function of learning. We tested effective connectivity models between V1, V2 and V3 in the three visual quadrants using Ancestral Graph theory (AGT, [4]). AGT derives directed connectivity from variability among trial responses rather than from variability in the time series, and fits connectivity patterns against the full class of graph theoretical models using an automated search method. Theoretical models were characterized as FF or FB according to an index using information about path length and connectivity strength in opposite directions, and weighted by model evidence (Bayes Information Criterion). We found that both FF and FB models increased in strength over the course of learning, with a small bias towards increased FB, but only for contrasts showing increased skill. Moreover, this result was more pronounced in the trained quadrant, less so in the exposed/ignored quadrants, and absent in the control quadrant. Our data indicate a correlation between enhanced inter-areal recurrent connectivity and enhanced skill in early visual cortex, supporting RRHT. [1] Karni & Sagi, PNAS, 1991; [2] Law & Gold, Nat. Neurosci., 2008; [3] Ahissar & Hochstein, Nature, 1997; [4] Waldorp et al., Neuroimage, 2011.

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Poster

824. Visual Learning, Memory, and Categorization

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Title: Modulation of spatial working memory by optogenetic stimulation in the nonhuman primate

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Abstract: In addition to modulating neural activity, optogenetic manipulation in the monkey has been shown to be effective in biasing saccadic choice behavior. In a previous study, we demonstrated that optogenetic activation in cortical area LIP could bias attention in a visuospatial discrimination task (Dai et al., 2014). Area LIP is also thought to be a key neural substrate for visual working memory, as neurons in this area can show persistent activity during the delay period of memory-guided saccade tasks. To further understand the functional contribution of LIP neural activity in visual memory encoding, saccade planning, and eye movement execution, we applied optogenetic activation as a temporal probe to explore its potential influence at various task stages. Monkeys were trained to perform a classic memory-guided saccade task, in which a cued location had to be remembered so that following a go cue, a saccade could be made to that memorized location for reward. We used the same viral construct employed in our earlier study (AAV5-CaMKIIa- C1V1(E122T/E162T)-TS-EYFP) to express green light sensitivity in LIP neurons. Optogenetic stimulation was applied randomly at the visual cue phase, during the memory phase, or at the saccade execution phase. Our data indicate that when the cue was presented at receptive field of neurons near the stimulation site, optogenetic activation during the memory phase could improve performance by ~10%. Weaker effects were observed for stimulation at the cue encoding phase. Thus far, no clear effects have been seen for stimulation at the execution stage. Further analysis on saccade trajectories indicated that stimulation at different task phases appears to differentially affect saccade landing position. Together, these results support the view that area LIP plays a central role in the registration and maintenance of locations of visual salience, but a less critical role in the direct execution of saccadic eye movements.

Disclosures: J. Dai: None. **D.L. Sheinberg:** None.

Poster

824. Visual Learning, Memory, and Categorization

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Topic: D.04. Vision

Support: CIHR

NSERC

CRC

Title: Absence of working memory coding in MT during a fine discrimination for motion task

Authors: *S. TORRES[1], J. MARTINEZ-TRUJILLO[2];
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Abstract: Working memory (WM), the ability to maintain and manipulate recently presented information over a short span of time in the absence of external sensory input, requires the functional build-up of neural circuitry operations, starting from low-level sensory processing to higher-level cognitive areas. Neurophysiological recordings in the brain of macaques have inferred neuronal correlates of WM in the sustained firing of feature-tuned neurons in multiple cortical areas, including the higher-order dorsolateral prefrontal cortex (dlPFC) as well as posterior parietal and inferior temporal cortices. Nevertheless, the precise role of earlier sensory areas in the maintenance of WM is still a matter of debate. Using a delayed Match to Sample (dMtS) task with broadly defined motion directions (90 degrees apart), neurons with robust WM-related responses were recently observed in medial superior temporal (MST), a multimodal area immediately down-stream from the strictly sensory area middle temporal (MT) (Mendoza et al., SFN abstracts). Using this same paradigm, MT activity decayed quickly after the offset of the sample stimulus. MT and MST are cortical areas at intermediate stages of the motion direction sensory processing, heavily interconnected and just one synapse away along the hierarchical stream of the dorsal visual pathway, and both areas have feedforward and feedback connections to dlPFC. However, fMRI studies using voxel-based pattern classification have challenged this idea by decoding WM information from early visual areas such as V1 (Harrison & Tong, 2009). One possibility is that MST signals encode the motion category (up, left, right, down) rather than the specific remembered direction, leaving the possibility that MT was not engaged in the task and could encode for the remembered motion direction. In order to verify that the monkeys were not categorizing, they performed a fine discrimination (15 degrees) motion direction DMTS task while recording from MT. MT activity was indistinguishable from baseline after the offset of the sample stimulus with similar decay latencies as previously reported, confirming the absence of WM signals in MT during a fine discrimination DMTS task. The absence of sustained activity during the WM period in MT suggests the sharp emergence of specialized neural circuitry for the maintenance of WM signals, and a functional threshold from early visual areas, encoding current visual input, to higher association cortices that additionally encode and maintain internally generated and relevant information.

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Poster

824. Visual Learning, Memory, and Categorization

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Support: Supported by the NIMH IRP

Title: Monkeys and humans share a similar neural structure for facial expression classification in the amygdala

Authors: *H. ZHANG, A. STACY, S. JAPEE, L. UNGERLEIDER;
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Abstract: Recognition and classification of facial expressions are crucial for effective social functioning. Both monkeys and humans can classify different facial expressions automatically and effortlessly. In our previous studies of monkeys (Zhang et al, SfN 2013) and humans (Zhang et al, SfN 2012), we reported that the amygdala plays a significant role in discriminating between fearful faces and non-fearful faces. Yet, it is still unclear whether or not the amygdala in both species share the same classification structure. In the current study, we investigated this issue. Two male macaque monkeys were injected with MION prior to being scanned at 4.7T in a slow event-related fMRI experiment. During scanning, they viewed images of 32 monkey faces belonging to eight identities and four expressions: fear grin (fearful), threat (aggressive), lip smack (submissive) and neutral. The 23 human subjects participated in a slow event-related fMRI experiment at 3T, in which they viewed images of 32 human faces belonging to eight identities and four facial expressions: fearful, angry, happy and neutral. In order to localize active regions in the amygdala, both monkeys and humans were also scanned in separate runs containing blocks of faces, objects and scrambled pictures. The one-versus-others Support Vector Machine (SVM)- based binary tree architecture was used to determine how well each class of facial expression was decoded from all other facial expressions. Accordingly, at the first level, the one-vs-three SVM classified faces as: fearful and non-fearful, angry and non-angry, happy and non-happy, and neutral and those expressing emotion. At the second level, the three expressions that did not have the highest accuracy of classification in the first level went into the one-vs-two SVM for classification. At the third level, the remaining two expressions that did not have the highest accuracy of classification in the second level went into a one-vs-one SVM for classification. Our results showed that, at the first level, classifying fearful and non-fearful faces had the highest accuracy of classification in the amygdala for both monkeys and humans. Similarly, at the second level, classifying neutral and the other emotional faces (threat/lip smack,

angry/happy) had the highest accuracy of classification for both monkeys and humans. At the third level, neither monkeys nor humans showed any significant accuracy at classification. Taken together, our results suggest that monkeys and humans share a similar neural structure for facial expression classification in the amygdala. Supported by the NIMH IRP.

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Poster

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Title: Visual perceptual training induces two dissociable learning processes

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Abstract: Our percept of a target stimulus can be affected by stimulus context. Previous studies on perceptual learning usually focus on improvement in sensitivity to the target; it is unclear whether training can also modify the interactions between the target and task-irrelevant context. In this study we examined learning-induced changes in percept of a visual target in the context of a surround stimulus. Human subjects were trained to discriminate a difference in orientation between two successively displayed grating patches. One was used as a reference at a constant orientation, and the other as a probe with small orientation deviations from the reference. The reference gratings were encompassed by gratings of a different orientation to induce a tilt illusion on the reference orientation. This design allowed for a simultaneous measure of the threshold for orientation discrimination and the magnitude of the tilt illusion based on the psychometric curve. Our data showed that not only the orientation threshold but also the illusion magnitude gradually decreased with training. These two processes were independent in time, as there was no correlation between their session-by-session changes. Moreover, by independently manipulating the orientations of the reference and its contextual surround, we found that the improved

orientation discriminability was specific to the orientation of the reference but not its surround, while the reduction in tilt illusion showed an opposite effect. This indicates that these two learning processes are orientation dependent and dissociable in space. We also observed that training to discriminate a small size difference between the reference and probe grating patches also decreased the tilt illusion but had no effect on orientation discrimination, indicating that the two learning processes are dissociable in task dependency. Taken together, our results indicate that perceptual training can induce two parallel learning processes, a task-specific process for enhancing task-related signals and a task-unspecific process for reducing contextual interactions. These two processes are subject to different interactions between bottom-up and top-down signals.

Disclosures: E. Zhang: None. **W. Li:** None.

Poster

824. Visual Learning, Memory, and Categorization

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 824.07/JJ2

Topic: D.04. Vision

Support: NEI Grant R01-EY014970

Title: A quantitative link between unsupervised neuronal plasticity in inferior temporal cortex and unsupervised human object learning

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Abstract: The inferior temporal (IT) cortex is thought to underlie visual object recognition. Neurons in IT cortex are selective to object identity and tolerant to variations in object size, position and pose. The temporal continuity of our natural visual experience has been proposed as one mechanism to form that tolerance. Consistent with this, physiological data in the IT cortex of macaque monkeys show that unsupervised viewing of swapped temporal statistics reshapes position and size tolerance of basic level objects. Similarly, psychophysical results from adult humans show that unsupervised viewing of altered temporal statistics reshapes the position and pose tolerance of subordinate level objects. If IT population codes are the basis of visual object recognition, then these experience-driven changes in IT neuronal tolerance should quantitatively

predict the changes in the tolerance of human object recognition. To test this hypothesis, we used our existing model that explains human object recognition from IT population responses. To ask if that model generalizes to predict unsupervised human object learning from neuronal plasticity in IT, we assembled neuronal and psychophysical unsupervised learning data with the same objects. First, we measured temporal-contiguity-induced human learning over a wide range of tasks (from subordinate to basic level). This revealed that unsupervised learning depends on the initial object discrimination difficulty: strong learning is found for object discrimination tasks that are of medium difficulty (initial d' of ~1.5), while little or no learning is found for very low and very high levels of object discrimination difficulty. Next, we created a simulated IT population based on real neural responses collected from monkey IT cortex to the same images as human learning tests. This simulated IT preserves the actual neural object selectivities and tolerances. Finally, we applied a neural learning rate measured with the same learning procedure in a previous study to all neurons in the simulated IT. We found that our model accurately predicted the observed magnitude of human learning and its development over additional experience, without any parameter tuning. One interesting caveat in our results is that, for easy discriminations, the neural population learning predicts stronger human perceptual changes than we observed, but our simulations show that this is to be expected due to the limits of psychophysical testing. Taken together, these results argue that IT population is responsible for human object perception, and we have a quantitative model that accurately links unsupervised IT neuronal plasticity to human object learning.

Disclosures: **X. Jia:** None. **J.J. DiCarlo:** None. **H. Hong:** None.

Poster

824. Visual Learning, Memory, and Categorization

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JST, CREST to Y.M.

JSPS Research Fellowships for Young Scientists 234682, 265926 to K.M.

Title: Dissociable memory formation processes within the macaque medial temporal lobe

Authors: *K. MIYAMOTO, Y. ADACHI, T. OSADA, T. WATANABE, R. SETSUIE, H. M. KIMURA, T. WATANABE, Y. MIYASHITA;
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Abstract: In humans, subsequent memory formation has been thought to occur in the medial temporal lobe (MTL) on the basis of functional MRI (fMRI) studies. However, precise anatomically-based localization and characterization of regions involved remains difficult because of limited knowledge on the demarcation of areas in the human MTL. To address the gap in knowledge bridging brain function and anatomy, fMRI investigations in macaque monkeys performing memory tasks (Miyamoto et al., 2013, Neuron 77, 787-799) have been beneficial, because the anatomical reference frame based on cytoarchitecture and axonal projections is available for monkeys. Here, we conducted fMRI experiments on two macaque monkeys performing a serial probe recognition task in a 4.7T MRI scanner, and, for the first time, identified monkey brain regions involved in the formation of memory traces that accurately predict subsequent memory performance (i.e., recognition or failure to recognize). Application of both multivoxel pattern analysis and conventional univariate analysis to high-resolution fMRI data allowed us to identify memory traces within the caudal entorhinal cortex (cERC) and area 36 of the perirhinal cortex (PRC), as well as within the hippocampus proper. Furthermore, activity in the cERC and hippocampus, which are directly connected, was responsible for encoding the initial items of sequentially presented pictures related to the primacy effect in the task, which may reflect recollection-like recognition, whereas activity in the PRC was not. Bilateral hippocampi were also activated when monkeys successfully recognized previously seen items, especially the initial items, as well as when they were encoding them. Moreover, the hippocampus was specifically responsible for successful retrieval based on actually encoded items, but not for false retrieval (Hit > False alarm). These results suggest that two qualitatively distinct encoding processes work in the monkey MTL, and that recollection-based memory is formed by interaction of the hippocampus with the cERC, a focal cortical area anatomically closer to the hippocampus and hierarchically higher than previously believed. Additionally, the memory signal formed in the hippocampus was suggested by the results to be critical for its retrieval. These findings will accelerate fine electrophysiological characterization of dissociable memory traces in the monkey MTL, and will advance the understanding of common memory systems between humans and monkeys.

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Poster

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Program#/Poster#: 824.09/JJ4

Topic: D.04. Vision

Title: Differential timing in the representation of different scene dimensions

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Abstract: Previous work on scene representations has emphasized the importance of several dimensions, including Content (manmade/natural), Depth (near/far), and Boundary (closed/open) (Oliva and Torralba, 2001; Torralba and Oliva, 2003). The spatial dimensions of Depth and Boundary are strongly evident in the responses of the early visual cortex (EVC) and the parahippocampal place area (PPA) (Kravitz, Peng, and Baker, 2011; Park et al., 2011), with EVC primarily grouping scenes by Depth and PPA by Boundary. Judgments of Content can be made very quickly (Joubert et al., 2007) and Content may be reflected in the response of the object-selective lateral occipital complex (Park et al., 2011). The objective of the present study was to determine whether there are different timecourses to the representation of these different scene dimensions, and, in particular, whether Boundary is represented more slowly than Content. Participants were asked to make judgments of the Content, Depth, or Boundary of probe scenes (drawn from Kravitz, Peng, and Baker, 2011) that had been preceded by a prime scene that was either congruent or incongruent with the probe along the relevant dimension. Each block required binary judgments of a single dimension (e.g., manmade or natural). The duration of prime was either 50 or 300 ms and followed by a random dot mask. Primes of 50 ms were effective only for the Content. The significantly better performance with the congruent than incongruent primes indicated that the prime duration of 50 ms was sufficient for the representations of Content to form. The Boundary and Depth priming occurred for the 300ms primes, leading to significant interactions between the dimension being judged and the duration of the primes. The later emergence of the representation of the spatial dimensions is in agreement with ongoing ERP work (Harel et al., in prep). Further an analysis of the image properties of these scenes, as well as other scene sets, indicates that the differences in Content may be captured by low-level visual statistics. In summary, our findings suggest that the representations of Boundary take a significant amount of time to form, perhaps reflecting a reliance on more complex visual statistics and later/more complex processing than Content.

Disclosures: H.K. Pyoun: None. **D. Kravitz:** None.

Poster

824. Visual Learning, Memory, and Categorization

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JST CREST

Title: Distribution of neurons responsible for visual stimulus-stimulus association memory in the macaque perirhinal cortex area 36

Authors: *K. W. KOYANO[1], M. TAKEDA[1], T. MATSUI[1,2], Y. OHASHI[1], T. HIRABAYASHI[1], K. KAKIZAWA[1], T. WATANABE[1], Y. MIYASHITA[1,2];
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Abstract: Brodmann area 36 of the perirhinal cortex (A36) is located in the medial temporal lobe and is critical for stimulus-stimulus associative memory. Previous single-unit studies using macaque monkeys showed that neurons in A36 exhibited activities related to mnemonic processing, such as memory retrieval. Here, we investigated the spatial laminar distribution of memory-related neurons along the depth direction within A36 by combining *in vivo* magnetic resonance imaging (MRI)-assisted single-unit recording (Matsui et al. 2007) and postmortem histological analysis. At the beginning of each recording session, a tungsten microelectrode was semi-chronically implanted to A36 of macaque monkeys, and then single-unit recording in the awake state and MRI scanning under anesthesia (a fast spin-echo sequence with a 4.7-T Bruker MRI scanner, TE/TR = 60/3000 ms) were performed alternately on successive days. The positions of the recorded neurons were reconstructed for each penetration from the microelectrode tip positions visualized on the MRI images. After all the recording experiments were finished, the monkeys were perfused and their brains were cut into 40-μm sections. The sections were stained for Nissl to show the laminar structure and reconstructed into three-dimensional histological volumes according to postmortem MRI images. The spatial coordinates of neurons localized in the *in vivo* MRI volume were affine-transformed into a histological volume with reference to elgiloy metal-deposit fiducial markers, which are detectable with either

MRI or histology (Koyano et al., 2011). As such, we reconstructed the positions of single-unit activities in two monkeys performing a pair-association task onto histological sections (n=1145 neurons). There was a cluster of neurons showing visual stimulus selectivity (one-way analysis of variance, $p<0.01$) in A36. Neurons in the infragranular layers of the cluster tended to show their stimulus selectivity not only during visual cue presentation but also during the subsequent delay period. Furthermore, a subset of these delay-selective infragranular neurons showed significant target-related recall activity. These results indicate that memory-related neurons in A36 were spatially organized according to their functional dissociation across the laminar structure.

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Poster

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Topic: D.04. Vision

Support: WELLCOME-DBT INDIA ALLIANCE

INDIAN INSTITUTE OF SCIENCE

COGNITIVE SCIENCE INITIATIVE, DST, GOVT OF INDIA

Title: Competitive interactions between rule and association learning during face categorization

Authors: ***H. KATTI**, N. C. PUNEETH, S. P. ARUN;
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Abstract: How do we learn new object categories? Two mechanisms have been proposed for category learning: (1) Low-level associations between each object and its category label and (2) Learning of a high-level rule that allows generalization to novel instances. Although there is evidence that associations and rules are both acquired during learning, it is not known whether the two processes interact or not. Here, we addressed this fundamental question by characterizing how humans learn a novel face category. We identified 23 subjects with poor performance on an ethnicity categorization task on Indian faces. Subjects underwent an intensive 5-day learning regime where they had to categorize 400 faces each day and were given feedback about the

correct category. On a fraction of the trials on each day, in addition to being asked to categorize the face, subjects were asked whether they recalled seeing the face on the previous day (each face was shown at most twice during learning). Thus, on each day, we were able to measure (1) the fraction of correctly categorized new and old faces; and (2) the fraction of correctly remembered faces. The main findings of our study are as follows: (1) Most subjects showed an increase in performance on novel faces across days, indicative of generalization. They were also more accurate on previously seen faces than novel faces, suggesting that they were also using their memory to categorize these faces. This increase in performance was accurately predicted using a mixture model that incorporated both rule and association learning. (2) Subjects that showed faster generalization tended to have decreasing recall across days, indicating an interaction between rule learning and associations; (3) The drop in recall may have occurred because subjects saw a large number of faces across days. To address this confound, we confirmed in a separate experiment that there was no drop in recall when subjects were tested only on recall without any learning. Taken together, our results show that both rule and association learning occur during category learning and exhibit competitive interactions.

Disclosures: H. Katti: None. **S.P. Arun:** None. **N.C. Puneeth:** None.

Poster

824. Visual Learning, Memory, and Categorization

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Topic: D.04. Vision

Title: Human Visual categorization is only consistent with Bayesian generative representations

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Abstract: In neuroscience, the generative framework of Bayesian Decision Theory has emerged as a principled way to predict how the brain has to act in the face of uncertainty (Ernst & Banks, 2002, Körding & Wolpert, 2004, Faisal et al., 2008). We hypothesize that the brain might also use generative Bayesian principles to implement its categorization strategy. Previous experimental work on human categorization shows data that is consistent with both discriminative and generative classification (Hsu & Griffiths, 2010) and did not allow confirming the implementation of one or the other. Therefore, we designed a novel experiment in

which subjects are trained to distinguish two classes A and B of visual objects drawn from Gaussian parameter distributions with equal variance. During two different experimental paradigms, we test how the subject's representation of the categories change after being exposed to outliers for only one of the categories, A, far from category B. Generative classifiers are by necessity sensitive to novel information becoming available during training, which updates beliefs regarding the generating distribution of each class. In contrast, discriminative classifiers are sensitive to novel information only if it affects the immediate discrimination of classes. In the first paradigm, we characterize the categorization boundary between the two classes and track the shift in the boundary after the introduction of outliers. A generative classifier will prompt to reconsider the variance of class A and shifts the boundary towards category B accordingly. However, the discriminative classifier will not react as there is no new information added to the boundary itself. Our second paradigm provides an even more stringent test for generative models: again, outliers for class A are presented far away from class B. Additionally, the two classes are selected to be close enough, such that a generative classifier would assume that class A's variance has increased significantly, thereby reaching across the region occupied by B. This will result in the emergence of a second classification boundary to the distal side of class B far away from class A. Again, the discriminative classifier would not change its behavior. Our results in both paradigms show that the introduction of the outliers for category A influences the subject's knowledge of the distribution associated with alternative categories. This can result in the introduction of additional boundaries, only predicted by our simulations of generative classifiers. These results give clear evidence that visual categorization is only consistent with generative and not discriminative classification mechanisms.

Disclosures: F. Mehraban Pour Behbahani: None. **A. Faisal:** None.

Poster

824. Visual Learning, Memory, and Categorization

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Topic: D.04. Vision

Title: Insights into the learning rule for visual familiarity memory through manipulations of sensory and cognitive drive

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Abstract: Visual familiarity memory - our capacity to remember whether we have previously encountered people, objects, and scenes - is virtually limitless. People can correctly identify whether they have seen an image before after viewing thousands of images, each only for a few seconds. Experimental and computational work suggests that this remarkable capacity is achieved via synaptic changes between neurons in high-level visual brain areas such as inferotemporal and perirhinal cortex. However, little is known about the specific manner in which these synaptic changes depend on the strength of the inputs driving these synapses or equivalently, the learning rule. To better understand the nature of this learning rule we measured how familiarity memory depends on manipulations known to modulate neural responsiveness, including changes in sensory drive (stimulus size) and cognitive drive (attention). In the first phase of these experiments, human subjects sequentially viewed hundreds of novel images as they performed a delayed match-to-sample (DMS) task in which they detected when a sample stimulus was repeated later as a target. Intervening distractor stimuli were repeated with the same frequency as targets. During a second phase subjects were presented with pairs of images - one novel and one familiar - and asked which they had seen in the first phase of the experiment. We found that manipulations of image size modulated visual familiarity memory with a near maximal dynamic range on this two alternative forced choice experiment, with memory peaking at an average of 94% across subjects for large sample images (9 degrees) presented as targets and declining to near chance levels at the smallest size (0.3 degrees). Visual familiarity memory was also profoundly modulated by the attentional demands of the DMS task: memory for distractors was reduced on average by 68% as compared to targets. These findings demonstrate that the synaptic changes that give rise to visual familiarity are driven by neurons whose responses are modulated by both sensory drive and task demands and they place quantitative constraints on the specific learning rules that the brain uses to store visual familiarity memories.

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Poster

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NIH R01 EY021755 (NTB)

Title: Repetition effects in ventral visual cortex after bilateral hippocampal loss

Authors: *J. G. KIM[1], E. GREGORY[3], B. LANDAU[3], M. MCCLOSKEY[3], N. B. TURK-BROWNE[1,2], S. KASTNER[1,2];
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Abstract: Repeated stimuli elicit attenuated responses in the visual system relative to novel stimuli, which can be considered a form of rapid learning. Such adaptation occurs not only when a stimulus is repeated immediately, but also when there is a lag in terms of time and other intervening stimuli before the repetition. Although immediate adaptation may reflect a refractory period within the local cortical area in which it occurs, the mechanisms of long-lag adaptation, which can span minutes or longer, remain unknown. Given its position at the top of the ventral visual stream and its mnemonic and temporal coding properties, the hippocampus is a candidate region for providing feedback to ventral visual cortex upon stimulus repetition that could induce adaptation. Here we examined this possibility in patient LSJ, a 64 year-old woman with severe anterograde and retrograde amnesia resulting from viral encephalitis. High-resolution anatomical MRI revealed that more than 98% of her hippocampus was lost bilaterally; she also has extensive damage to other medial temporal lobe and anterior temporal structures, mainly in the left hemisphere. Although LSJ is profoundly impaired on memory tasks (e.g., Wechsler Memory Scale General Memory index < 0.1 percentile), her basic sensory and language abilities are spared. To measure adaptation, we first defined regions of interest in ventral visual cortex using functional localizers for objects and scenes: lateral occipital complex (LOC) and parahippocampal place area (PPA), respectively. We then performed four experiments in each region, varying the lag between repetitions. The first experiment used a block design in which stimuli repeated several times back-to-back. The other experiments used a rapid event-related design, but differed in terms of the interval between the first and second presentation of each stimulus: 30 seconds, 3 minutes, and 6 minutes. We expected immediate repetitions to produce adaptation in LSJ, and indeed this was observed for both objects and scenes in LOC and PPA, respectively. Strikingly, in all of the other lags, LSJ showed identical results to age-matched controls: reliable adaptation was found for both categories at 30-second and 3-minute lags, but not at the 6-minute lag. Together, these findings suggest that the hippocampus is not necessary for long-lag repetition effects in ventral visual cortex. Future work will examine what other brain systems might be responsible, and whether the hippocampus is involved in normal controls, with LSJ recruiting other compensatory systems.

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Poster

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Topic: D.04. Vision

Support: NIH Grant EY023384-01

Title: Objective mental images

Authors: ***T. NASELARIS**[1], K. LYNAM[2];
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Abstract: Advances in brain-reading technology have raised the possibility of reconstructing mental images from fMRI measurements of human brain activity. Mental imagery cannot be precisely manipulated, so it would be difficult to assess the accuracy of any reconstruction of a mental image from brain activity. We present a paradigm for estimating an objective ground-truth mental image from simple behavioral responses to mental imagery probes. In our experiments subjects study a reference photograph until they feel familiar with the inventory and placement of the objects it depicts. Subjects then view a blank gray screen onto which they project a mental image of the photograph. Mental imagery probes are then flashed on the screen, one at a time. Each probe is a cluster of white pixels covering a variable area of the screen. Subjects indicate the number of objects in the imagined photograph that are occupied by each probe. Approximately one thousand responses are collected in an hour. From these probe/response pairs we infer the parameters of a generative model of the mental image. The model reveals the location and boundaries of the imagined objects while capturing the intrinsic variability of the inspection process. The model is easily validated by comparing its predicted responses to novel probes with the subject's actual responses. Preliminary results from two subjects suggest that mental images can be highly distorted relative to a reference photograph. Objective ground-truth mental images may thus be critical for benchmarking algorithms that attempt to reconstruct mental images from brain activity.

Disclosures: **T. Naselaris:** None. **K. Lynam:** None.

Poster

824. Visual Learning, Memory, and Categorization

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Topic: D.04. Vision

Support: BMBF 01GQ0923

BMBF 0315581B

DFG GZ WI830/10-1

Title: Experience-dependent structural plasticity in entorhinal cortex following monocular deprivation in adult rats

Authors: *S. GULL[1], S. SCHMIDT[1], J. REICHENBACH[2], C. GASER[3], O. W. WITTE[1]; [1]Hans Berger Dept. of Neurol., [2]Dept. of Diagnos. and Interventional Radiology I, Med. Physics Group, [3]Dept. of Psychiatry, Structural Brain Mapping Group, Jena Univ. Hosp., Jena, Germany

Abstract: *Objective:* Monocular deprivation (MD) has been established as an experimental model to study the plasticity of the adult brain. In interaction with the visual cortex, deprivation of one eye modulates as yet unknown pathways to enable enhanced sensitivity of the non-deprived eye. To search for the brain areas mainly involved in this sensory adaptation, we longitudinally tracked the changes of gray matter macro-structure *in-vivo* by magnetic resonance imaging (MRI) and deformation-based morphometry (DBM). Next, we validated our findings in cross-sectional experiments on the microscopic level. *Methods:* T2-weighted brain magnetic resonance images were acquired longitudinally at baseline and 3, 7 and 10 days following MD (male wistar rats, 2 months) and processed by DBM. Spatial frequency sensitivity of the optokinetic response (visual acuity [VA]) was monitored for the open eye. Changes in astrocytic morphology, neuronal activity and spine pool were analyzed by using immunohistochemistry (GFAP, Arc) and Golgi impregnation. *Results:* VA of the non-deprived eye increased rapidly during the first 3 days of MD and stabilized at ~35 % above baseline around day 7. Fast regional expansion of gray matter ranging 2-6 % evolved in entorhinal cortex followed by renormalization. Entorhinal expansion recapitulated the MD-induced “learning-curve” and was accompanied by increased neuronal activity and reduced astrocytic complexity. Normalization of entorhinal volume manifested with an enlarged spine pool and increased complexity of astrocytes. *Conclusions:* Changing binocular experience following abrupt loss of one eye’s visual field is reflected in the brain by transient swelling of the entorhinal cortex - the main relay station in a widespread network for spatial memories and navigation. Entorhinal expansion

corresponds to daily amount of sensory gain and is accompanied by enhanced neuronal activity and depletion of the static cytoskeleton of GFAP-positive astrocytes. Subsequent volume renormalization manifests with an enlarged spine pool and increased complexity of astrocytes.

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Poster

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Support: NHMRC APP1008287

HFSP CDA

Title: Adaptation decorrelates neuronal activity in visual cortex in a tuning-dependent manner

Authors: *N. S. PRICE, E. ZAVITZ, S. HAGHGOOIE, H.-H. YU, A. DAVIES, M. ROSA; Physiol., Physiology, Monash Univ., Clayton, Australia

Abstract: Prolonged exposure to an "adapting" visual stimulus improves the detection and discrimination of subsequently viewed "test" stimuli that have similar properties to the adaptor. These changes in perceptual sensitivity are often accompanied by reductions in the firing rates and shifts in the tuning curves of sensory neurons. Here, we characterise "noise" or "spike-count" correlations (rSC) between the responses of pairs of multi-unit neuronal recordings obtained in four cortical visual areas in anaesthetised marmosets (Callithrix jacchus). Six marmosets were anaesthetized with sufentanil (8 μg/kg/h) and N2O (70% in oxygen), and extracellular recordings were obtained using 96-channel microelectrode arrays (Blackrock Microsystems), inserted into V1, V2, the dorsomedial visual area (DM) or the middle temporal area (MT). We recorded the responses to sine-wave gratings moving for 0.5 seconds in one of 24 possible directions. Gratings were presented after 1-2 seconds of a blank grey screen, or 1-2 seconds of sustained motion in a single adapting direction (0, 45 or 90deg). At the single neuron level, the effects of adaptation depended on the cortical area. In all areas, neuronal gain, or the peak firing rate evoked by a test stimulus, was reduced following adaptation, regardless of the relationship between a neuron's preferred direction and the adaptation direction. In V1/V2,

adaptation produced “repulsive” changes in tuning, such that the post-adaptation preferred direction was shifted away from the adaptation direction. Surprisingly, in DM and MT, no consistent changes in direction tuning were observed following adaptation. To characterise effects at the population level, for each pair of neurons we determined spike-count correlations (rSC), the Pearson correlation between the spike counts evoked by multiple repetitions of an identical stimulus. Before adaptation, rSC was highest when the neuron pair had similar preferred directions and the greatest level of overlap in their receptive fields. After adaptation, average rSC across the population did not change significantly, but the structure of correlations changed in a tuning-dependent manner. rSC decreased for neurons with similar direction preferences, leading to a smaller range of correlations across the population. This tuning-dependent change in correlations after adaptation may optimise sensitivity by only affecting a limited range of inputs to a population of neurons, notably those that are shared locally, between neurons with similar preferences, rather than those that are globally shared across all neurons in an area.

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Poster

825. Sensory Coding, Perception, and Navigation

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Topic: D.07. Vestibula

Support: NIH R01 DC04158

Title: How many decision boundaries contribute to human vestibular decisions?

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Abstract: Decision-making is fundamental to a broad range of fields including neuroscience, engineering, psychology, economics, medicine, etc. The two predominant theoretic approaches underlying the study of decision-making - signal detection theory and sequential analysis (“drift diffusion”) - differ fundamentally. Signal detection theory assumes a single decision boundary

that cleaves the decision variable space into two regions (e.g., left/right), while sequential analysis assumes two decision boundaries that cleave the decision variable space into three regions (e.g., left/right as well as undecided). Which model best represents human decision-making? Earlier investigations into this fundamental issue were inconclusive, so we chose to utilize vestibular decision-making to investigate this question. Self-motion threshold tasks provide an excellent way to study decision-making since vestibular decision-making is fundamental (e.g., Which way am I falling?) and since vestibular circuitry is relatively simple when compared to other sensory systems (e.g., vision). To investigate this question, we tested human subjects using whole-body self-motion in the dark. Standard direction-recognition forced-choice methods were utilized. Stimuli magnitude was chosen using standard adaptive staircase methods. While control data using other tasks are presented, for our primary experiments, we used a two-stage task implemented on an iPad. First, the subjects provided a standard binary response indicating perceived motion direction (e.g., left versus right). This was immediately followed by a second binary response indicating whether the first response was a guess ("uncertain") or not. Binary thresholds were determined by fitting a signal detection model having a single decision boundary to the binary data (i.e., first response). Trinary thresholds were determined by fitting a model having two decision boundaries to the data whereby the binary response was changed to uncertain when the subject's second response indicated that they were uncertain regarding their first binary response. Underlying noise was assumed Gaussian for both models. Results suggest little difference in the thresholds estimated by the two models. Theoretical analyses show that this is the expected outcome if humans can make decisions using a single decision boundary when given that task but can also make decisions using two decision boundaries when given that task.

Disclosures: D.M. Merfeld: None. **T.K. Clark:** None. **Y. Yi:** None. **R. Galvan-Garza:** None. **M.C. Bermúdez Rey:** None.

Poster

825. Sensory Coding, Perception, and Navigation

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Title: Whole-body tilt thresholds outperform static maximum-likelihood sensory integration

Authors: *K. LIM[1,2], F. KARMALI[1,3], K. NICOUCAR[1,3], D. M. MERFELD[1,3];
[1]Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Harvard-MIT Div. of Hlth. Sci. and Technol., MIT, Cambridge, MA; [3]Otology and Laryngology, Harvard Med. Sch., Boston, MA

Abstract: The vestibular system effectively senses dynamic head motion of moving vertebrates. Here we propose that the human brain utilizes its knowledge of the dynamics of sensory organs and body to maximize the precision of motion perception (i.e., provide minimal thresholds). Humans have been shown to integrate static (i.e., not time-varying) noisy multisensory information in a manner consistent with simple inverse variance maximum likelihood (ML) estimators. However, it is still unknown how dynamic cues are combined. To study how the brain combines dynamic sensory signals, we measured perceptual roll tilt and roll rotation thresholds between 0 (DC) and 5Hz in 6 normal human subjects. More specifically, roll tilt thresholds were measured at 12 frequencies (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 1, 2, and 5Hz), and roll rotation thresholds were measured at 6 frequencies (0.1, 0.2, 0.5, 1, 2, and 5Hz). Average DC roll tilt threshold across subjects was 1.91 deg, and its standard error of the mean (SEM) was +0.20/-0.16 deg. Roll rotation velocity thresholds decreased with increasing frequency, showing high-pass-like dynamics. When fitted to a first-order high-pass filter model, the average cutoff frequency was 0.44Hz (+0.11/-0.07) with a plateau velocity of 0.42 deg/s (+0.10/-0.06). As our primary finding, it was found that roll-tilt thresholds at 5 frequencies between 0.2 and 0.5Hz were significantly lower than predicted by a inverse variance ML estimator ($p<0.01$ for all subjects, Chi-square test; $p=0.016$, Wilcoxon ranked sign test). To investigate how vestibular dynamics contributes to the observed performance enhancement, a Kalman filter internal model was combined with an inverse variance ML estimator to predict dynamic roll tilt perception thresholds. We also investigated how noise correlations between the roll rotation and tilt cues affect the combined thresholds. Simulations showed that dynamic sensory integration using a Kalman filter qualitatively and quantitatively explained the human performance.

Disclosures: K. Lim: None. **F. Karmali:** None. **K. Nicoucar:** None. **D.M. Merfeld:** None.

Poster

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Topic: D.07. Vestibula

Support: CIHR

Title: Regular and irregular otolith afferents use different coding strategies to encode linear self-motion

Authors: ***M. JAMALI**, J. CARRIOT, K. E. CULLEN;
Physiol., McGill Univ., Montreal, QC, Canada

Abstract: Understanding how sensory neurons transmit information about relevant stimuli is a major challenge in neuroscience. Accordingly, we took advantage of the otolith system which is well-defined anatomically and physiologically and encodes easily characterized sensory stimuli (i.e., head acceleration). The otolith afferents, at the earliest stages of processing, encode linear motion and provide this information to higher order brain areas. Importantly, these afferent fibers have a broad diversity in their spontaneous discharge regularity. Here, we employed gain and coherence measures to probe the impact of background discharge regularity on the encoding of linear acceleration by otolith afferents. Specifically, we investigated how sensory information is processed in the otolith afferents by recording from utricular fibers in alert macaques while stimulating each unit along its preferred direction during translations with broad band (0-15 Hz) Gaussian noise linear accelerations. We first used the traditional gain measure to characterize the response dynamics of otolith afferents and found an increase in the response gain for both regular and irregular afferents as a function of the stimulus frequency; the gain enhancement was more prominent for irregular units. Next, to explore the impact of afferents' discharge variability on linear motion encoding, we computed the coherence between stimuli and responses. Irregular unit responses were more coherent with the stimuli at higher frequencies (>7Hz), whereas regular afferents displayed relatively greater coherence at low acceleration frequencies (≤2Hz). These findings suggest that while highly sensitive irregular afferents are more advantageous for transient and dynamic stimuli, the regular units can provide accurate information when the stimulus is less dynamic (e.g. static tilt). Finally, to investigate whether spike timing plays a role in encoding linear motion we recorded neuronal responses to repetitions of the same stimuli (i.e., frozen noise). In contrast to regular afferents, irregular units displayed coherence in their responses at temporal frequencies outside of those contained in the stimuli. These "outband coherences" were significantly reduced by the addition of small spike timing jitter to afferent spike trains. This result suggests that information is contained in the fine temporal structure of irregular afferent spike trains, whereas regular afferents use a rate coding scheme to encode the stimuli. Taken together, our results indicate that regular and irregular afferents act as parallel channels with different coding strategies to encode linear self-motion.

Disclosures: **M. Jamali:** None. **J. Carriot:** None. **K.E. Cullen:** None.

Poster

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Topic: D.07. Vestibula

Title: A sense of self-motion or vection created by a moving soundscape

Authors: ***D. GRENET**[1,2], R. C. FITZPATRICK[1,2];
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Abstract: Illusory movement or vection produced by a moving visual field is a well-known phenomenon. Here we demonstrate a sense of auditory vection and a novel method to create this illusion. Subjects' ears (pinnae) were cast in silicone rubber. The silicone ears were mounted on a mannequin head and microphones were placed under them at the position of the entrance to the external auditory canal. The ambient sounds of the laboratory and a variety of sound stimuli created a soundscape that was picked up by these microphones, amplified and played to the subject through headphones in real time as they sat in a dark booth within the laboratory to isolate them from the direct path of the sound. Thus, the subject heard the soundscape as if he or she was at the position of the mannequin head and with the sound filtered by the conduction through the subject's own pinnae. All subjects perceived the sounds as external to their head and could accurately localise their sources. We rotated the mannequin head in yaw at 4 speeds (5, 10, 20 and 50 degrees/s) and asked subjects to report their perceptions of self-rotation by clicking a button every time they had rotated 45 degrees. All subjects reported a sense of self-motion as a yaw rotation. Overall, this illusion was observed in 81% of trials with a mean onset time of 14 s from presentation of the stimulus and lasting for the duration of the stimulus. Subjects reported gains (response to stimulus speed) of between 0.2 and 1.7 with the highest gains being reported at the 5 degrees/s stimulus speed. Based on these data, the gains of the vection were similar to those reported for visual vection, although subjective reports were that the experience did not have the same strength or immersive quality. We conclude that the CNS uses the auditory soundscape to feed the sense of self-motion and orientation rather than simply to localise external objects.

Disclosures: **D. Grenet:** None. **R.C. Fitzpatrick:** None.

Poster

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Topic: D.07. Vestibula

Support: NHMRC Australia

Title: Motion reduces perceptual responses and balance reflexes to vestibular stimulation

Authors: *R. C. FITZPATRICK[1], S. WATSON[2], F. EMILSON[3];
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Abstract: With the hypothesis that vestibular sensitivity is tuned to optimise responsiveness across a range of behavioural motion conditions, we explored the effects of passive whole-body motion on perceptual and balance reflex responses to vestibular stimuli. In ten normal subjects, balance reflexes and perceptual responses to vestibular stimulation were measured before and after a period of imposed passive motion. Rapid vestibulospinal balance reflexes during standing were evoked by galvanic vestibular stimulation (GVS) and measured as the characteristic biphasic shear reaction force. Perceptual tests measured thresholds for detecting angular motion, the perceived size of imposed suprathreshold rotations, and the perception of rotation evoked by constant GVS. The imposed conditioning motion was 10-minutes of stochastic rotation (0.5-2.5 Hz; peak acceleration ~300 deg.s-2) about a head-centred vertical axis with subjects sitting blindfolded. This motion conditioning markedly reduced both reflexive and perceptual responses. The medium-latency GVS-evoked reflex (300-350 ms) was halved in amplitude (mean 48%; $P = 0.011$), but the short-latency response was unaffected. Thresholds for detecting imposed rotation more than doubled (mean 248%; $P < 0.001$)) and were still significantly elevated after 30 minutes. Whole-body rotations of 30° - 180° over 5 s were over-estimated by 41.1% (mean) before conditioning but this was reduced to 21.5% afterwards ($P = 0.033$). Sensations of illusory rotation evoked by GVS, which have a pure vestibular origin (mean 113º for 10 s @ 1 mA) were reduced 44% by conditioning and remained low (24%) after one hour ($P <0.01$ and $P < 0.05$ respectively). We conclude that a system of vestibular sensory autoregulation exists and that this most likely involves central and peripheral mechanisms. We propose that failure of these regulatory mechanisms at different levels could lead to disorders of movement perception and balance control during standing.

Disclosures: **R.C. Fitzpatrick:** None. **S. Watson:** None. **F. Emilson:** None.

Poster

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Topic: D.07. Vestibula

Support: EU FP7-FET project SpaceCog (600785)

Title: Reference frame of inhibition-of-return during whole-body motion

Authors: ***J. J. TRAMPER**, P. MEDENDORP;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: When an attention-grabbing cue briefly appears in the periphery, processing of a visual target at that location is initially facilitated. However, after a cue-target onset asynchrony (CTOA) of about 250 ms, processing is inhibited. This effect, which is called inhibition-of-return (IOR), has been explained as an adaptive mechanism to guide our attention during visual search. Here, we investigate the reference frame of IOR during whole-body motion, distinguishing between an allocentric and egocentric reference frame. Subjects had to maintain fixation at a central stimulus while a cue was briefly flashed either 15 cm to the left or to the right from a central world-fixed light. Next, after a lateral body displacement of 30 cm (duration of 1 s) with gaze fixed at the world-fixed light, a target was presented either at the same allocentric location or at the same egocentric location of the cue (CTOAs between 1 and 2 s). Subjects had to respond as quickly as possible to the target onset. As a comparison, we tested the same subjects in a stationary IOR-task without body motion. If subjects update a previously attended location, we expect larger reaction times if both the cue and the target appeared at the same allocentric location. Alternatively, if they do not update the attended location, they will perceive the location as being fixed to their body. In that case we expect larger reaction times if both the cue and the target were presented at the same egocentric location. Preliminary results show that responses are inhibited when the cue and target appeared at the same allocentric location. This suggests that previously attended locations are updated across body movements, and mapped into an allocentric reference frame.

Disclosures: **J.J. Tramper:** None. **P. Medendorp:** None.

Poster

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Topic: D.07. Vestibula

Support: EU-ERC 283567

Title: Cortical integration of vestibular signals for spatial updating of world-fixed and gaze-fixed targets

Authors: *T. P. GUTTELING, W. P. MEDENDORP;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: In everyday life, it is important to keep track of the objects in our surroundings as we move through the world. This 'spatial updating' of locations has been widely studied during eye movements, showing that the retinal location of an object is remapped according to the executed eye movement to maintain spatial constancy. This phenomenon has been studied less for whole body movements, requiring other sources of information to compute the update, including vestibular information. Recently, we showed, using EEG, that target locations during whole body motion are represented by lateralized alpha desynchronizations in parieto-occipital areas, which are remapped trans-hemispherically, taking into account the geometry of the environment. While this points to interactions of vestibular motion signals and retinotopic target information, it is still unclear how the integration comes about. Here we further investigated this issue by examining the effect of the target's reference frame on spatial updating. During body motion, the retinotopic representation of a world-fixed target location must be updated, using vestibular signals, whereas the representation of a gaze-fixed target must remain constant, ignoring the vestibular inputs. Subjects were linearly accelerated using a linear sled, while 88-channel EEG was recorded. Subjects peripherally viewed either world-fixed or gaze-fixed LEDs, the location of which had to be maintained during the motion in which gaze was kept central. Preliminary findings indicate that the updating of world-fixed targets, which is based on vestibular information only, is significantly underestimated. Updating of gaze-fixed targets, requiring a suppression of the vestibular input, is currently being tested. In combination, per-trial estimates of updating performance of both gaze- and world-fixed targets, will be used to isolate the spectral modulations that contribute to the quality of spatial updating based on vestibular information.

Disclosures: T.P. Gutteling: None. **W.P. Medendorp:** None.

Poster

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Support: NIH Grant K23 DC011298

Trilogical Career Scientist Award

Title: Coordinates of human visual and vestibular heading estimation

Authors: *B. T. CRANE;
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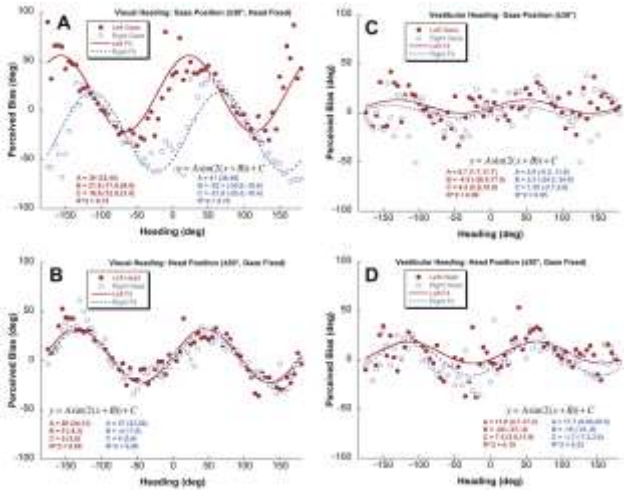
Abstract: The head and eyes are free to move relative to the body which influences the relative direction of motion sensed by the labyrinth, optic flow sensed by the retina, and somatic sensation from the body. It remains unclear how these multiple coordinate systems are reconciled across sensory systems. One hypothesis is that a common reference frame is used for all sensory modalities. The alternative hypothesis is that the sensory reference frames remain independent. This was investigated for visual and inertial motion while varying eye and head position. Subjects experienced a 2s, 16 cm translation (peak velocity 16 cm/s). Trial blocks used either visual or inertial motion. In each test condition 72 translations (360° in 5° increments) were delivered in random order. After each movement the subject oriented a mechanical dial in the direction of perceived motion. Some trial blocks included interleaved conditions were tested to look at influences of ±30° of gaze direction and ±30° of head position. For each set of test condition the overall average bias and phase shift were determined by fitting a curve to the responses. For visual motion changes in gaze yield a perceptual bias 87% of the size of the gaze shift such that for rightward gaze all heading estimates were shifted to the left by the almost the same amount and vice versa (Fig. 1A). A similar result was obtained when gaze was shifted the same amount by moving the head (not shown). However shifting the head position while maintaining forward gaze did not cause any overall shift or phase shift in estimates (Fig. 1B). This finding suggests that visual headings are perceived in retina based coordinates. When heading stimuli were delivered with a vestibular stimulus gaze position did not bias the perceived headings (Fig. 1C). Vestibular headings were slightly biased by changes in head position such that the perceptual bias was 15% of head angle (Fig. 1D). Thus vestibular headings were sensed in a body centered reference frame. The current findings provide conclusive evidence that a common coordinate system is not used in human visual and vestibular perception.



Disclosures: **B.T. Crane:** None.

Poster

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the Koetser Foundation for Brain Research

the Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

Title: Gravity influences the visual line bisection task

Authors: ***A. A. TARNUTZER**, G. BERTOLINI, C. J. BOCKISCH, D. STRAUMANN; Univ. Hosp. Zurich, Zurich, Switzerland

Abstract: Background: The line bisection task (LBT) is sensitive to perceptual biases of visuospatial attention, showing a slight leftward error in healthy human subjects. While various parameters including age, sex and handedness are known to modulate the LBT, the impact of gravity has not been systematically studied. The LBT is solved in an allocentric frame of reference, without any obvious need for graviceptive input. Noteworthy, for other visual line adjustments, such as the subjective visual vertical, otolithic input seems to be integrated

whenever available - even for tasks solved in an egocentric (i.e. non-gravicentric) frame of reference. We hypothesized that graviceptive input is integrated when performing an allocentric line bisection task as. We made the following predictions: (1) The precision of bisecting lines is reduced when subjects are roll-tiled as otolith variability increases with head roll; (2) the accuracy of bisecting lines in sustained roll-tilted positions is affected by adaptation and drift of estimated direction of gravity; (3) upon return to upright after prolonged roll, line bisection accuracy is influenced by the previous roll-tilt position and line bisection precision is reduced. Methods: Healthy human subjects (n=8) repetitively bisected lines that were either earth-fixed (session 1) or body-fixed (session 2) in darkness. Recordings were obtained before (baseline), during (±45deg and ±90deg whole-body roll) and after roll-tilt for periods of 5min each. Results: At baseline, line bisections showed significant ($p<0.05$) drift over 5min in 8 out of 16 runs. For earth-fixed lines, accuracy was biased significantly towards roll positions of ±90deg (difference=0.4deg; $p=0.03$) and precision decreased by up to 50% with increasing head roll angle, confirming predictions 1 and 2. At post-tilt, accuracy was not affected and precision was only reduced slightly compared to baseline, not confirming prediction 3. For body-fixed lines, accuracy and precision did not modulate with head-roll position and were unchanged after prolonged roll. Conclusions: Our findings further support the hypothesis that the brain integrates graviceptive input when solving tasks that are performed in egocentric or allocentric reference frames. A strategy that takes into account all sensory input when solving a specific task can explain this behavior. The impact of graviceptive input, however, seems to depend on the spatial orientation of the line to bisect, being present only if it is earth-fixed. Furthermore, fluctuations and drift over time of visuospatial attention may bias the LBT as well.

Disclosures: A.A. Tarnutzer: None. **G. Bertolini:** None. **C.J. Bockisch:** None. **D. Straumann:** None.

Poster

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ZIHP University of Zurich

Title: Determinants of motion sickness in tilting train simulations

Authors: *G. I. BERTOLINI, M. A. DURMAZ, K. FERRARI, M. AFTHINOS, D. STRAUMANN;
Dept. of Neurology, Univ. Hosp. Zurich, Zurich, Switzerland

Abstract: Motion sickness is a syndrome elicited in healthy subjects by ongoing passive self-motion that contains certain dynamic and kinematic properties. Because passive motion (car, bus, train, plane) are abundant in modern life, motion sickness is a frequent problem. Motion sickness symptoms range from drowsiness to vomiting and apathy. Tilting the car bodies of trains partly compensates for the centripetal acceleration during turns, allowing higher velocities. Unfortunately, passengers in tilting trains often develop symptoms of motion sickness. Understanding of the mechanism underlying motion sickness is essential to develop technical solutions. The prevalent theory of motion sickness states that it occurs when individuals are exposed to a mismatch between two sensory inputs or between a sensory input and previous experience. During turns in tilting trains, passengers are exposed to complex motion stimuli. The centripetal acceleration adds to the gravity vector resulting in a sustained tilt the gravito-inertial acceleration with respect to the angular velocity vector. Tilt of the car bodies aligns the gravito-inertial vector with the body vertical. The dynamic of the vestibular system response causes further misalignment of the sensed angular velocity depending on the timing between the onset of the curve and the tilt, inducing a known provocative stimulation to the vestibular system (cross-coupling stimulus). Although the angular velocity are usually small, it has been shown that motion sickness worsen when using a tilt-system not closely synchronized with yaw rotation (Cohen et al. 2011). Segregation of the determinants of the sensory conflict is needed to verify the actual source of motion sickness increase. We simulated a train ride using a 3D rotating chair. 8 subjects were exposed to two sequences of 6 curves simulating the average yaw angular velocity (4°/s), accelerations ($2°/s^2$) and tilt profile (amplitude: 8°, peak velocity: 4°/s, acceleration: $10°/s^2$) recorded from a tilting train during rides on a curvy track. The directions of the turns were randomized. Condition with no delay and 3 s delay of the tilts were tested on each subject in different session, randomizing the order of presentation. Motion sickness score and nausea level were assed using a questionnaire. Variation in both nausea and motion sickness were very mild, with 2 subjects showing no effect, but mean scores were higher after the 3 s delay condition than after no delay (Ratios: 2.0 and 1.5 respectively). We concluded that the delay in tilt of cars is critical for motion sickness induced by tilting trains and that cross-coupling can be nauseogenic even at very low angular velocity.

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Poster

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Topic: D.07. Vestibula

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Title: Real time and low noise tracking of torsional eye movements with automatic eyelid detection

Authors: *J. OTERO-MILLAN[1], D. C. ROBERTS[2], A. LASKER[2], D. S. ZEE[2], A. KHERADMAND[2];
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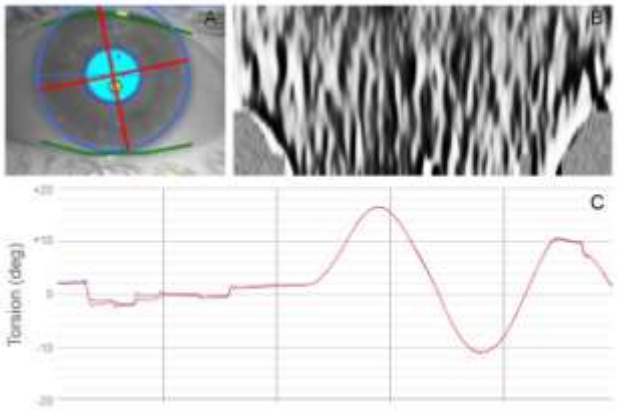
Abstract: Torsional eye movements are rotations of the eye around the line of sight and are relevant to diagnosis of vestibular and neurological disorders. In video-oculography systems, horizontal and vertical eye movements are usually measured by tracking the pupil. Measuring torsion is more challenging and it requires using information from the iris or the scleral vessels. Previous methods do not work in real time or require input from the user to select the visible segments of the iris. We have developed a new method to measure torsional eye movements that uses the entire visible iris pattern and automatically masks the parts of the iris occluded by the eyelids. To implement the method we used open source computer vision software (opencv) and commercial head mounted infrared cameras (Micromedical, RealEyes). The eyelid detection uses the Hough transform to detect line segments belonging to the eyelid rims near the pupil and the torsion tracking uses polar template matching of optimized iris patterns. Currently, the method can operate binocularly at 100Hz and achieves levels of noise below 0.1 degrees. The Figure shows an example of torsion measurement. A) Sample image of the eye. Red cross represents the torsion angle relative to a reference image of the same eye. Green lines represent the automatic tracking of the eyelids and light blue the dark pixels identified to be part of the pupil. Dark blue circles represent the pupil and the iris. B) Optimized iris pattern in polar coordinates. Top and bottom correspond with inner and outer contours of the iris respectively. Areas masked with noise in the bottom corners correspond with each half of the top eyelid covering part of the iris. Note how rich the pattern becomes after image optimization. C) Example of 5 seconds of binocular recordings showing the torsion associated with a sequence of horizontal or vertical saccades (first half) and the ocular counterroll during roll vestibulo-occular reflex (second half).



Disclosures: J. Otero-Millan: None. **D.C. Roberts:** None. **A. Lasker:** None. **D.S. Zee:** None. **A. Kheradmand:** None.

Poster

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Support: Regio13/Reha@home

Title: New high acceleration automatic whole body impulse test reveals VOR asymmetries not detectable with manual head impulse testing

Authors: K. EIBENBERGER[1,2], M. SCHUBERT[2], B. G. EIBENBERGER[2], D. C. ROBERTS[3], *T. P. HASLWANTER[1], J. P. CAREY[2];
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Abstract: The angular vestibulo-ocular reflex (aVOR) stabilizes gaze by generating compensatory eye movements for rotational head movements. The head impulse test (HIT) is a clinical standard tool to assess the function of the three Semicircular-Canals (SCCs). However, due to the manual execution of the HIT, the informative value is limited. Our goal was to perform HIT with a controlled and repeatable movement profile. To enable this, we customized a floor mounted motor (torque 2100 Nm, Rexroth Bosch, Gerlingen, Germany) on which a fiber

glass chair was mounted. Eight normal humans (age 33.3 ± 7.6 y, mean ± STD) underwent horizontal rotation (acceleration <7046 °/sec², 15° amplitude) in the dark while fixating a LED target at 185 cm distance. Head clamp and bite bar restricted any head movement. On the same subjects, manual HIT tests were performed by a trained ENT clinician. 3-dimensional (3D) eye, head and chair positions were recorded using binocular magnetic search coils, using a customized C-N-C (C-N-C Engineering, Seattle, WS, USA) system with our own digital recording unit. Horizontal velocity gain was calculated as eye above head velocity. Horizontal, RALP and LARP gains were calculated after projecting head and eye orientation in the plane of the SCCs. To our knowledge, this is the first time that is has been possible to systematically investigate the VOR response to rapid head impulses in 3D, with accelerations similar to those elicited by manual HIT. Our results are consistent with previous observations by other groups: we observed inter-ocular gain differences for the adducting eye for higher accelerations of up to 13.9% (Weber et al. 2008). We also found a decrease in gain with higher accelerations (3000 vs 4000°/sec²). The correlation between eye- and head-velocity as was extremely high, with inner-subject correlation coefficients of over 0.9996 between eye and head velocity over all trials for the automatic HIT, and an inter-trial correlation of 0.9985 for eye velocity. In addition, we found a subject who showed a clear reduction in gain only for accelerations above 3000 °/s² (horizontal canal gain 0.99 ± 0.01 at 3000 °/s² vs. 0.52 ± 0.01 at 4000 °/s², mean ± STD). This indicates an undiagnosed vestibular asymmetry that could not be clearly seen on the manual HIT, but which could be detected with this new approach. In the future, the application of our new testing method may be especially interesting for e.g. controlled treatment of Meniere's patients undergoing Gentamicin treatment, where a controlled reduction of vestibular function is desirable for minimizing the impairment from Meniere's disease.

Disclosures: K. Eibenberger: None. **M. Schubert:** None. **B.G. Eibenberger:** None. **D.C. Roberts:** None. **T.P. Haslwanter:** None. **J.P. Carey:** None.

Poster

825. Sensory Coding, Perception, and Navigation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 825.13/JJ25

Topic: D.07. Vestibula

Support: Rozsa Foundation

Title: Human horizontal angular vestibulo-ocular reflex with and without otolith stimulation

Authors: E. W. BLOCK, W. A. FLETCHER, B. J. LANGE, *G. MELVILL-JONES;
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Abstract: Introduction: Routine laboratory tests of the vestibulo-ocular reflex (VOR) in humans, such as caloric irrigation and horizontal (yaw) rotation about an earth-vertical axis, examine only the function of the horizontal semicircular canals. The same yaw rotation about the earth-horizontal axis also stimulates the otolith organs, primarily the utricles, through a perceived rotating gravity vector. In this study, we examine the hypothesis that the utricular signals produced during rotation around an earth-horizontal axis are used to improve the human horizontal angular VOR. Methods: Five normal subjects (ages 21 - 64 years) participated. Torsio infrared video goggles and software were used to record eye position. An inertial gyroscope was used to measure angular (yaw) head velocity. A prototype 2-D rotary chair was used to produce whole-body rotation about the earth-vertical and earth-horizontal axes. Subjects were secured by a 5-point harness and an adjustable welder's headband anchored to the chair. Four trials of sinusoidal rotation, each comprising 10 cycles of +/- 90° amplitude, 60°/sec peak velocity and 0.11 Hz frequency, were performed in darkness. One trial provided rotation about the earth-vertical axis and 3 trials about the earth-horizontal axis - centered around the supine, supine-right and supine-left positions. Slow phase eye velocities were obtained by differentiating eye position and removing all fast phases. The slow phase velocities were fitted with a sine function ($y = A * \sin(wt) + C$) using a least-squares error method. The mean peak slow phase velocity (A) and offset (C) for each trial were obtained from the fitted curve. Gain was calculated as A/peak head velocity. Asymmetry was calculated as C/A x 100%. Results:

Trial	**Gain Mean (SD)**	**p-value***	**Asymmetry (%) Mean (SD)**	**p-value***
Earth-vertical	0.55 (0.07)	---	0 (0)	---
Earth-horizontal supine	0.75 (0.16)	0.03	1.6 (1.8)	0.07
Earth-horizontal supine-right	0.71 (0.13)	0.03	3.0 (4.2)	0.14
Earth-horizontal supine-left	0.70 (0.14)	0.05	1.7 (1.7)	0.06

*earth-vertical vs earth-horizontal Conclusion: Humans show a significant increase in horizontal aVOR gain for rotation about the earth-horizontal axis compared to that for rotation about the earth-vertical axis. A previous study (Bockisch et al, 2005) showed no difference in the VOR gains for earth-horizontal and earth-vertical sinusoidal rotations, but the peak velocities used were much smaller. We propose that the observed increase results from the effect of the rotating gravity vector on the utricles.

Disclosures: **E.W. Block:** None. **G. Melvill-Jones:** None. **W.A. Fletcher:** None. **B.J. Lange:** None.

Poster

825. Sensory Coding, Perception, and Navigation

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 825.14/JJ26

Topic: D.07. Vestibula

Support: CIHR

Title: Vestibular coding strategies for representing natural self-motion

Authors: ***J. CARRIOT**, M. JAMALI, M. CHACRON, K. E. CULLEN;
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Abstract: During every day activities, self-motion is sensed by the vestibular system, which in turn contributes to an impressive range of brain functions from the most automatic reflexes to spatial perception and motor coordination. It is generally assumed that sensory systems efficiently encode natural stimuli using coding strategies that are adapted to the statistics of the environment in which the organism lives. However, to date, sensory coding has been primarily studied using artificial stimuli. Accordingly, we established the statistics of multidimensional natural vestibular stimuli experienced by human subjects during everyday activities. We found that preneuronal processing by i) the biomechanical properties of the body and ii) self-generated head motion increase the power of behaviorally relevant frequencies, thereby significantly altering the statistics of natural vestibular stimuli. Next, we characterized the response of Vestibular Only (VO) neurons receiving input from otolith and/or semicircular canal afferents during complex stimuli with trajectories similar to natural motion. We recorded from central vestibular neurons in response to i) unidimensional stimuli (rotation or translation), ii) multidimensional stimuli with trajectories similar to natural motion, and iii) while the monkey was generating self-motion. Consistent with previous results, we found that the firing rate response to unidimensional stimuli was accurately reproduced by a typical linear model, which could account for up to ~95% of the variance in the data. In contrast, during multidimensional motion VO neurons sub-additively integrated the translational and rotational inputs rather than simply adding the otolith and canal afferents information. Interestingly, the weights corresponding to the translational and rotational components of the combined movements depended on the frequency of the stimuli. Finally, we found that neuronal responses were

significantly attenuated (~70%) during self-generated vs passively applied movements. Taken together, these findings provide insights into the underlying processing required for the integration of rotational and translational inputs in the vestibular system and are key for understanding the neuronal mechanisms required for accurate posture and motor control, as well as perceptual stability, during everyday life.

Disclosures: **J. Carriot:** None. **M. Jamali:** None. **M. Chacron:** None. **K.E. Cullen:** None.

Poster

825. Sensory Coding, Perception, and Navigation

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Topic: D.07. Vestibula

Support: Foundation for Physical Therapy PODS II Scholarship

Title: Vestibular loss: Gaze stability differences between active and passive walking

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Abstract: Individuals with vestibular loss (VL) often complain of oscillopsia, the apparent motion of the stable environment, during head motion. Active (rather than passive) head movements have been suggested as a more natural probe of vestibular function, which may better characterize functional impairments for individuals with VL. Previous investigations into gaze stability during walking assumed that only angular head motion contributed to observed eye movements. Characterizing gaze stability using all head motions as inputs during walking may provide a window for assessing functional limitations to daily activities in a more natural way for individuals with VL. Individuals with VL and healthy subjects walked on a treadmill at 2 km/hr while fixating a target 2.2m in front of them. Head kinematics were recorded (Optotrak) and converted offline to control motion of a chair mounted on 6-degree of freedom platform (Moog, Inc). All subjects sat on the Moog and passively experienced their sagittal plane walking head trajectories (3-degrees of freedom) while performing the same fixation task. Head and eye velocity were recorded (EyeSeeCam) while walking and on the Moog. Frequency response functions (FRFs) were calculated to describe the input/output relationships that characterize gaze stability. A multiple input single output (MISO) gaze stabilization system including angular and

linear velocity and the relationship between each head movement was compared to more traditional single input single output (SISO) FRFs. Active and passive MISO FRFs were compared to determine if walking enhances gaze stability in a way not predicted by passive motion. During "seated passive walking" individuals with VL demonstrated gains close to unity, but had increased phase lag with increasing frequency. Eye movements during "seated passive walking" demonstrated an under-estimation of low frequency (< .3 Hz) gaze stability compared to treadmill walking. There may be a low-frequency augmentation to gaze stabilization that occurs when walking but not in passive vestibular testing. Individuals with VL may have better gaze stability during locomotion than predicted by passive testing.

Disclosures: E.R. Anson: None. **T. Kiemel:** None. **J.P. Carey:** None. **J. Jeka:** None.

Poster

825. Sensory Coding, Perception, and Navigation

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Topic: D.07. Vestibula

Support: COTREL Grant

Title: Are vestibular tests useful to identify senior at risk of fall?

Authors: *C. DE WAELE[1], E. CHIAROVANO[1], G. LAMAS[2], P.-P. VIDAL[1];
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Abstract: Objectives To investigate the vestibular receptors function and the rotatory perception sensation during caloric tests in seniors. We tested the hypothesis that vestibular dysfunction and/or misuse of vestibular information at the central level could be used to detect senior at risk of fall. Methods Data were obtained in 50 patients over 65 years of age in the ENT department of the Pitie-Salpetriere hospital. Vestibular function was assessed using caloric, vHIT test and VEMPs. During caloric stimulation, patients were asked to describe their sensation of movement (nothing, dizzy, rotation on the left or on the right). 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 and to detect the risk of falls. Patients were asked to fill in the Dizziness

Handicap Inventory (DHI) questionnaire. 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%). Finally, abnormal Equitest (falls in condition 5 or 6) was more often observed in patients with low scores to DHI and/or in patients with no sensation of rotation during calorics. Interestingly these patients kept a normal horizontal slow phase velocity during the irrigation. Conclusion This study showed an alteration of the function of some vestibular receptors with age: utricle, saccule and posterior canal. In addition, some patients failed to detect any rotation during caloric tests although their ocular responses were normal. These results suggested that falls in seniors are not only related to dysfunction of vestibular sensors but also to an inadequate cortical treatment of vestibular information.

Disclosures: **E. Chiarovano:** None. **C. de Waele:** None. **P. Vidal:** None. **G. Lamas:** None.

Poster

825. Sensory Coding, Perception, and Navigation

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Topic: D.07. Vestibula

Support: NIH R90DA033462-03

NSF EFRI 1137229

Title: Directional acuity of whole-body perturbations during standing balance

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Abstract: Little is known about whether deficits in motion perception contribute to impaired balance in individuals with sensory integration deficits, which is common in Parkinson's disease and stroke. In standing balance, proprioceptive, cutaneous, vestibular, and visual systems all contribute to our ability to sense the direction and magnitude of an impending fall and to appropriately activate muscles to restore balance. However, perception tests of heading direction and verticality have typically been performed during seated motion, which does not actively

engage the motor system. Here our goal was to measure directional acuity as a way to assess kinaesthetic perception of the body's motion and direction in space during perturbations to standing balance. We sought to validate methods for quantifying directional acuity in response to linear support-surface perturbations during standing, and to establish sensory threshold values for a young adult population. To avoid use of visual and auditory cues, subjects were blindfolded and wore headphones with a white noise masking stimulus. In each trial, two ramp and hold perturbations of identical magnitude (7.5 cm, 15 cm/s, and 0.1 m/s2) were applied, with 3 seconds between perturbations to allow recovery of balance. The first perturbation was in the backward direction, causing forward displacement of the center of mass (CoM), and the second perturbation was deviated by 0-30° to the right or left. After each pair of perturbations, subjects pressed a button to indicate whether they perceived the perturbations to be in the same or different direction. Sensory thresholds identified using an adaptive algorithm, the Parameter Estimation by Sequential Testing (PEST), were comparable to those estimated from full psychometric curves. Future work is aimed at determining thresholds in the lateral and diagonal directions and to compare perceptual and motor responses. Deficits in directional acuity during behaviorally-relevant conditions may be an important and understudied aspect of many sensorimotor impairments.

Disclosures: M.J. Puntkattalee: None. **C. Shephard:** None. **G. Stanley:** None. **L. Ting:** None.

Poster

825. Sensory Coding, Perception, and Navigation

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 825.18/JJ30

Topic: D.07. Vestibula

Title: Functional imaging of human vestibular cortex activity elicited by skull tap and auditory tone burst

Authors: *F. NOOHIBEZANJANI[1], C. KINNAIRD[1], S. WOOD[2], J. BLOOMBERG[3], A. MULAVARA[4], R. SEIDLER[1];
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Abstract: The aim of the current study was to characterize the brain activation in response to two modes of vestibular stimulation: skull tap and auditory tone burst. The auditory tone burst

has been used in previous studies to elicit saccular-mediated colic Vestibular Evoked Myogenic Potentials (cVEMP). Some researchers have reported that air-conducted skull tap elicits both saccular and utricle-mediated ocular VEMPs, while being faster and less irritating for the subjects. However, it is not clear whether the skull tap and auditory tone burst elicit the same pattern of cortical activity. Both forms of stimulation target the otolith response, which provides a measurement of vestibular function independent from semicircular canals. This is of high importance for studying vestibular disorders related to otolith deficits. Previous imaging studies have documented activity in the anterior and posterior insula, superior temporal gyrus, inferior parietal lobule, pre and post central gyri, inferior frontal gyrus, and the anterior cingulate cortex in response to different modes of vestibular stimulation. Here, we hypothesized that skull taps elicit the similar pattern of cortical activity as the auditory tone bursts. Subjects put on bilateral MR compatible skull tappers and headphones inside the scanner, while lying in the supine position, with eyes closed. All subjects received both forms of the stimulation, however, the order of stimulation with auditory tone burst and air-conducted skull tap was counterbalanced across subjects. Pneumatically powered skull tappers were placed bilaterally on the cheekbones. The vibration of the cheekbone was transmitted to the vestibular system, resulting in the vestibular cortical response. Auditory tone bursts were also delivered for comparison. To validate our stimulation method, we measured the ocular VEMP outside of the scanner. This measurement showed that both skull tap and auditory tone burst elicited vestibular evoked activation, indicated by eye muscle responses. Our preliminary analyses showed that the skull tap elicited activation in medial frontal gyrus, superior temporal gyrus, postcentral gyrus, transverse temporal gyrus, anterior cingulate, and putamen. The auditory tone bursts elicited activation in medial frontal gyrus, superior temporal gyrus, superior frontal gyrus, precentral gyrus, inferior and superior parietal lobules. In line with our hypothesis, skull taps elicited a pattern of cortical activity similar to one elicited by auditory tone bursts. Further analysis will determine the extent to which the skull taps can enhance assessment of both vestibular evoked behavioral and brain imaging responses.

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Poster

826. Trigeminal Processing of Pain

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Support: CAPES

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FAPESC

PRONEX

Title: Hyperalgesic effects of endothelins in trigeminal neuralgia and their relationship with satellite glial cell activation in trigeminal ganglion

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Abstract: Trigeminal neuralgia is a debilitating orofacial neuropathic pain state that often results from trigeminal nerve injury. Endothelins (ETs) and their ETAR and ETBR receptors have been shown to contribute to neuropathic nociception triggered by trigeminal injury (TI) in rats. They are also involved in the intercellular interactions which occur in peripheral sensory ganglion in altered nociceptive states. As it appears that trigeminal neurons can release ETs, and satellite glial cells (SGC) in trigeminal ganglion (TG) display ETBR, this study aims to assess the involvement of the ETs system in SGC activation that occurs after TI in mice. Experiments were conducted on 2 month old male Swiss mice. All the protocols were previously approved by the Committee on Ethical Use of Laboratory Animals of Universidade Federal de Santa Catarina (PP00851). Trigeminal nerve injury was induced by constriction of the right infraorbital nerve (CION).The latency of responses of constricted or sham-operated mice to thermal radiant heat stimulation of the snout was tested before surgery and then once daily for 10 days. Endothelin-1 (ET-1) (0.3, 1 or 3 pmol) and the selective ETBR agonist Sarafotoxin (S6c) (3, 10 or 30 pmol) or vehicle (PBS, 0.5 µl) were injected via infraorbital foramen directly into the right TG of naive mice. On day 5 after surgery, mice were given a single intraganglionar TG injection of the peptidic ETBR antagonist BQ-788 (0.05 or 0.5 nmol) or vehicle and the SGC activation in the ipsilateral TG was assessed 3 hours after administration by immunohistochemistry. Additional CION mice received daily systemic treatments with Bosentan (ETAR/ETBR antagonist; 100 mg/kg p.o.) or vehicle, from day 5 to day 8 after surgery and the latency of responses was tested prior to surgery and then once daily over the next 10 days. Intraganglionar injection of ET-1 (1 or 3 pmol) and S6c (10 or 30 pmol) into the TG of naïve mice induced thermal hyperalgesia lasting up to 4 and 2 h after administration, respectively. The thermal hyperalgesia present on day 5 after CION was significantly attenuated by intraganglionar injections of BQ-788 at either 0.5 or 5 nmol, however, the same treatment was not able to reduce the SGC activation. The repeated daily treatments with Bosentan reduced thermal hyperalgesia from days 6 to 9. Thus, TI induces prolonged orofacial thermal hyperalgesia and activation of SGC in the TG. Furthermore,

the ET system is involved in promotion of the thermal hyperalgesia, but a causal relationship between this action and SGC activation still remains to be elucidated.

Disclosures: **L.O. Gomes:** None. **J. Oliveira:** None. **G. Rae:** None.

Poster

826. Trigeminal Processing of Pain

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Title: Increased 5-HT2A receptor activity and decreased 5-HT1A and 2C receptor activity contribute to enhanced excitatory synaptic transmission in the dorsal horn and the maintenance of neuropathic pain

Authors: Y.- . CHU[1], M. LI[1], J. LIU[1], W. GUO[1], A. CASTRO[2], K. REN[1], A. KELLER[2], R. DUBNER[1], *F. WEI[1];
[1]Neural and Pain Sci., Dent. Sch, & Prog. Neurosci. Univ. Maryland, BALTIMORE, MD;
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Abstract: We recently demonstrated that 5-HT3 receptors in primary afferent central terminals mediate 5-HT-dependent descending facilitation and central sensitization during the maintenance but not induction of mechanical hyperalgesia after nerve injury (Kim et al., Neuron 81:1-15, 2014). Here, we further extended our findings to examine whether other 5-HT receptor subtypes in the medullary dorsal horn, including 5-HT1A, 2A and 2C, are involved in regulation of excitatory synaptic transmission and the maintenance phase of neuropathic pain after unilateral chronic constriction injury of the infraorbital nerve (CCI-ION). We studied the consequence of disrupting these receptors on CCI-induced secondary hyperalgesia/allodynia and negative reinforcement on conditioned place preference (CPP) at 14 d after trigeminal nerve injury. Intra-ipsilateral Vc microinjection of the 5-HT2AR antagonist 4F4PP (50 fmol/0.5 μl) significantly attenuated secondary hyperalgesia and promoted CPP in CCI-injured mice. In contrast, selective activation of 5-HT1AR or 5-HT2CR by intra-Vc microinjection of the agonists 8-OH-DPAT (15

nmol) or RO60-0175 (25 nmol), respectively, also significantly blocked secondary hyperalgesia and induced positive CPP in CCI-treated animals. Next, we performed whole-cell patch clamp recordings from lamina II neurons from adult mouse trigeminal subnucleus caudalis (Vc) slices and analyzed excitatory synaptic transmission between primary afferent terminals and the identified Vc neurons in which electrical stimulation of the trigeminal nerve evoked monosynaptic Aδ or C responses. Compared with naïve and sham-treated animals, both frequency and amplitude of sEPSCs recorded from lamina II neurons were increased at 14 d after CCI-ION. Bath application of 4F4PP (60 μM) suppressed CCI-induced increase of the frequency but not the amplitude of sEPSCs and the amplitude of evoked EPSCs (eEPSCs). In addition, application of 8-OH-DPAT (10 μM) significantly attenuated the enhanced frequency of sEPSCs but not the amplitude of sEPSCs and eEPSCs in Vc neurons from CCI-treated animals. RO 60-0175 (1 μM) reversed nerve injury-induced increases in both the frequency and amplitude of sEPSCs, as well as the amplitude of eEPSCs. Taken together, and consistent with behavioral findings, these results suggest that enhanced 5-HT2AR activity and reduced inhibitory tone from both 5-HT1ARs and 5-HT2CRs on the central terminals of primary afferent fibers and/or postsynaptic dorsal horn neurons in the Vc are involved in central mechanisms underlying 5-HT-dependent descending facilitation and the maintenance of neuropathic pain.

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Poster

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AR057194

AR063228

Title: Anatomical evidence of pruriceptive trigeminothalamic and trigeminoparabrachial tract neurons in mice

Authors: *E. E. CARSTENS[1], E. CURTIS[1], T. NGUYEN[1], M. IODI CARSTENS[1], T. AKIYAMA[2];
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Abstract: Itch is relayed to higher centers by projection neurons in the spinal and medullary dorsal horn. We presently used a double-label method to map the ascending projections of pruriceptive and nociceptive trigeminal and spinal neurons. The retrograde tracer fluorogold (FG) was stereotaxically injected into the right thalamus or lateral parabrachial area (LPb) in mice under pentobarbital anesthesia. Seven days later, mice received intradermal (id) microinjection of histamine (50 μg/10 μl), chloroquine (100 μg/10 μl), capsaicin (30 μg/10 μl), or vehicle (saline, 7% Tween-80) into the left cheek under pentobarbital anesthesia. Animals were perfused transcardially 2 hr later with 4% paraformaldehyde. Frozen sections of diencephalon, caudal medulla and upper cervical spinal cord were processed for Fos immunofluorescence (FITC) and imaged under fluorescence microscopy to identify FG injection sites and retrogradely labled neurons (340-380 nm) and Fos-positive neurons (490-525 nm). Id histamine, chloroquine and capsaicin elicited similar distributions of Fos-positive neurons in the medial aspect of the superficial medullary and spinal dorsal horn from the trigeminal subnucleus caudalis to C2. Vehicles resulted in very few Fos-positive cells. Projection neurons were observed in superficial and deep medullary and upper cervical spinal dorsal horns. Of the neurons that were retrogradely labeled from the thalamus, 44, 11 and 67% were Fos-positive following id histamine, chloroquine or capsaicin. Of the Fos-positive neurons following pruritic or capsaicin stimuli, ~1.2% were retrogradely labeled with FG. Trigeminoparabrachial projection neurons exhibited a higher incidence of double-labeling in the superficial dorsal horn. Of the neurons retrogradely labeled from LPb, 31, 38 and 63% were Fos-positive following id injection of histamine, chloroquine and capsaicin, respectively. Of Fos-positive neurons elicited by id histamine, chloroquine and capsaicin, respectively, 1.1, 1.8 and 4.8% were retrogradely labeled from LPb. The present results indicate that, overall, relatively small subpopulations of pruriceptive (~1.8%) and/or nociceptive (~4.8%) neurons innervating the cheek project to thalamus or LPb. This confirms a previous double-label study reporting that 2-4% of cervical spinal neurons expressing Fos following id injection of serotonin projected to thalamus or LPb in rats. These results also imply that the vast majority of pruritogen- and algogen-responsive spinal neurons are likely to function as interneurons that presumably play an important role in the segmental modulation of itch and pain transmission.

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Poster

826. Trigeminal Processing of Pain

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Title: Sensitization of trigeminal brainstem neurons during persistent tear insufficiency

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Abstract: Dry eye (DE) is a multifactorial disease associated with tear dysfunction, hyperosmolarity and ocular discomfort. The role of CNS plasticity in DE is not known. In a rat model for tear insufficiency, we measured evoked eyeblink behavior, orbicularis oculi activity (OOemg) and single neuron activity at the trigeminal interpolaris/caudalis transition (Vi/Vc) and caudalis/upper cervical junction (Vc/C1, lamina I-II and V) regions, to hypertonic saline (0.15-5M NaCl, 20ul) applied to the ocular surface. In male rats, the left exorbital gland was removed 14d prior to recording. Ocular-responsive trigeminal neurons were recorded under isoflurane and OOemg activity under urethane. Spontaneous tear volume was reduced to 35-50% of sham in 14d DE rats. Saline-evoked eyeblink behavior was enhanced 2 days after gland removal and persisted for 14d. Saline-evoked neural activity at the Vi/Vc transition and Vc/C1 junction (both lamina I-II and V) regions encoded the concentration of hypertonic saline and was enhanced compared to units from sham rats ($p<0.01$). Saline-evoked OOemg also increased in proportion to saline concentration and was markedly enhanced in DE compared to sham rats ($p<0.01$). To determine the roles for the Vi/Vc transition and Vc/C1 junction regions in mediating saline-evoked eyeblink, OOemg was recorded after synaptic blockade of Vi/Vc transition or Vc/C1 junction regions by $CoCl_2$ microinjection (100mM, 100nl). Saline (2.5M) -evoked OOemg was reduced by >95% and >70% by 10 min after blockade of the Vi/Vc transition and Vc/C1 junction regions ($p < 0.01$), respectively. These results suggest that persistent tear deficiency in DE is sufficient to significantly modify the encoding properties of ocular-responsive trigeminal brainstem neurons and. osmotic-evoked OOemg activity. Based on changes in encoding properties of Vc neurons and OOemg responses to local synaptic blockade, tear insufficiency results in widespread central sensitization of trigeminal brainstem neurons at both the Vi/Vc transition and the Vc/C1 junction regions. These data suggest that persistent alterations in tear

film integrity likely are accompanied by long-term functional changes in CNS neurons and may help explain why peripheral ocular approaches often fail to adequately treat the discomfort in chronic DE.

Disclosures: **M.M. Rahman:** None. **K. Okamoto:** None. **A. Katagiri:** None. **R. Thompson:** None. **S. Kazunari:** None. **D.A. Bereiter:** None.

Poster

826. Trigeminal Processing of Pain

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 826.05/JJ35

Topic: D.08. Pain

Support: R01 NS061571 NINDS/NIH

R21 NS058695 NINDS/NIH

K05-AA017261 NIAAA

Title: Repeated infusion of prostaglandin E2 alone onto the dura induces chronic trigeminal sensitivity via TRPA1 channels

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Abstract: To identify the essential components for the transition to chronic trigeminal hypersensitivity, we investigated the effect of each component of the widely used inflammatory soup on the transition to chronic trigeminal hypersensitivity. Numerous models of trigeminal sensitization, including allodynia and migraine, have relied on the infusion of an inflammatory soup (IS) containing histamine, serotonin, bradykinin, and prostaglandin E2 at low pH onto the dura of rats. A single application of the IS induces afferent activation and central sensitization for >5 hours while repeated infusion 3x/wk of 10 infusions induces chronic trigeminal hypersensitivity which outlasts the final infusion for >3 months. These sensitized rats feature numerous behavioral and physiological characteristics similar to that observed in migraineurs, including a hypersensitivity to chemical headache triggers such as glycerol trinitrate (GTN). Individual soup components or IS were infused onto the dura of awake rats through a cannula 3X/wk for 10 infusions. Periorbital nociceptive pressure thresholds were determined with von Frey monofilaments. Rats that had transitioned to chronic periorbital sensitivity had thresholds of

<4.0g at least 1 week following the last infusion. Naive rats or rats who did not transition had thresholds of 8-10g. Infusion of an IS with physiological pH (7.4), not low pH, was sufficient to induce chronic trigeminal hypersensitivity. Infusion of PGE2 alone produced similar chronic trigeminal sensitivity. Both IS and PGE2-infused rats showed hypersensitivity to GTN. Concurrent infusion of a TRPA1 receptor antagonist, with PGE2 prevented behavioral sensitization. Infusion of 0.1mM PGA2, another prostaglandin that is formed by the dehydration of PGE2 that has an higher affinity for TRPA1 channels, also produced the transition to chronic trigeminal hypersensitivity. Infusion of other individual components of the inflammatory soup; bradykinin, serotonin, or histamine alone did not produce trigeminal hypersensitivity. The only IS component needed to the transition to chronic trigeminal hypersensitivity is PGE2, which activates TRPA1 channels on peripheral nociceptors. PGE2's transitioning effect is blocked with concurrent infusion of a TRPA1 antagonist, suggesting that PGE2's action is via TRPA1 channels. PGA2, similar in structure to PGE2 but with greater affinity for TRPA1 channels, also produces chronic trigeminal sensitivity, further suggesting a role of TRPA1 on peripheral trigeminal nociceptors in the transition to chronic trigeminal pain.

Disclosures: **N.T. Fried:** None. **M.L. Oshinsky:** 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.; St. Jude Medical Inc., Medtronic Inc., Electrocore LLC.. **M. Cooper:** None.

Poster

826. Trigeminal Processing of Pain

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Support: NIH Grant DE017805

Title: Elevated TNF-alpha levels in the TMJ promotes transient peripheral sensitization of trigeminal nociceptive neurons

Authors: *P. L. DURHAM[1], Z. L. DURHAM[2];
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Abstract: Objective: The goal of this study was to better understand the cellular mechanisms involved in mediating the nociceptive effects of TNF-α on trigeminal ganglion neurons that provide sensory innervation to the TMJ. Background: Temporomandibular joint disorder (TMJD) and temporomandibular disorders (TMD) are prevalent and debilitating pain conditions in the United States, especially among younger women. Since TMJD affects the trigeminal nerve and has high co-morbidity with migraine headache and sinus headache, it is associated with significant social and economic burdens. Levels of the cytokine TNF-α are elevated in the TMJ capsule and positively correlate with reported pain levels. Methods: Male Sprague-Dawley rats were injected bilaterally with 100 ng/ml TNF-α or vehicle control and changes in nocifensive responses to mechanical stimulation evaluated using calibrated von Frey filaments applied to the cutaneous area over the masseter (V3 region) at 2 and 24 hours post injection. In addition, levels of 29 cytokines were determined in trigeminal ganglia by protein array analysis at 2 and 24 hours. Results: Two hours following TNF-α injection into both TMJ capsules, the rat's nocifensive response to mechanical stimulation was significantly elevated over naïve and vehicle controls. After 24 hours, rats injected with TNF-α did not exhibit significantly different responses to the von Frey filaments than naïve or vehicle controls. Both vehicle and TNF injection resulted in an increase in the levels of several cytokines in the trigeminal ganglion when compared to levels in naïve animals. While eight cytokines were significantly elevated above vehicle control levels 2 hours post injection (CNTF, fractalkine, IL-1α, IL-1β, IL-6, IP-10, thymus chemokines, and VEGF), only CNTF and thymus chemokines remained at increased levels after 24 hours. In contrast, the levels of CINC-1, IFN-γ, IL-6, MIP-3α, and TIMP-1 were significantly decreased at 24 hours when compared to vehicle control levels. Conclusion: Our results provide evidence that elevated levels of TNF-α in the joint capsule promotes transient sensitization of primary trigeminal nociceptors via a mechanism that temporally correlates with increased levels of cytokines in the ganglion. Thus, therapeutic strategies that can selectively target the inflammatory and nociceptive cellular events mediated by TNF-α in the TMJ are likely to be beneficial in the acute treatment of individuals suffering from TMJD.

Disclosures: P.L. Durham: None. **Z.L. Durham:** None.

Poster

826. Trigeminal Processing of Pain

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 826.07/KK1

Topic: D.08. Pain

Title: The role of trigeminal spinal subnucleus caudalis in dry-tongue pain

Authors: *Y. NAKAYA[1], A. OKADA[2], Y. TSUBOI[3], M. SHINODA[3], Y. IMAMURA[2], K. IWATA[3];
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Abstract: Some patients suffering from dry mouth complain of tongue pain. It's important to know the mechanisms underlying tongue pain associated with dry mouth, in order to develop the appropriate treatment for tongue-pain patients. Therefore, we intended to elucidate the pathological pain associated with dry tongue. The dry-tongue model was developed in the rats receiving tongue exposure in the air for 2 hours per day for 14 days under light anesthesia (2% Isoflurane). Mechanical or heat nocifensive behavior, phosphorylated extracellular signal-regulated kinase (pERK) immunohistochemistry and single neuronal activity in the trigeminal spinal subnucleus caudalis (Vc) were precisely analyzed in the dry-tongue rats on days 7 and 14. Head-withdrawal threshold (HWT) to mechanical but not heat stimulation of the tongue significantly decreased on day 7 compared with sham rats. The mechanical but not heat responses of Vc nociceptive neurons were significantly enhanced on day 7 compared with sham rats. The pERK-immunoreactive (IR) cell expression significantly increased in the Vc following noxious stimulation of the tongue compared with sham rats at 7 days after tongue dry. The decrement of the mechanical HWT was reversed during intrathecal (i.t.) administration of MEK1 inhibitor PD98059. The Vc neuronal activity and the number of pERK-LI cells following noxious mechanical stimulation of the tongue was also significantly decreased following i.t. administration of PD98059. This is the first documentation that mechanical but not heat hyperalgesia occurs in the dry-tongue rats and suggests that mechanical tongue pain associated with dry mouth is caused via ERK phosphorylation in Vc nociceptive neurons.

Disclosures: Y. Nakaya: None. **A. Okada:** None. **Y. Tsuboi:** None. **M. Shinoda:** None. **Y. Imamura:** None. **K. Iwata:** None.

Poster

826. Trigeminal Processing of Pain

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Program#/Poster#: 826.08/KK2

Topic: D.08. Pain

Support: NIH Grant EY021230

Title: The effect of acute dry eye on the properties of rat corneal primary afferent cold receptors and ocular inflammation

Authors: M. KUROSE[1], J. WIERSMA[2], A. HATTA[1], S. BARTON[2], N. MECUM[3], K. YAMAMURA[1], *I. D. MENG[2];
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Abstract: Cold sensitive neurons innervating the cornea regulate ongoing tearing by monitoring the ocular surface fluid status. Previously, we demonstrated the sensitization of corneal cold receptors eight weeks after induction of dry eye by lacrimal gland excision in the rat. In the present study, cold receptors were characterized one week after lacrimal gland excision. Additionally, corneal tissue from dry eye and sham control animals was examined one week after surgery for expression of inflammatory mediators and growth factors using ELISA. Extracellular single-unit recordings were performed in urethane-chloralose anesthetized rats one week following excision of the infra- and extra-orbital lacrimal glands and in age-matched controls. Electrodes positioned in the trigeminal ganglion were used to isolate and characterize cold-sensitive corneal neurons. Cold and heat responses were evoked by a contact thermode before and after the application of menthol (10 - 500 μM), hyperosmotic artificial tears and their respective vehicles. The short-term dry eye condition increased cold receptor sensitivity to menthol and hyperosmotic stimuli when compared to control animals. Furthermore, dry eye increased corneal expression levels of several pro-inflammatory cytokines and chemokines, including IL-1alpha, IL-1beta, MCP-1, and MIP-1alpha, and increased levels of vascular endothelial growth factor (VEGF) and nerve growth factor (NGF). Since ocular inflammation has previously been shown to inhibit corneal cold receptors, it is hypothesized that the sensitization of corneal cold receptors in dry eye is mediated by growth factors such as NGF.

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Poster

826. Trigeminal Processing of Pain

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Topic: D.08. Pain

Support: KAKEN 26861799

KAKEN 24659916

Title: Morphological and physiological alterations evoked by orthodontic force in rats

Authors: ***A. SASAKI**[1], N. HASEGAWA[2], K. TAKAHASHI[3], G. YUN[3], T. NAGAO[4], N. SUDA[2], H. SAKAGAMI[4], K. ADACHI[4,5,6];
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Abstract: The orthodontic treatment is applied to the patients who complain about dental displacement to dissolved functional and/or aesthetic issues, however, it could be a source of painful stimulation. Although non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used to treat orofacial pain in dentistry, those analgesics might affect the treatment of dental displacement. Since inhibition of cyclooxygenase by NSAIDs provides both analgesic effect and reduction of osteoclast activity. Recently, we have established the animal model for quantitative evaluation of the orthodontic force-induced pain and revealed that the hypersensitivity occurred immediately after application of the orthodontic force and it lasted for three days and then returned to normal level in seven days. In the present study, we investigated morphological and physiological alterations evoked by orthodontic force in periodontal tissues of rats. Under general anaesthesia with isoflurane (2.0 %), Wister rats (10 weeks) were applied to orthodontic brace and Ti-Ni coil spring between right maxillary first molar and bilateral incisors to load the continuous orthodontic force (50 g). One (Day 1), three (Day 3) and seven (Day 7) days after applying orthodontic brace (n = 5 in each group), rat was anaesthetised with isoflurane (0.9-1.1 %) and pairs of electromyographic (EMG) electrodes were implanted into bilateral anterior digastric muscles to evaluate jaw-opening reflex (JOR) excitability. Another pairs of electrodes were placed bilateral maxillary first molar gingiva in buccal-palatal direction for local electrical stimulation. Currents (200 μs) were applied to define the threshold for inducing JOR to left and right maxillary first molar for the comparison. The expression of activated satellite glial cells (SGC) in the trigeminal ganglion (TRG) were identified by glial fibrillary acid protein-immunoreactivity and moving distance of the first molar was indirectly evaluated by digital microscopic model analysis. At Day 1, the expression of activated SGC was observed in right side TRG, and then expanded to bilateral TRG at Days 3 and 7. Loading of the continuous orthodontic force induced the medial movement of the first molar, which was increased with the progression of postoperative days (Day 1: 0.12 ± 0.14 mm, Day 3: 0.19 ± 0.05 mm, Day 7: 0.42 ± 0.02 mm). Interestingly, moving distance of the first molar was negatively related with JOR threshold alteration, which is also the case in human patients treated orthodontically. These results suggest the applicability of this animal model for the exploration of new analgesics that do not alter bone resorption.

Disclosures: **A. Sasaki:** None. **N. Hasegawa:** None. **K. Takahashi:** None. **G. Yun:** None. **T. Nagao:** None. **N. Suda:** None. **H. Sakagami:** None. **K. Adachi:** None.

Poster

826. Trigeminal Processing of Pain

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Topic: D.08. Pain

Support: NIH NINDS NS072204

NIH NIGMS GM102575

Migraine Research Foundation

Title: Brainstem BDNF contributes to hyperalgesic priming in a rat model of migraine

Authors: *G. O. DUSSOR, C. BURGOS-VEGA, T. PRICE;
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Abstract: Migraine is the most common neurological disorder although little is known about the mechanisms contributing to migraine pain. Migraine attacks are episodic but whether one attack increases the likelihood for subsequent attacks is not known. Previous studies in our laboratory have shown that application of interleukin-6 (IL-6) to the rat dura produces cutaneous allodynia that persists for up to 24 hours. The purpose of these studies was to investigate whether sensitization of the dural afferent system remains following resolution of IL-6 allodynia. Male Sprague-Dawley rats were implanted with dural cannulas and allowed to recover for 7 days. Following stimulation of the dura with IL-6, facial and hindpaw allodynia were measured for 5 hours and at 24-hour intervals until allodynia resolved. Animals remained at baseline for 24 hrs and were then given a normally subthreshold pH stimulus (pH 6.8 or 7.0) onto the dura. Allodynia was tested 2-hours following low pH stimulation. TrkB/FC, a BDNF sequestering agent, was injected into the cisterna magna 24 hours post IL-6 or animals were given intracisternal injections of BDNF to determine whether brainstem BDNF contributes to IL-6 sensitization. Dural application of IL-6 produced facial and hindpaw allodynia that resolved by 48 hours. After allowing rats to remain at baseline for an additional 24 hours, dural application of the normally subthreshold pH stimuli of pH 6.8 and 7.0 produced robust facial and hindpaw allodynia in IL-6 but not vehicle-treated animals. IL-6 sensitization to low pH was blocked by injection of TrkB/FC into the cisterna magna 24 hours after IL-6. Injection of 1 pg BDNF into the cisterna magna produced cutaneous allodynia similar to that observed with dural IL-6 and this allodynia followed a similar timecourse. Twenty-four hours following resolution of BDNF-induced allodynia, dural application of pH 6.8 or pH 7.0 produced robust cutaneous facial and hindpaw allodynia that was not present in animals given vehicle injections into the cisterna

magna. These data show that dural afferents can be primed to normally sub-threshold stimuli via prior administration of IL-6 onto the dura. BDNF signaling within the nucleus caudalis contributes to priming as sequestration of BDNF in the brainstem eliminates priming and exogenous administration of BDNF into the brainstem can prime the system in the absence of afferent input. These findings indicate that dural stimulation produces BDNF-dependent sensitization at central synapses that primes the trigeminal system to subsequent subthreshold events, a mechanism that may contribute to the pathophysiology of migraine headache.

Disclosures: G.O. Dussor: None. **C. Burgos-Vega:** None. **T. Price:** None.

Poster

826. Trigeminal Processing of Pain

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Topic: D.08. Pain

Support: NRF-2008-0062282 (MSIP)

Title: Expression of P2X3 receptor in the astrocytes and postsynaptic dendrites in the rat brain stem

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Dept. of anatomy and neurobiology, Sch. of Dentistry, Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: P2X3, a purinergic receptor, is a nonselective cation channel which is implicated in neuropathic and inflammatory pain. Its expression has been presumed to be confined to primary nociceptive afferent terminals. Here, we report, for the first time, expression of P2X3 in astrocytes and postsynaptic dendrites in the superficial lamina of the rat trigeminal caudal nucleus (Vc) and their possible role in neuropathic pain by use of electron microscopic immunoperoxidase and immunogold labeling methods and animal model of infraorbital nerve ligation (CCI-ION) . Immunoreactivity of P2X3 was consistently observed in somata and process of astrocyte, and postsynaptic dendrites besides round vesicles containing endings which are presumed to be primary afferent terminals (PATs). P2X3 expression was increased in the PATs, dendrites, and astrocytes in the Vc of CCI-ION rats, implying the involvement of P2X3 receptor in pain perception following nerve injury. Supporting this observation, intracisternal administration of P2X3 and P2X2/3 receptor selective antagonist, A-317491, significantly

ameliorated the mechanical allodynia in CCI-ION rats. Moreover, MPEP, an antagonist of metabotrophic glutamate receptor 5 (mGluR5), significantly reduced the expression of P2X3 in astrocytes and dendrites and ameliorated mechanical allodynia in CCI-ION rats. These results raise the possibility that the activation of mGluR5 mediated signaling pathway is associated with the increased expression of P2X3 in astrocytes, and implicate the role of astrocytic P2X3 in mechanical hypersensitivity. Our findings provide ultrastructural evidence of P2X3 expression in astrocytes of the Vc, and suggest the role of astrocytic P2X3 receptor in neuropathic pain.

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Poster

826. Trigeminal Processing of Pain

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Topic: D.08. Pain

Support: JSPS KAKENHI 25462908

Title: Kappa opioid receptor-mediated antinociception of temporomandibular joint -responsive neurons in superficial laminae at spinomedullary junction in female rats depends on estrogen status

Authors: *A. TASHIRO, Y. NISHIDA, Y. NISHIDA;
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Abstract: Chronic painful temporomandibular joint disorders (TMD) occur more often in women than men and are difficult to manage. 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 determine if estrogen mediate the variation in kappa opioid analgesia of TMJ unit in female, single units were recorded in laminae I-II at the spinomedullary (Vc/C1-2) junction from ovariectomied female rats (OvX) treated with high E2 (40 μg/d, HE2), low E2 (4 μg/d, LE2). Under isoflurane anesthesia TMJ units were activated by ATP (1 mM, 20μl) injected into the joint space before and during cumulative doses of U50,488H (selective KOR agonist; 0.03- 3mg/kg, iv) given at 20 min intervals. ATP-evoked responses of TMJ units in HE2 were enhanced versus LE2 ($p \leq 0.05$). U50,488H caused a doses related

inhibition of ATP-evoked unit activity in HE2 rats, while units in LE2 rats displayed inconsistent effects. BNI (selective KOR antagonist; 1mg/kg) caused at least partial reversal of kappa opioid inhibition. These results suggest that estrogen status plays a significant role in kappa opioid analgesia of TMJ units.

Disclosures: A. Tashiro: None. **Y. Nishida:** None. **Y. Nishida:** None.

Poster

826. Trigeminal Processing of Pain

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Topic: D.08. Pain

Support: NIH DA02110

Title: Mechanistic studies on the effects of Botulinum toxin in Migraine: Role of transport in trigemino-somatic and trigemino-vascular afferents

Authors: *R. RAMACHANDRAN, T. L. YAKSH, C. LAM;
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Abstract: Background and Aim: Migraine pain secondary to meningeal input is referred to extracranial regions innervated by somatic afferents that project to homologous regions in the trigeminal nucleus caudalis (TNC). Reported efficacy of extracranial botulinum toxin (BoNT) in treating migraine is surprising since a local extracranial effect of BoNT cannot alone account for its effect upon migraine pain. We hypothesize that the intradermal BoNT effects occur as a result of a central transport and *trans-synaptic* movement of the cutaneous afferent BoNT to block the meningeal responses (see Marino, Pain. 2014 155:674-84). Based on this background, we examined whether unilateral supraorbital injection of BoNT-B can cleave VAMP protein in the ipsilateral (ipsi) trigeminal ganglion (TG) and peripheral afferents in TNC and block nocisponsive behavior and activation of cFOS in ipsi- TNC otherwise evoked by supraorbital *or* meningeal capsaicin. **Methods:** Briefly anesthetized C57Bl/6 mice (male) received unilateral supraorbital injections of BoNT-B (1.5 U/40 µl) or saline. 3 days later, mice received ipsi-supraorbital capsaicin (2.5 µg/ 30 µl) or meningeal capsaicin, though a small cranial burr hole (4ul of 1µg/ml) and were analyzed for nocisponsive behavior (measured by number of ipsi-wiping of the face), VAMP (SNARE protein) in the ipsi- TG/ TNC and activation of cFOS in the ipsi- TNC. **Results:** We observed that pre-treatment with ipsi supraorbital BONT-B i) decreased

the nocicsponsive behavior on the ipsi side following ipsi supraorbital capsaicin ii) cleavage of VAMP only in the V1 region of ipsi TG iii) reduced activation of cFOS in ipsi TNC following ipsi supraorbital capsaicin iv) and importantly reduced cFOS activation in ipsi TNC secondary to ipsi meningeal capsaicin. **Conclusion:** The present study shows that BoNT-B is taken up by the peripheral afferents and transported to the central terminals where it blocks transmitter release resulting in decreased activation of second order neurons evoked by capsaicin. Further, this study provides evidence of trans-synaptic movement of the BoNT from the cutaneous trigeminal afferent to either the second order neurons (which receives convergent input from the meningeal afferent) or the terminal of the converging meningeal afferent. The present study provides insights relevant to the development of therapies for migraine as well as to other debilitating craniofacial disorders. (Migraine Research Foundation)

Disclosures: R. Ramachandran: None. **T.L. Yaksh:** None. **C. Lam:** None.

Poster

827. Spinal Cord Injury and Plasticity II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 827.01/KK8

Topic: D.12. Kinematics and EMG

Support: NIH grant HD032571

Title: Locomotor activity of feline ankle extensors and kinematics during level and slope walking after removal of stretch reflex from soleus and lateral gastrocnemius by self-reinnervation

Authors: *R. J. GREGOR[1], B. PRILUTSKY[2];
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Abstract: Previous reports have emphasized the importance of muscle stretch response contributions to ankle extensor locomotor activity in the cat (Stein et al. 2000; Yakovenko et al 2004); however, cats with self-reinnervated ankle extensors (i.e., with no stretch reflex; Cope et al. 1994) demonstrate normal joint kinematics during level and upslope walking, and only modestly increased ankle yield during stance of downslope walking (Abelew et al. 2000; Maas et al 2007). Thus, the role of stretch reflex in locomotor activity of ankle extensors needs further investigation. The goal of this study was to document changes in EMG locomotor activity of major ankle extensors during the first 14 weeks after cut and repair of the nerve innervating

soleus (SO) and lateral gastrocnemius (LG) muscles. Based on the previous studies we hypothesized that activity of reinnervated SO and LG during locomotion will be reduced due to the absence of the stretch reflex, and normal walking kinematics would be achieved by increased activity of the intact synergists medial gastrocnemius (MG) and plantaris (PL). Six cats were trained to walk on a walkway inclined at 0 deg and +/-27 deg using food rewards. EMG fine wire electrodes were implanted into SO, LG, MG and PL. After recording baseline EMG activity and walking mechanics, the LG-SO nerve was cut and repaired using fibrin glue (Gregor et al. 2006; Prilutsky et al., 2011). Data collection resumed within 3-5 days after nerve injury and continued weekly or biweekly for 14 weeks. Our results indicate that EMG magnitude of self-reinnervated SO and LG recovered to pre injury values at weeks 9-10 and exceeded them at weeks 12-14. EMG magnitude of intact MG and PL muscles increased after LG-SO nerve injury and returned to the control by 12-14 weeks. EMG of all muscles was higher during stance in upslope walking than in level or downslope conditions. EMG of self-reinnervated SO and LG prior to stance onset was not affected by slope but EMG of MG and PL was affected by slope. EMG burst onset prior to paw contact started earlier in downslope walking than in level or upslope conditions, but burst duration was the longest during upslope walking in all muscles. Enhanced ankle yield in stance was still present by week 12-14 indicating incomplete recovery of walking kinematics. We concluded that the loss of stretch reflex from SO and LG did not affect their mean EMG activity or EMG magnitude of their synergists, however, ankle joint kinematics did not fully recover 14 weeks after SO-LG nerve repair. Length-dependent feedback from proximal muscles and force-dependent feedback from all ankle extensors appeared to contribute to regulation of muscle activity during slope walking.

Disclosures: R.J. Gregor: None. **B. Prilutsky:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NS17323

DFG Fellowship HU 1963/1-1

Title: Effects of spinal cord injury on the intrinsic firing properties of spinal motor neurons examined with perforated patch recordings from adult mice

Authors: A. HUSCH[1], *B. R. JOHNSON[2], R. M. HARRIS-WARRICK[1];
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Abstract: Spinal neurons in locomotor networks lose their activating and modulatory signals from higher brain regions after spinal cord injury (SCI). As a result, their intrinsic excitability properties and transmitter sensitivity may change. This secondary effect may contribute to the loss of locomotor function after spinal cord injury and may inhibit recovery of locomotor control. We investigated the effects of SCI on the intrinsic firing properties of lumbar and sacral motor neurons (MNs) from adult mice (at least 8 weeks old). Perforated patch recordings were made from lumbar and sacral MNs in longitudinal spinal cord slices prepared from mice four to six weeks after a complete transection of the thoracic or lumbar spinal cord, and from age matched control mice. SCI mice showed rear leg paralysis and behavioral deficits consistent with complete SCI. We identified MNs in the most ventral spinal cord slices from ChAT-GFP transgenic mice by: 1) GFP labeling, 2) ventrolateral soma location and 3) large soma size compared to spinal interneurons. MNs were isolated from fast synaptic input. Similar to V2a interneurons (Husch et al., 2012), MNs from SCI mice were more sensitive to serotonin than control MNs. SCI MNs responded with depolarization and spontaneous firing onset or firing rate increases to concentrations as low as 10 nM 5-HT, in contrast to control MNs which depolarized only with 10 μM 5-HT. Surprisingly, neither lumbar nor sacral MNs with either thoracic or lumbar lesions showed greater bistability compared to control MNs, when tested with step or ramp current injections from different holding potentials. In control and SCI MNs, depolarizing currents typically evoked tonic firing with spike frequency adaptation. SCI and control MNs showed a similar hysteresis on current ramps and no increase of long lasting firing upon depolarizing stimulation. More negative holding current was needed to hold MN membrane potentials at -70 mV in SCI than in control mice, despite similar input resistance values at -70 mV for both groups. This indicates a more depolarized membrane potential in SCI MNs. In control spinal cord slices, 8% (12/13) of lumbar MNs fired spontaneous action potentials with no holding current; in SCI treated slices, 50% (8/16) of MNs were spontaneously active at rest. Our experiments did not demonstrate the increases in MN excitability after SCI seen in other rodent SCI preparations, despite our behavioral and physiological evidence of complete SCI. We are presently exploring explanations for differences between our results and those previously reported from rodent SCI preparations.

Disclosures: A. Husch: None. **B.R. Johnson:** None. **R.M. Harris-Warrick:** None.

Poster

827. Spinal Cord Injury and Plasticity II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 827.03/KK10

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NJCSCR CSCR13FEL009

Title: Standing biomechanics of the knee and effect of electrical stimulation on muscle response in sci adults

Authors: *M. B. BAYRAM, M. DAMCOTT MITCHELL, R. PILKAR, P. BARRANCE, G. F. FORREST;
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Abstract: Introduction: Musculoskeletal deterioration and its prevention in Spinal Cord Injury (SCI) have been investigated extensively. One such method is mechanical loading intervention, which in SCI, has been explored during stance and neuromuscular stimulation (NMS). The aim of this study is to explore the development of instrumentation and protocols to quantify the external knee pad (Fx, Fy, Fz) forces applied to the bone across loading interventions and provide insight to allow determination of the optimal parameters to reduce bone loss. **Methods:** For our preliminary study, 1 individual with motor complete SCI (mcSCI) and 1 able-bodied (AB) subject participated. NMS **(35 Hz, 300 μs)** was applied to the rectus femoris muscle while the subject was positioned in a standing frame with the knee flexed. The force produced by electrically stimulated contraction was measured by a load cell on anterior aspect of the patella. EMG was collected unilaterally using electrodes placed on rectus femoris and lateral aspect of the rectus femoris. Six degrees of freedom movement sensors were placed unilaterally above and below knee to ensure total isometric contraction. For the initial trial, NMS started at a minimum threshold and ramped up by 2mV increments, to the predetermined maximum contraction on site. In second trial, the protocol was reversed. For mcSCI, an additional high frequency (80Hz) NMS was tested for both trials. **Results:** Knee pad Fz (compressive) forces were consistent over 3 repetitions for the first and second trial for both AB and mcSCI. Knee pad forces for AB were 13.90±0.60 and 12.61±0.59 percent of the body weight, for trial 1 and 2 respectively. Knee pad forces for mcSCI were 13.51±0.31 and 14.24±0.23 percent of the body weight, for trial 1 and 2 respectively. Fatigue was not observed for each trial. **Conclusion:** The NMS parameters (35 Hz, 300 μs) have been reported to be effective on attenuating bone loss during a standing protocol for chronic motor complete SCI. These preliminary results for knee pad forces for trial 1 and 2 were less than reported in the literature for a similar experimental setup. Potentially these knee pad force data provide important insight for optimizing NMS stimulation parameters during a standing protocol for chronic motor complete SCI.

Disclosures: M.B. Bayram: None. **M. Damcott Mitchell:** None. **R. Pilkar:** None. **P. Barrance:** None. **G.F. Forrest:** None.

Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation

Christopher and Dana Reeve Foundation

Title: The effect of therapeutic deep brain stimulation of the mesencephalic locomotor region on structural plasticity of brainstem-spinal tracts in mice

Authors: *L. C. BACHMANN[1,2], N. A. GOOD[1,2], M. E. SCHWAB[1,2];
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Abstract: Deep brain stimulation (DBS) of the mesencephalic locomotor region (MLR) improves gait when applied in chronically spinal cord injured rats. This effect is presumably mediated by signals carried by spared parts of the reticulo-spinal tract. Functional improvements are pronounced during, and immediately after the stimulation. After damage to the brain or spinal cord, structural plasticity, i.e., sprouting of axonal projections, is believed to be one mechanism for functional recovery. We have previously found specific parts of the medullary reticulo-spinal tract to sprout in response to stroke or incomplete spinal cord injury. Here we addressed the impact of MLR DBS during the recovery from large, incomplete spinal cord injuries (lateral hemisections) on sprouting of brainstem-spinal fibers in the mouse. Stimulation in intact animals caused an increased strength of locomotor output with increasing stimulation intensity. After T9-10 lateral hemisection, animals were stimulated in daily intervals for ten days. The time course of the functional gains was assessed by kinematic measurements. Four weeks after injury, the number of neurons with a lumbar midline crossing projection into the ipsilesional hemicord was assessed in 26 spinally projecting brain areas.

Disclosures: L.C. Bachmann: None. **N.A. Good:** None. **M.E. Schwab:** None.

Poster

827. Spinal Cord Injury and Plasticity II

Location: Halls A-C

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Program#/Poster#: 827.05/KK12

Topic: D.12. Kinematics and EMG

Support: ACSM Grant

AHA Fellowship

Title: Inducing plastic changes in lower motoneurons and functional improvement in stroke survivors

Authors: *B. TAHAYORI, D. KOCEJA;
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Abstract: Release in spinal inhibitory mechanisms following a cerebrovascular accident is regarded as an interfering factor to volitional execution of movement. The aim of this study was to examine the possibility of inducing plastic changes (through operant conditioning) at the level of alpha motoneurons and investigate its functional consequences. A human-computer interface was developed to train the subjects (n=3) in a task-oriented operant conditioning protocol over three weeks (3 sessions/week). Subjects demonstrated the ability to down-regulate the soleus H-reflex. The rate of success in this down-regulation was on average 80.1±9.96%. This success rate was in strong agreement with improvement in both gait symmetry and gait velocity. The duration of treatment in our protocol falls within the first phase of plastic changes associated with reflex down-regulation. During this phase, plastic changes are reversible and do not cause a long lasting depression in the amplitude of the reflex. Nonetheless, functional improvements were observed in this first phase. Neural correlates were observed after the termination of treatment, suggesting that this treatment is accompanied by an increase in presynaptic control of Ia fibers. Our electrophysiological examination suggests an increase in presynaptic control of Ia afferents due to this treatment. An increase in presynaptic control of Ia afferent could prevent the interruption of cortical drive and hence encourage volitional motor commands. We combined computer technology and basic neuroscience knowledge to provide a novel treatment method for clinical rehabilitation of stroke survivors. Our data strongly suggest the ability of these patients to down-regulate the amplitude of the H-reflex and induce a plastic change at the level of alpha motoneurons. This method, in all likelihood, provides better volitional control over the lower motoneurons without changing their excitability level and/or spasticity level. Inducing plastic changes at the level of lower motoneurons seems to be a very promising treatment strategy to decrease the interference with cortical drive at the level of final common pathway.

Disclosures: B. Tahayori: None. **D. Koceja:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Program#/Poster#: 827.06/KK13

Topic: D.12. Kinematics and EMG

Support: AHA Fellowship

Title: The interaction of Post activation depression and Presynaptic inhibition in the spinal circuits

Authors: *D. M. KOCEJA, B. TAHAYORI;
Indiana Univ., Bloomington, IN

Abstract: The mechanism and functional significance of post activation depression is not well understood. Current evidence suggests that the depression only affects the activated Ia fibers, acts presynaptically and is due to the depletion of neurotransmitter. Such assumptions severely limit the functional significance of this mechanism. However, being a presynaptic modulator, this mechanism might be used for controlling the synaptic strength from other sources. Here, in a series of experiments, by manipulating the amplitude of H- and H-prime independently, we showed that in healthy individuals by increasing the amplitude of the second H (H2) and conditioning it with a constant H-reflex, a nonlinear modulation of H2 occurred. Therefore, a small size preceding H-reflex had a substantial effect on a large portion of alpha motoneurons. Also, by applying the two inhibitory mechanisms of post activation depression and presynaptic inhibition a substantial reduction in inhibition was observed. This double inhibition protocol resulted in an H-reflex that was substantially larger than either the presynaptically inhibited H or H-prime. This effect was absent for a double inhibition protocol using disynaptic reciprocal inhibition. This suggests that the cancellation of these two inhibitory sources occurs presynaptically. In stroke patients similar results were observed regarding the nonlinear relation in H2 only when the second H was elicited within 200 ms after the first H. However, increasing the interval between the two reflexes dissociated this relation and caused a facilitatory effect on H2. The double inhibition protocol did not produce the cancellation of inhibition observed in healthy individuals. These findings suggest that post activation depression might be used as a feedback signal to regulate reciprocal presynaptic inhibition. Impairment of this feedback loop might be a part of the pathophysiology of spasticity.

Disclosures: D.M. Koceja: None. **B. Tahayori:** None.

Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

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Title: Overexpression of CCL2 enhances capacity of axon regeneration in DRG sensory neurons

Authors: *M. J. KWON[1,2], H. SHIN[1,2], J. CHOI[1,2,3], D. HWANG[1,2], B. G. KIM[1,2,3];
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Abstract: Regeneration of the central branches of the dorsal root ganglia (DRG) sensory neurons is induced if the peripheral branches are injured before the central injury (conditioning injury, CI). We have previously shown that macrophages play an essential role in the CI-induced axon regeneration. We also identified a novel chemokine signaling mediated by CCL2 that links regenerating neurons and macrophage activation with proregenerative phenotypes in the CI model. In the present study, we provide evidence that overexpression of CCL2 can mimic CI effects on the long-lasting axon regeneration. Intraganglionic injection of recombinant CCL2 resulted in an increase of macrophages infiltration in the DRGs and enhanced neurite growth of the DRG neurons taken at 7 days after the injection. Injection of either fractalkine or CCL3 failed to enhance neurite outgrowth while both chemokines were sufficient to increase the number of macrophages in the DRGs. The differential effects are probably due to differential macrophage polarization because only CCL2 drove M2 polarization whereas M1 polarization was dominant by the other two chemokines in primary macrophage cultures. Macrophages in the DRGs after CI were also polarized into M2 phenotype. The enhanced capacity of neurite growth by intraganglionic CCL2 injection was significantly diminished by 4 weeks after the injection. Therefore, we tested if adeno-associated virus serotype 5 (rAAV5) mediated overexpression of CCL2 can lead to long-lasting enhancement of axon regeneration capacity. Intraganglionic rAAV5-CCL2 injection led to long-term neuronal expression of CCL2 for up to 4 weeks. Neurite outgrowth of the DRG neurons taken at 4 weeks was significantly increased by the intraganglionic rAAV5-CCL2 injection compared to control rAAV5-GFP injection. This study suggests that chronic elevation of CCL2 level in DRG neurons can enhance long-lasting

regenerative capacity. Manipulation of CCL2 signaling and the neuron-macrophage interactions may lead to a novel therapeutic approach to promote axon regeneration after CNS injury.

Disclosures: **M.J. Kwon:** None. **H. Shin:** None. **J. Choi:** None. **D. Hwang:** None. **B.G. Kim:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Program#/Poster#: 827.08/KK15

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Do strategies to enhance performance of a skilled walking task change when sensory stimulation is combined with motor practice?

Authors: *A. E. CHISHOLM[1,2], T. LAM[1,2];
[1]Intl. Collaboration On Repair Discoveries, Vancouver, BC, Canada; [2]Sch. of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Background: Many people with a spinal cord injury (SCI) can recover some basic walking ability; however they experience difficulty when performing more skilled walking tasks (e.g. stairs and obstacles). Improved motor function and re-learning motor skills after injury strongly depends on the presence of appropriate sensory input. A training program that enhances both motor and sensory pathways may further improve motor performance, and activate appropriate networks to develop effective locomotor strategies to re-learn functional walking skills. The purpose of this study is to evaluate changes in the locomotor control strategies utilized in the acquisition of a skilled walking task when sensory stimulation is added to motor practice. Methods: Able-bodied adults performed a skilled walking task focused on foot height during swing with the Lokomat robotic-gait orthosis. They were presented with real-time visual feedback of their foot height along with a virtual target that they were instructed to match during the swing phase. The target height changed randomly for each of the 30 steps during the pre- and post-training tests. For the training bout, subjects were randomized to receive one of 4 different practice conditions: 1) no sensory stimulation, 2) proprioceptive only, 3) cutaneous only and 4) paired proprioceptive and cutaneous. Proprioceptive stimulation was delivered as a Lokomat-applied resistance against hip and knee flexion during swing. Cutaneous stimulation was applied by brief trains of electrical impulses to the sural nerve. Foot trajectory error was measured as the vertical distance between the target and actual foot height. We measured lower limb muscle

activity and sagittal joint angles during the pre- and post-training tests. Results: The paired stimulation group demonstrated stronger positive correlations post-training between target height and biceps femoris activity at pre-swing (r= 0.44 pre, r= 0.59 post). Medial gastrocnemius activity at pre-swing was positively correlated to target height after training in the proprioceptive only group (r= -0.12 pre, r= 0.25 post). All groups had greater correlations between target height and peak knee angle during swing after training (r= 0.4-0.62 pre, r= 0.54-0.76 post). Conclusions: Preliminary findings indicate that sensory stimulation may enhance the coordination of the knee flexor muscle activity to match the target foot trajectory. Further data collection is warranted to confirm the benefits of enhanced sensory stimulation on skilled locomotor performance, and to apply this combined training method with individuals who have a SCI.

Disclosures: A.E. Chisholm: None. **T. Lam:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: ERC grant

Title: Muscle spindle feedback drives locomotor recovery and circuit reorganization after spinal cord injury

Authors: *A. TAKEOKA[1,2], I. VOLLENWEIDER[3], G. COURTINE[3], S. ARBER[4,2];
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Abstract: Spinal cord injuries alter motor function by disconnecting descending pathways from spinal circuits, rendering sensory afferents a primary source of direct external drive to neuronal networks caudal to lesion. Here, we studied mice specifically lacking functional muscle spindle feedback to determine the role of this sensory channel in locomotor control and in gait recovery after thoracic lateral spinal cord hemisection injury. High-resolution kinematic analysis revealed proficient execution with specific impairments in intact mutant mice during basic locomotor tasks, but poor performance of skilled locomotion. After injury, wild-type mice spontaneously

recovered basic locomotor function, whereas mice with deficient muscle spindle feedback failed to regain control over the ipsi-lesional hindlimb. Virus-mediated tracing demonstrated that mutant mice exhibit defective rearrangements of circuits projecting to deprived spinal segments during the recovery period. Together, our findings reveal an essential role for muscle spindle feedback in driving basic locomotor recovery and facilitating circuit reorganization after spinal cord injury.

Disclosures: **A. Takeoka:** None. **I. Vollenweider:** None. **G. Courtine:** None. **S. Arber:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation

NIH Grant NS54894

Title: Combination therapies of robot rehabilitation and viral delivery of brain-derived neurotrophic factor (BDNF) to lumbar cord promote large functional gains after a complete transection SCI in adult rats

Authors: ***J. LEE**[1], V. J. TOM[2], S. F. GISZTER[2];
[2]Neurobio. & Anat., [1]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Spinal cord injury (SCI) disrupts the normal, healthy architecture of the central nervous system, leading to impaired locomotor function. In the rat model, complete thoracic spinal cord transection at T8-T10 is a common model for studying SCI. In our previous work, using trunk-based robotic rehabilitation and treadmill training, we showed that rats spinalized as neonates can significantly recover locomotor function with robotic intervention at the pelvis, whereas rats transected as adults do not exhibit the same level of recovery. We believe this is due to the absence of autonomous reflex hindlimb stepping patterns in adult transection, resulting in an inability to incorporate and benefit from robot support. Previous work by Boyce and Mendell has demonstrated that use of adeno-associated virus-5 (AAV5) viral delivery of neurotrophic factors, such as BDNF and NT-3, to enable reflex hindlimb stepping in the rat, Boyce and Lemay demonstrated related gains in the cat model. We thus propose a combined treatment approach,

using a neurotrophin intervention combined with robot training to induce autonomous stepping and achieve greater locomotor recovery. We prepared two groups of rats (n=4 per group) with microinjections (final volume: microliters) caudal to transection site into the ventral horn of the spinal cord: one group receiving AAV5-BDNF and another receiving a sham AAV5 virus expressing green fluorescent protein. Following post-operative recovery, animals were treadmill trained with robotic pelvic rehabilitation therapy for six weeks. We used the Antri, Orsal, and Barthes (AOB) bipedal stepping scale and robot data measuring the interactive force between the rat and the robot to characterize improvement and recovery of locomotor function. We found that animals that received AAV5-BDNF and robot-assisted treadmill training showed significantly improved recovery over the course of training in both AOB ($p<<0.0001$) and overall robot interactive force ($p=0.002$), compared to those that received sham virus, which did not. Comparing across both groups, locomotor recovery assessed on the final day of therapy also showed significant improvement in the experimental group, as compared to the control group, with respect to AOB scores ($p=0.0001$) and robot interactive force ($p=0.0002$). This work provides a foundation upon which to investigate further combinations of biological and bionic therapies for treating SCI. This work is sponsored by the Craig H. Neilsen Foundation and the NS 54894.

Disclosures: **J. Lee:** None. **V.J. Tom:** None. **S.F. Giszter:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Shriners Postdoctoral Research Grant

Title: Activation of mTOR and Stat3 intrinsic mechanism combined with Chondroitinase and Neurotrophin 3 expressions enhances injured CST axons growth

Authors: *Y. LIU, J. HONG, X. TANG, G. SMITH;
Shriners Hosp. Pediatric Res. Ctr., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: The adult mammalian CNS is extremely limited in its ability to regenerate axons following injury. Failure of these axons to regenerate is caused by a multiplicity of factors, including both extrinsic inhibitory factors surrounding injured axons and the reduced intrinsic ability of neurons to regenerate. Treatments targeting these mechanisms individually have resulted in some axon regrowth and functional recovery after spinal cord injury. However, the number of regenerating axons is generally limited and functional recovery modest. Therefore the combined stimulation of intrinsic growth capacity of the neuron and reducing the inhibitory milieu of damaged axons may lead to successful regeneration of injured CNS axons. Previous studies shown that concurrent activation of mammalian target of rapamycin (mTOR) and signal transducers and activators of transcription (Stat3) signaling pathways by deletion of both phosphatase and tensin homolog (PTEN) and SOCS3 (suppressor of cytokine signaling 3) in retinal ganglion cells synergistically promoted significant long-distance axon regeneration after optic nerve lesions. In this study, we enhanced the intrinsic regenerative capacity of injured neurons by expressing Ras homolog enriched in brain (Rheb), a direct activator of mTOR, and Stat3 in the sensorimotor neurons. We tested whether a combinatorial therapy of corticospinal tract (CST) regeneration by both activating the intrinsic capacity of neuronal regeneration , reducing the extrinsic inhibitors (expression of Chondroitinase) and expressing NT3 in the injury site could enhance the growth of injured CST axons and pass the lesion site. We found that adeno-associated virus-mediated transduction resulted in transgene expression *in vitro* and *in vivo* and activation of mTOR and Stat3 signaling pathways. Furthermore, we observed that the combination of methods enables the CST axons to grow and pass across the lesion site in the mid-cervical spinal cord, although not at a very high density. These results suggest that stimulate the intrinsic growth potential of neurons, while reducing the inhibitory environment surrounding the injury site may offer new therapeutic strategies for the treatment of human spinal cord injuries.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation

Title: Development and validity of the pediatric neuromuscular recovery scale

Authors: E. ARDOLINO[1], M. MULCAHEY[2], S. A. TRIMBLE[3,4], M. BIENKOWSKI[5], C. MULLEN[5], L. C. ARGETSINGER[3,4], *A. L. BEHRMAN[6,7,4];
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Abstract: Objective: The Neuromuscular Recovery Scale (NRS) was developed to assess the capacity of adults post-spinal cord injury (SCI) to perform functional tasks without compensation and relative to performance one day prior to injury. Thus, achievement of the task is not only assessed, but how the task is achieved is valued. For each item, scores reflect skills from the lowest score (1A; unable) to the highest score (4C; fully recovered). Within and across items, scores reflect neuromuscular capacity within a "hierarchical" manner of task difficulty and recovery. This new outcome measure is particularly relevant with the onset of activity-based therapies and research in neuroplasticity, regeneration, and physiological strategies to activate muscles below the lesion for adults and children. The purpose of this study was to adapt the NRS for use with the pediatric population with SCI and to establish content validity for children ages 1-12 years. Methods: This study was conducted in 3 phases. In Phase 1, the investigative team with SCI clinical and research expertise developed a draft Pediatric NRS by reviewing the current adult version, modifying items for the pediatric population or eliminating items inappropriate for children, and examining current pediatric outcomes for relevant motor items. Phase 2 used a Delphi method for review and input to the draft Peds NRS by 12 clinical pediatric experts (5 OTs, 5 PTs, 3 MDs). Any item on the scale that did not reach 80% agreement was revised based on the comments provided by the experts. In Phase 3, the investigative team tested the revised Peds NRS on a sample of children with SCI (n=5) and without (n=2). Results: The initial Peds NRS draft consisted of 13 items each scored on a 12 point scale: 10 conducted off the treadmill (over ground) and 3 on the treadmill. With completion of the Delphi survey via 4 rounds of questions and responses, the scoring for 7 items was modified. After round 4, all items had reached 80% agreement. All items were maintained and no new items were added. After field-testing of the Peds NRS, several items required modifications for tester instructions, item consistency and difficulty. Standardized equipment list and instruction manual for execution and scoring were finalized. Conclusion: In this study, we carried out a systematic, iterative process using mixed methods to develop and examine the content validity of the pediatric NRS. The work represents the first effort towards development of a pediatric SCI scale that evaluates neurorecovery, in the context of pediatric function, without reliance on devices or compensation.

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Poster

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Kentucky Spinal Cord and Head Injury Research Trust

Commonwealth of Kentucky Challenge for Excellence

NIHINIGM8-P30GM103507

Title: Training strategies based on segmental versus descending circuitry following spinal cord injury: Impact on recovery

Authors: *D. R. HOWLAND[1,2,3,4,5], A. C. RISING[2,3,5], K. A. CHEFFER[2,3,5], K. L. WHYLAND[2,3,5], W. A. O'STEEN[2,3,5], A. L. BEHRMAN[3,4,5];
[1]Res. Service, [2]Robley Rex VA Med. Ctr., Louisville, KY; [3]Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY; [4]Kosair Charities Ctr. for Pediatric Neurorecovery, Louisville, KY; [5]Neurolog. Surgery, Univ. of Louisville, Louisville, KY

Abstract: Rehabilitation is likely to be a key part of any future, successful, combinatorial treatment for enhancing recovery following spinal cord injury (SCI). The current, clinical Locomotor Training (LT) approach shows that spared circuitry caudal to a SCI can respond to task specific training. This activity-based intervention emerged from animal studies that focused on bipedal hindlimb stepping in quadrupeds and is designed to activate the spinal circuitry by providing a sensory-appropriate experience for walking. There also is some evidence to suggest that training of locomotor activities affected by supraspinal input can enhance locomotor recovery. The current study was designed to understand if separate strategies for locomotor features that depend upon segmental and/or descending pathways will be required to promote maximal recovery following SCI. Twenty-one adult cats with low thoracic, lateral hemisection injuries were divided into five groups: 1) untrained; 2) trains on wide runway; 3) step trains on a treadmill and wide walkway; 4) trains on treadmill, wide walkway and multiple challenging walkways; and 5) trains on all walkways, but not treadmill. Challenging walkways, which include obstacle negotiation, a narrow beam, horizontal ladder, and peg walkway, require

multiple adaptive features including changes in limb trajectories, stride length, step height, paw placements, interlimb coordination, trunk alignment and balance. All animals were tested periodically across all tasks to determine performance of trained and untrained tasks and locomotor features. Cats also were tested on novel tasks to further understand the potential for skill transfer to new situations for all groups. Due to notable recovery following injury, the low thoracic hemisection provides a good model in which to assess more adaptive walking skills. However, it is critical to note that permanent deficits in basic motor components also are seen post-hemisection. Qualitative and quantitative analyses are used and include 3D kinematics. Adaptive locomotor features which are critical components of home and community ambulation are not well addressed in current rehabilitation. The results from this work will help determine if a rehabilitation strategy that targets training of more challenging or skilled locomotor tasks can enhance recovery of adaptive components of locomotion, if these adaptive components require task specific training, and if skills achieved can be transferred for negotiation of new complex environments.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: University of Louisville Kosair Charities Center for Pediatric Neurorecovery

Title: Segmental trunk control improves for children with spinal cord injury and locomotor training

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Abstract: Objective: In contrast to physical rehabilitation that relies on compensatory strategies which use muscles innervated above the level of a spinal cord injury (SCI), this study assessed

whether an intervention targeting activation of the neuromuscular system below the level of injury promotes development of trunk control using muscles innervated caudal to the lesion. Methods: Five children suffered SCI (birth - 4 yrs 10 mos). The children began an activity-based therapy, locomotor training (LT), at ages 2.5 - 6 years. The therapy, delivered 1.5 hours/day, 5x/week, consisted of: 1) step training on the treadmill with partial body weight support (BWS) as trainers provided sensory cues consistent with walking and standing; 2) assessment and transfer of skills gained to off the treadmill; 3) incorporation of new skills into daily activities for home/community. During step training, children were encouraged to reciprocally swing and coordinate their arms during facilitation of upright head and trunk alignment. The BWS was set at the minimum for the child to maintain normal postural alignment during trainer facilitation of pelvis and leg positions and BWS decreased as control improved. During stand training (on the treadmill), children repetitively practiced activities to activate the trunk (e.g. reaching overhead for a ball, rotating to hit a tennis ball, and regaining alignment after trunk perturbation). Off the treadmill, trunk activity was facilitated in assisted sitting and standing. For example in sitting, trunk flexion and extension was practiced in incremental ranges. In standing, the trunk was progressively challenged during activities without arm support. Parents were instructed in ways to challenge trunk control in home and community activities. Results: Children received a mean of 97 LT sessions. All children improved trunk and head alignment during standing on treadmill, Segmental Assessment of Trunk Control scores (assessed every 20 sessions) during sitting (mean increase 5.7 increments), decreased BWS (mean 5%), and by parent report of improved sitting balance. Conclusion: Improved trunk control below the level of the lesion occurred in children following LT. With 100% of children injured before age 5 developing scoliosis, the impact of enhancing trunk control on the quality of life for children with SCI is especially important as it may dramatically alter the trajectory of outcomes.

Disclosures: S.A. Trimble: None. **L.C. Argetsinger:** None. **D.R. Howland:** None. **A.L. Behrman:** None.

Poster

827. Spinal Cord Injury and Plasticity II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: 8 P30 GM103507

Rebecca F. Hammond Trust

KSCIRC

Title: Trunk control and pulmonary function in children with spinal cord injuries: a pilot study on the effects of locomotor training

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Abstract: In children under age 12 with spinal cord injury (SCI), the prevalence of cervical lesions is as high as 80%. Cervical injuries cause paralysis or weakness of trunk and respiratory muscles which are related to a high incidence of scoliosis (100% when injured < 5 years of age), pneumonia, and other respiratory complications. To date, the primary treatment to prevent scoliosis is passive stabilization via a thoraco-lumbar-sacral-orthosis. Abdominal strapping is commonly used to improve expiratory function and coughing after SCI, though these have not been effective tools. Locomotor training (LT) is an activity-based therapy designed to re-train the spinal cord circuitry by activating the neuromuscular systems above and below the lesion. While benefits of LT have been reported in adults post-SCI for improved walking, cardiovascular and pulmonary function and activation of trunk musculature below the lesion , they have not been examined in children with SCI. The purpose of this study is to assess the effect of LT on activation of trunk musculature across and below the spinal cord lesion level during 1) sitting activities which challenge balance, 2) respiratory maneuvers and 3) cough. LT is provided 5x/week during 1.5 hour session and includes 1) cued stepping and standing in the treadmill environment with partial body weight support and challenge to trunk control, 2) transfer of skills to activities off the treadmill, and 3) use of skills in the home and community. Methods: Two non-ambulatory children with SCIs were assessed while enrolled in an LT program. These children had T1 AIS C and T3 AIS B injuries, were 8 and 5 years old, and 7 and 1.3 post-injury respectively. Neural activation of trunk and neck muscles was assessed via EMG and kinematic recordings during the “Segmental Assessment of Trunk Control” (SATCo) test and respiratory maneuvers (i.e. spirometry, maximal respiratory pressure and coughing). Baseline values were captured and performance re-assessed after every 30 sessions of LT. Outcomes: Both participants improved in their trunk motor control (assessed by the SATCO) and respiratory maneuvers values. Discussion: Children undergoing LT show higher scores for the SATCo indicating better trunk motor control. Additionally, they showed improvements in maximal forced respiratory tasks. Better expiratory function may support an improved ability to cough, and lead to a decreased risk of pneumonia and other respiratory infections. Thus, in addition to the targeted gait-related benefits, this small ongoing study suggests that LT enhances trunk

control and respiratory function which may diminish the risk of developing scoliosis and pneumonia in children with SCI.

Disclosures: D.G. Terson de Paleville: None. **S. Trimble:** None. **L. Argetsinger:** None. **M. Love:** None. **M. Kloby:** None. **D. Howland:** None. **A. Behrman:** None.

Poster

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Topic: D.12. Kinematics and EMG

Support: NSERC

CFI

Title: Examining changes in spinal excitability during dual-task performance

Authors: *D. DAY, M. BOIVIN, A. ADKIN, C. TOKUNO;
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Abstract: A dual-task paradigm, in which individuals simultaneously perform a cognitive and balance task, is often used to examine how attentional resources are shared between two tasks. Prior research has investigated how performance measures are affected during dual-task performance but few studies have examined the neural strategies used to compensate for the decreased availability of attentional resources. Recent work by Weaver et al. (2012) indicates that during simple dual-task situations, individuals reduce the neural excitability at the spinal level so that more resources can be allocated to the cognitive task. However, it is currently not known whether a similar strategy is employed in progressively more difficult dual-task situations. Therefore, the purpose of this study was to investigate whether spinal excitability is scaled to the difficulty of the dual-task situation. Twenty adults performed nine dual-task conditions, with each condition requiring the simultaneous performance of a cognitive and balance task. The cognitive task required participants to use a controller to respond to moving targets on a monitor, while the balance task had participants stand upright on a stability platform that could tilt in the sagittal plane. A combination of changes in cognitive and balance task difficulty created the nine dual-task conditions. Cognitive task difficulty was increased by manipulating the order in which the controller buttons corresponded to the moving targets.

Balance task difficulty was altered by changing the amount of resistance to make the platform more or less stable. To establish whether spinal excitability was scaled to the difficulty of the dual-task situation, Hoffmann reflexes (H-reflexes) were elicited in the soleus muscle through the percutaneous electrical stimulation of the posterior tibial nerve. Results confirm that balance and cognitive task difficulty was manipulated as intended. Balance task performance, as measured by increases in the root mean square amplitude of the platform angle, and cognitive task performance, as measured by decreases in the accuracy of hitting the moving targets, were affected by 56% and 10%, respectively, from the easiest to the hardest task difficulty levels. Despite the progressive increase in dual-task difficulty, the soleus H-reflex amplitude was not found to be different between the three balance task ($p=0.687$) and the three cognitive task ($p=0.232$) difficulty levels. These results suggest that individuals must rely on neural strategies that occur beyond the spinal level when challenging dual-task situations are encountered.

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Poster

827. Spinal Cord Injury and Plasticity II

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Topic: D.12. Kinematics and EMG

Support: NIH 5R24HD050821-09

Title: Long-latency reflexes of elbow and shoulder muscles display reciprocal excitation of flexors and reciprocal excitation of extensors

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Abstract: An intrinsic complexity of body motion is that a load applied to one body segment induces motion of connected body segments. This complexity is addressed by the arm's long-latency reflexes (LLR, 50-100ms after an arm displacement) as they integrate motion information from several joints to counter the underlying torque. For example, the shoulder extensor's LLR is evoked by flexion of just the elbow which is appropriate to counter underlying flexion torque applied to the shoulder (and elbow). Here we test whether the elbow's LLRs respond to elbow and shoulder motion appropriate to counter the underlying elbow torque. Eleven healthy subjects participated (median age = 24 yro; 6M & 5F). Surface EMG was

obtained from their elbow muscles (brachioradialis & triceps lateral) and shoulder muscles (pectoralis major & posterior deltoid). A programmable robot (KINARM, BKiN Technologies) applied different background torques (±1-2 Nm) and torque pulses (100 ms) to evoke responses during postural maintenance. Experiment 1 displaced the subject's elbow and shoulder (n = 8) in eight directions with nearly equal magnitude (≈1° @ 50ms) and separation (≈45°) in joint space. Planar fits of the evoked LLR versus elbow and shoulder displacement revealed the preferred joint motion (PJM) of each arm muscle. The shoulder extensor's LLR was maximally excited by shoulder-elbow flexion (22 ±2°) while the shoulder flexor's LLR was maximally excited by shoulder-elbow extension (206 ± 5°). This pattern is consistent with compensation of the underlying shoulder torque (18° and 197°) rather than the local shoulder motion (0° and 180°). Similarly, the elbow flexor's LLR was maximally excited by shoulder-elbow flexion (242 ± 9°) while the elbow extensor's LLR was maximally excited by shoulder-elbow extension (35 ± 6°) which is appropriate to compensating the underlying elbow torque (225° and 45°) rather than local elbow motion (270° and 90°). Experiment 2 (n = 3) utilized a physical brace to block elbow motion. Thereby, we tested if elbow LLRs responded to motion of just the shoulder. In fact, shoulder extension evoked elbow flexor activity while shoulder flexion evoked elbow extensor activity. The multi-joint LLR by a monoarticular likely reflects stretch of other monoarticulars. LLRs of shoulder and elbow flexors are evoked by extension of either joint which implies an excitatory reflex connection between these two muscles. Similarly, LLRs of shoulder and elbow extensors are evoked by flexion of either joint implying an excitatory reflex connection between these two muscles. Reciprocal excitation between flexors and between extensors may be a core network for postural stabilization of the arm.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: KAKEN Grant-in-Aid for Scientific Research（C）

Title: Implantation of human mesenchymal stem/progenitor cells (hMSCs) from bone marrow suppresses spinal cord injury mediated by pituitary adenylate cyclase activating polypeptide (PACAP)

Authors: *H. OHTAKI[1], T. TSUMURAYA[1], A. SATO[1], Z. XU[1], J. WATANABE[1], M. MATSUMOTO[1], N. MURAI[1], H. HASHIMOTO[3], Y. HIRAIZUMI[2], T. NAKAMACHI[1,4], S. SHIODA[1];
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Abstract: Mesenchymal stem/progenitor cells (MSCs) from bone marrow are a promising tool for cell therapy to regenerate and rescue diverse damaged tissues including central nervous system (CNS). However, the neuroprotective mechanism of MSCs has not understood in detail. We determined in present study that implantation of human MSCs (hMSCs) decreased spinal cord injury (SCI) partially mediated by a neuropeptide, pituitary adenylate cyclase activating polypeptide (PACAP) using by PACAP gene deficient (KO) mice. Under inhalation of anesthesia, mice either PACAP+/+ (wild) or +/- (KO) were subjected to spinal cord transection by razor at level of Th9 - 10 intervertebral spinal cord. One day later, the mice were injected hMSCs (5x10^5 cells /0.5 μL) or vehicle one caudal the intervertebral spinal cord. The mice were scored locomotor activity detected by Basso Mouse Scale and evaluated injury area with GFAP surrounded area at 7 days post operation. We also examined mouse PACAP and PACAP specific receptor (PAC1R) gene expressions after the implantation. WT mice implanted hMSCs into spinal cord ameliorated significantly locomotor activity and injury volume to compare with vehicle-treated one. The protections were canceled by repeated freeze-thawing produced inviable hMSCs implanted into WT mice and by viable hMSCs implanted into KO mice. Spinal cord implanted hMSCs expressed an increase of mouse PACAP gene, but did not change PAC1R. These results suggest that hMSCs suppress neural cell death mediated by PACAP gene expression.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

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Title: Variable gradient of intermuscular inhibition as preliminary evidence for spinal mediated modulation of task dependent limb behavior

Authors: *M. A. LYLE, N. E. BUNDERSON, C. TUTHILL, I. F. NIAZI, T. R. NICHOLS; Sch. of Applied Physiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: Animals and humans have a remarkable ability to rapidly adapt limb mechanics to accommodate different tasks (e.g. walk, run, land) and environmental surfaces (e.g. Daley, 2007; Farley, 1998), but the neural control processes responsible remain unknown. We hypothesize that task dependent modulation of the relative gradient of intermuscular force feedback (e.g. inhibition greater from proximal to distal muscles) is a potential rapid spinal mediated neural candidate. Here, we tested the feasibility of this hypothesis by characterizing the gradient of intermuscular force dependent feedback across decerebrate cats. Intermuscular force feedback was evaluated by stretching tendons of selected muscles in isolation and in pairwise combinations and then measuring the resulting force dependent intermuscular interactions. Several muscle pairs were analyzed to determine the relative gradient between muscles and symmetry across limbs and muscle pairs. We are currently evaluating the functional importance of observed experimental data with a computational model of the cat hindlimb using Neuromechanic. Three main features of inhibitory force feedback were observed across cats (inhibition stronger from distal to proximal muscles, the converse, and fairly symmetric). Usually, a gradient observed for a given cat was present in both limbs; moreover, the gradient of inhibitory force feedback often extended to several muscle combinations within a limb. The variable distribution of force feedback across decerebrate cats provides preliminary evidence that inhibitory force feedback could be a regulated control variable, and extends recent findings demonstrating that intermuscular inhibition appears to be fixed from distal to proximal muscles in cats with acute and chronic hemisection (i.e. a potential default state potentially responsible for behavioral deficits). A prediction, currently being evaluated with the computational model, is that the various gradients observed experimentally provide important task dependent affordances (e.g. directionally appropriate joint compliance, joint coupling, and compensation for nonuniform inertia). This study was supported by F32NS080393 to MAL and HD32571 to TRN.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

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Dept of VA (RR&D)

Rebecca F. Hammond Trust

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Title: Reorganization of inhibitory force feedback to and from hind limb toe flexors after chronic spinal cord injury in the cat

Authors: I. F. NIAZI[1], *T. NICHOLS[2], D. R. HOWLAND[3];
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Abstract: Using electrophysiological methods, R.M. Eccles and A. Lundberg (1959) showed that inhibitory transmission in the spinal cord is suppressed in the decerebrate preparation and released upon spinal transection. In contrast, more recent work from the Nichols laboratory using natural stimulation indicated that force dependent inhibition can be substantial in the control decerebrate animal, but that its distribution is quite variable among preparations. Inhibition between the flexor hallucis longus (FHL) and gastrocnemius (G) muscles is often stronger from G to FHL (particularly during stepping), sometimes stronger from FHL to G, or balanced between the two muscles. In our most recent studies of animals with chronic spinal hemisection, the inhibition was amplified and always stronger from FHL to G and weak or undetectable from G to FHL. Here we report an investigation of the magnitudes of force feedback for other muscle combinations in order to derive a more global understanding of the changes in distribution of force feedback due to spinal injury, with the goal to determine how these changes contribute to the observed disorders of movement and posture. Animals with chronic spinal hemisection were decerebrated under deep surgical anesthesia. Intermuscular force feedback was evaluated by stretching tendons of selected muscles in different combinations and then measuring the resulting force responses. We found that FHL was a particularly strong source of inhibition to other muscles, such as soleus and plantaris, and that inhibition from these other muscles to FHL was undetectable or much weaker than observed in control decerebrate animals. The effect was bilateral, although stronger on the side of the lesion. Lesions were either complete or slightly less than complete for the ventral funiculus. We are currently assessing the integrity of serotonergic and other pathways at the site of the lesion to determine which pathways might likely regulate

the strength and distribution of force feedback. We conclude that the powerful inhibition projecting from FHL to more proximal muscles is likely to contribute to motor deficits after spinal cord injury. **Support:** NIH HD32571, Dept of VA (RR&D), Rebecca F. Hammond Trust and NIHINIGM8-P30GM103507

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Poster

827. Spinal Cord Injury and Plasticity II

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Topic: D.10. Spinal Cord Injury and Plasticity

Title: Astrocytic TNFR2 is essential for recovery of locomotor function following spinal cord injury

Authors: *L. H. FUNK[1], A. HACKETT[2], J. BETHEA[3], J. LEE[2];
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Abstract: Currently there are approximately 273,000 people in the U.S. living with disability due to spinal cord injury (SCI) and approximately 12,000 new cases occur each year. SCI pathology is described by an initial hemorrhagic necrosis due to mechanical trauma followed by a gliotic response whereby astrocytes upregulate pro-inflammatory gene expression, proliferate, hypertrophy and form a boundary surrounding the injury site. Among the upregulated genes is Tumor Necrosis Factor (TNF), a pleiotropic cytokine that has been shown to promote pro-inflammatory changes, apoptosis, neurotoxicity and gliosis following SCI. Two forms of TNF exist, soluble TNF (solTNF), which selectively binds TNF receptor 1 (TNFR1), and transmembrane TNF (tmTNF), which binds either TNFR1 or TNFR2. The cell specific effects of TNFR1 and TNFR2 signaling in the context of SCI pathophysiology are not known. To elucidate the role of TNFR2 in the astrocytic response, GFAPcreER-TNFR2f/f mice were generated to genetically delete TNFR2 from astrocytes. Following administration of tamoxifen, GFAPcreER-TNFR2f/f and TNFR2f/f littermate controls underwent contusive SCI at the T8 level and were evaluated for locomotor recovery using the Basso mouse scale (BMS). As early as 7 days following the injury, the GFAPcreER-TNFR2f/f mice had significantly lower BMS scores than their littermate controls. These data demonstrate the importance of astrocytic TNFR2 for recovery following SCI despite the known role of TNF in neurotoxicity and inflammation.

Understanding the therapeutic mechanism of astrocytic TNFR2 is important for the development of TNF modulatory therapies for SCI.

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Poster

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Wings for Life Foundation WFL-US-013/13 (KM)

Title: Loading-related spinal plasticity assume the contribution of functional recovery after spinal cord injury

Authors: *K. MORIOKA[1,2], T. TAZOE[2,3], X. MA[1], C. F. GUANDIQUE[1], L. VANCITTERS[1], J. R. HUIE[1], J. C. BRESNAHAN[1], M. S. BEATTIE[1], S. TANAKA[4], A. R. FERGUSON[1], T. OGATA[2];
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Abstract: Limb loading assumes a pivotal role in locomotor recovery and re-organization of spinal reflex circuits through facilitating the central pattern generator for locomotion after spinal cord injury (SCI). Immobilization such as long-term bed rest after SCI is considered to produce maladaptive spinal plasticity which can impair recovery of function through the development of spasticity and exaggerated nociceptive withdrawal reflexes. However, little is known about the specific mechanisms and biological consequences by which loading and unloading shape spinal plasticity after SCI. In this study, we investigated the effect of hind-limb unloading (HU) early after SCI using a multidisciplinary approach. Adult female SD rats were subjected to mild bilateral contusive injury (impact force 50 kdyn) to the lower thoracic (T9) spinal cord using the

Infinite Horizon impactor device. Early after injury, subjects were assigned to two experimental groups: 1) chronic HU by tail suspension, and 2) normal loading control. After 2 weeks, the HU group was returned to normal loading conditions for the duration of the study. All animals were observed until 8 weeks post-injury. Locomotor recovery was assessed using the BBB scale and kinematic analysis. The effect of HU early after SCI on the spinal reflex modulation was assessed using H-reflex testing of the plantaris muscle at 8 weeks. Spinal cord tissue was assessed postmortem using biochemistry and unbiased high-resolution robotic confocal microscopy for plasticity-related changes by HU in the spinal cord. Our results demonstrate that HU early after SCI impaired locomotor recovery and produced over-excitation of spinal reflex circuits. Our findings indicates the possibility that complete limb unloading early after SCI produces maladaptive spinal cord plasticity which can impair locomotor recovery. Biochemical and confocal microscopic studies into the substrates of this plasticity are ongoing. Our data has great potential to provide novel evidence of loading-related spinal maladaptive plasticity early after SCI, leading to advances in neurorehabilitation for clinical acute SCI.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Title: Electrophysiological characterization of cortical reorganization after C4/C5 lateral hemisection in common marmosets

Authors: *A. KOSUGI[1], T. KONDO[2], K. YOSHINO-SAITO[2], H. J. OKANO[4], M. NAKAMURA[3], H. OKANO[2], J. USHIBA[5];
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Abstract: Spinal cord injury results in impairment of motor and sensory functions below the level of the lesion. Spontaneous recovery of motor function comes after weeks to months in non-

human primates, but neurophysiological mechanisms underpinning the recovery are not fully understood. Several studies suggested that functional reorganization of the primary motor cortex (M1) is accompanied by spontaneous recovery, but the cortical map assessed by intracortical microstimulation (ICMS) only reflected the result from passive stimulation. It is still questionable that these related neurons are functionally recruited and appropriately activated during voluntary movements. Therefore, a causal relationship between the cortical reorganization and the functional recovery of voluntary movements still remains unclear. In the current study, we evaluated the changes in cortical activities during voluntary movements after spinal cord injury. We in this study employed epidural electrocorticography (ECoG) to record voluntary M1 activity while attempting paralyzed hand reaching in C4/C5 laterally hemisected common marmosets. Epidural ECoG signals were placed on the sensorimotor cortex. During the recording the maromosets performed reach-and-grasp movements to retrieve a pellet with the intact and the affected hand respectively. Since the behavioral assessment of open field scoring reached the ceiling (i.e., spontaneous recovery reached a plateau) around 13 weeks after the lesion, we started ECoG recording thereafter. During movements with the intact hand, power-increase in higher frequency bands of ECoG signal including the high-γ (80-150 Hz) band was observed on several channels in the contralateral sensorimotor cortex. According to evoked movements of the intact hand by ICMS of the contralateral M1 in the awake subjects, we estimated that these channels were located around the M1 hand area. On the other hand, during movements with the affected hand, no significant difference of ECoG signals compared to the rest was detected in the contralateral sensorimotor cortex. Furthermore, though the size of the hand area estimated by ICMS was reduced, affected hand movements were still evoked by the stimulation in some parts of contralateral M1. These results suggest that activities of M1 cortical neurons associated with voluntary movements were impaired after spinal cord injury possibly because of asynchronous timing of each neuron firing or the decrease in firing rate, even though the neurons could respond to passive stimulation.

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Poster

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Mayo Clinic Center for Regenerative Medicine

Gene Therapy Resource Program (GTRP) of the NIH NHLBI

Title: Glutamatergic NMDA receptor expression in phrenic motoneurons following cervical spinal cord injury

Authors: H. M. GRANSEE[1], G. F. MARTINEZ-GALVEZ[1], M. A. GONZALEZ PORRAS[1], J. C. DIAZ SOTO[2], J. M. ZAMBRANO[1], W.-Z. ZHAN[1], G. C. SIECK[1], *C. B. MANTILLA[2]; [1]Physiol. & Biomed. Engin., Mayo Clin., Rochester, MN; [2]Anesthesiol., Mayo Clin., ROCHESTER, MN

Abstract: Cervical spinal hemisection at C2 (SH) interrupts descending ipsilateral bulbospinal pathways to phrenic motoneurons causing diaphragm muscle paralysis. The time course of changes in glutamatergic receptor expression at phrenic motoneurons correlates with the spontaneous recovery of ipsilateral phrenic activity post-SH. Intrapleural treatment using adeno-associated virus (AAV) serotype 7 encoding the full-length TrkB receptor targets phrenic motoneurons, enhancing recovery of rhythmic phrenic activity. We hypothesized that expression of glutamatergic receptors in phrenic motoneurons after SH correlates with the extent of recovery of ipsilateral phrenic activity. Adult male Sprague-Dawley rats were instrumented for chronic diaphragm EMG recordings and absence of ipsilateral phrenic activity was confirmed 3 days after SH. During eupnea, a subset of animals displayed recovery of ipsilateral diaphragm EMG activity over time post-SH. The proportion of animals displaying recovery increased with AAV-TrkB treatment compared to untreated and AAV-GFP treated animals. Retrogradely-labeled phrenic motoneurons were sampled using laser capture microdissection for quantitative real-time RT-PCR analyses. Greater NMDA receptor expression was evident in animals displaying recovery by 14 or 21 days post-SH than in those that did not recover. Peak root-mean-square EMG amplitude correlated with NMDA receptor expression in phrenic motoneurons, regardless of treatment group. Enhancing phrenic motoneuron expression of glutamatergic NMDA receptors (e.g., via intrapleural delivery of AAV7-TrkB) promotes recovery of diaphragm activity.

Disclosures: H.M. Gransee: None. **G.F. Martinez-Galvez:** None. **M.A. Gonzalez Porras:** None. **J.C. Diaz Soto:** None. **J.M. Zambrano:** None. **W. Zhan:** None. **G.C. Sieck:** None. **C.B. Mantilla:** None.

Poster

827. Spinal Cord Injury and Plasticity II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 827.25/KK32

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH grant R01 HL96750

Title: Impact of glutamatergic NMDA signaling on recovery of phrenic activity after cervical spinal cord injury

Authors: C. B. MANTILLA[1], W.-Z. ZHAN[2], L. G. ERMILOV[2], H. M. GRANSEE[2], *G. C. SIECK[2];
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Abstract: Cervical spinal cord injury at C2 (SH) disrupts descending excitatory drive to phrenic motoneurons paralyzing the ipsilateral diaphragm muscle. Over time post-SH, there is spontaneous recovery of ipsilateral phrenic activity reflecting neuroplasticity. Recent studies indicate that SH-induced neuroplasticity in phrenic motoneurons comprises increased NMDA receptor expression and that the time course of recovery correlates with changes in phrenic motoneuron NMDA receptor expression. We hypothesized that recovery of ipsilateral phrenic activity post-SH requires NMDA receptor signaling. Adult male Sprague-Dawley rats implanted with bilateral diaphragm EMG electrodes for chronic EMG recordings were examined for evidence of recovery of ipsilateral phrenic activity up to 28 days post-SH. In all animals, absence of ipsilateral activity was verified at 3 days post-SH. At 28 days, ~60% of animals displayed recovery of eupneic diaphragm EMG activity. The extent of recovery was calculated by peak root-mean-squared (RMS) EMG activity. For animals displaying recovery at 28 days post-SH, peak RMS EMG activity during eupnea was ~100% compared to pre-injury anesthetized (ketamine-xylazine) measurements. Diaphragm EMG activity was recorded during exposure to hypoxia-hypercapnia (10% O_2-5%CO_2) and intranasal capsaicin (30 microM, 10 microL) to induce sneezing. The cervical spinal cord segments containing the phrenic motor nucleus (C3-5) were then surgically exposed and the NMDA receptor antagonist D-AP5 (100 mM, 30 microL) was instilled intrathecally. Treatment with D-AP5 acutely reduced ipsilateral RMS EMG activity during all diaphragm motor behaviors (~50%). Our results support the importance of glutamatergic NMDA signaling to recovery of phrenic activity after cervical spinal cord injury.

Disclosures: **C.B. Mantilla:** None. **W. Zhan:** None. **L.G. Ermilov:** None. **H.M. Gransee:** None. **G.C. Sieck:** None.

Poster

827. Spinal Cord Injury and Plasticity II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 827.26/LL1

Topic: D.10. Spinal Cord Injury and Plasticity

Support: MED 142 FACULTAD DE MEDICINA UNIVERSIDAD DE LA SABANA

COLCIENCIAS No 377-2009

Title: Olfactory ensheathing cells and aFGF transplant promotes functional recovery and remyelination in the chronic transected rat spinal cord

Authors: *L. BOTERO[1], R. M. GOMEZ[2], G. K[2], O. CHAPARRO[3];
[1]Univ. Nacional De Colombia, Bogota, Colombia; [2]Univ. de la Sabana, Chia, Colombia; [3]Univ. Nacional de Colombia, Bogota, Colombia

Abstract: Spinal cord injury (SCI) can lead to paraplegia or quadriplegia. Although there are no fully restorative treatments for SCI, many cellular and molecular therapies have been tested in animal models. Olfactory ensheathing cells (OECs) are known to enhance axonal regeneration and to produce myelin after transplantation and have become a prime candidate for cell-mediated repair following a variety of CNS lesions. Some grown factors like aFGF are used to potentiate this effect. This study evaluated the effect of OEC+aFGF transplantation on spinal cord lesions in rat. Fifteen Wistar rats underwent a T8-T 10 complete spinal cord section. Sixty days post injury, nine rats were injected directly into the injury with OECs+aFGF, and 6 rats were used as controls. Functional outcome was measured using the Basso-Beattie-Bresnehan score and inclined grid test 24 hour after the treatment and up to seventy-five days after transplantation when the animals were sacrifized. All animal procedures followed the NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the National University of Colombia. Samples of spinal cord tissue were studied for ultrastructural changes. The results showed a clear and progressive functional recovery of the animals treated with OEC+aFGF, compared to controls, the BBB locomotion and inclined grid test scores of transplanted group were better than those of the non-transplanted group. Ultrastructural evaluation exhibited fewer axonal and myelin changes in OEC+aFGF transplanted rats as compared with non-trasplanted rats. There were foci of remyelinated axons that were not observed in non-trasplanted rats. These

results suggest that OEC+aFGF transplant induces axon regeneration and remyelination and functional recovery in chronic injured rats.

Disclosures: L. Botero: None. **R.M. Gomez:** None. **G. K:** None. **O. Chaparro:** None.

Poster

827. Spinal Cord Injury and Plasticity II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 827.27/LL2

Topic: D.12. Kinematics and EMG

Title: Control mechanisms underlying an involuntary movement: The effect of resistive and assistive perturbation on the Kohnstamm phenomenon

Authors: J. A. DE HAVAS[1], S. ITO[2], *H. GOMI[2], P. HAGGARD[1];
[1]Univ. Col. London, London, United Kingdom; [2]Human Info Sci. Lab., NTT Communication Sci. Labs, Kanagawa, Japan

Abstract: The Kohnstamm phenomenon (where a sustained isometric contraction produces an involuntary aftercontraction of the same muscle upon relaxation; Kohnstamm, 1915) has been studied for over a century yet the control mechanisms remain unknown. It has been argued that this involuntary movement is caused by a central adaptation interacting with a positive, load-dependent sensory feedback loop (Parkinson & McDonagh, 2006). We tested this for the first time by applying resistive and assistive perturbations via the use of a manipulandum at the shoulder joint. Participants (n=7) pushed at 70% MVC for 30 seconds and then relaxed. They experienced an aftercontraction causing a shoulder extension in the horizontal plane. After the arm had completed 20 degrees of angular displacement either a constant assistive (0.5Nm), resistive (0.5Nm), or no perturbation was applied. Electromyography (EMG) was recorded from the posterior deltoid. It was found that resistive perturbation produced a trend towards increased EMG relative to baseline. This was the case when the data was sampled in the angle and time domain. However, inter-subject variability was large, with some participant's arms being stopped by the resistive perturbation while others showed increased movement velocity. Amongst those displaying continued movement, there were individual differences in the latency of the increased EMG, indicating variability in the putative central integrators. No effect of assistive perturbation was observed. Taken together, the results suggest that the Kohnstamm phenomenon is caused by load-dependent sensory feedback modulating a central adaptation within the motor system.

Disclosures: J.A. De Havas: None. **S. Ito:** None. **H. Gomi:** None. **P. Haggard:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.01/LL3

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CCSU Faculty Research Grant

Title: Corticosterone modulation of the crayfish neuromuscular system

Authors: *M. E. JACKSON;
Biol., Central Connecticut State Univ., NEW BRITAIN, CT

Abstract: Stress is a powerful modulator of memory in most animal species. In Crayfish, the tail-flip escape response can be used to study habituation to stressful stimuli, but little is known about the effect of neuromodulators on the neurons in this circuit, including the effect of the stress hormone corticosterone. We recorded neurons innervating the superficial flexor nerve-muscle system of the crayfish abdomen. Neurons were exposed via standard dissection and pinned out in a Slygard dish and maintained in crayfish saline. Baseline extracellular recordings were made using suction electrodes, and spontaneous and stimulated action potentials were recorded. Sensory stimulation was obtained by direct manipulation of the tail fan. The normal saline was replaced with saline containing 100-300 μM corticosterone. Recordings of spontaneous and stimulated action potentials continued for one hour under corticosterone before washout by normal crayfish saline. We report that corticosterone modulated both spontaneous and stimulated activity of neurons in the crayfish neuromuscular system, with both facilitation and inhibition seen.

Disclosures: M.E. Jackson: None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.02/LL4

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant MH46742

University of Missouri Research Board

Title: Identification and characterization of neuromodulator/neurotransmitter receptor expression in the crustacean stomatogastric nervous system

Authors: *D. J. SCHULZ[1], K. M. LETT[1], E. MARDER[2];
[1]Div. of Biol. Sci., Univ. Missouri, COLUMBIA, MO; [2]Biol. Dept. and Volen Ctr., Brandeis Univ., Waltham, MA

Abstract: The crustacean stomatogastric nervous system is one of the premiere model systems for studying the physiological effects of neuromodulation on neural network dynamics. Dozens of neuromodulators and neurotransmitters have been identified that modify network parameters such as ionic conductances and synaptic strength to generate highly variable output from a small number of identified neurons. Yet very little is known about the receptor subtypes that mediate these responses. We have used a bioinformatics approach to identify putative receptor subtypes in the neural transcriptome of the crab Cancer borealis and the lobster Homarus americanus. We then examined receptor expression patterns in identified neurons of the stomatogastric ganglia (STG) of these species through single cell RT-PCR. Our bioinformatics screening has identified multiple receptor subtypes for serotonin receptors (5HTR1A, 5HTR1B, 5HTR2, and 5HTR7), dopamine receptors (DAR1 and DAR2) as well as other biogenic amine receptors for octopamine and tyramine. Expression analyses reveal cell-type specific expression patterns of these receptors across identified neurons of the STG. Our analysis also includes receptors for small molecule transmitters such as glycine, glutamate, and GABA. We have also identified a single putative muscarinic acetylcholine receptor and a family of at least 9 different nicotinic alpha subunits from both C. borealis and H. americanus. In addition, we have identified multiple types of putative receptors neuropeptides, including CCAP (crustacean cardioactive peptide), FLRFamide, RPCH (red pigment concentrating hormone), and allatostatin. Taken together, the identification and classification of neuromodulator receptor sequences in this model system will greatly complement the already well-established body of literature on the physiological effects of these compounds, allowing for a synthesis of approaches to further understand the dynamics of neuromodulation of neural networks.

Disclosures: D.J. Schulz: None. **K.M. Lett:** None. **E. Marder:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.03/LL5

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant DC012918

NIH Grant MH060358

Title: Entrainment of beta band power in behaving macaque S2

Authors: *Y. KAJIKAWA[1], S. HAEGENS[1,2], C. SCHROEDER[1,2];
[1]Nathan Kline Inst., Orangeburg, NY; [2]Psychiatry, Columbia Univ., New York City, NY

Abstract: Beta band oscillation in EEG and LFPs at around 20 Hz is observed in Basal Ganglia (BG) and sensorimotor cortical systems and is involved in sensorimotor control. Abnormal beta elevation in BG is an electrophysiological signature of Parkinson's disease. Aberrant beta patterns in sensory systems may be related to schizophrenia. This study concerns beta band oscillations associated with manual response to a target stimulus in the secondary somatosensory area (S2) of macaque monkeys. We trained macaques to perform audiovisual (AV) tasks in which monkeys pulled a lever to initiate each trial. During a trial, they monitored a repetition of AV stimuli to detect oddballs in either auditory or visual modalities. Upon detection of an oddball, monkeys responded by releasing the lever quickly to obtain reward. Sequence of stimuli were composed of 500 ms audiovisual clips interleaved with 600~1200 ms of silence. During electrophysiological recordings with linear array electrodes targeting the lower bank of the lateral sulcus (LS), we had opportunities to simultaneously record activity in the upper bank of LS, in S2. In S2, we found task-related beta band activity with spectral peaks near 20 Hz. Its local generation in S2 was indicated by laminar current source density (CSD) configurations of adjacent sinks and sources with identical time courses. Beta band power increased when monkeys pulled a lever to initiate trials, and decreased when monkeys released the lever upon detection of oddball stimuli. During series of stimuli, beta band power entrained to stimulus events: it elevated gradually towards the onset of AV clips, then decreased after the onset, and repeated the pattern as stimulus repetition proceeded. We also found that concomitant multiunit activity (MUA) amplitude was anti-correlated with the beta band envelope pattern. MUA gradually decreased before stimuli and kept decreasing until ~400 ms post stimulus onset. In association with the motor response, MUA increased while beta band was decreasing. Average reaction time of manual responses after the onset of oddball AV stimuli was about 500 ms. Our results suggested that S2 is another generator of beta band oscillations, and that the local impact is a suppression of activity. S2 activity may reflect the preparation for manual responses. Its

entrainment to the sequence of stimuli suggested its involvement in sensory expectation or cognitive control of sensory-driven motor behaviors.

Disclosures: Y. Kajikawa: None. **S. Haegens:** None. **C. Schroeder:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.04/LL6

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIMH 64711

Title: Protein synthesis is required for endogenous bursting after long term decentralization

Authors: S. EISENBACH[1], *J. P. GOLOWASCH[2];
[1]Fed Dept Biol. Sci., [2]Dept Biol. Sci., NJIT, NEWARK, NJ

Abstract: Motor rhythm-generating networks depend on neuromodulatory inputs to regulate the network activity. The pyloric network of the *Cancer borealis* stomatogastric ganglion (STG), a rhythmic motor pattern generating network, requires modulatory inputs to generate this activity. When neuromodulatory inputs are removed (which we term decentralization), the pyloric network falls silent. However, patterned pyloric activity recovers spontaneously in about 24 hours in organ culture (Golowasch et al., 1999). In the same network from the lobster *Jasus lalandii* it has been shown that transcription in STG pyloric neurons during a critical early time window (5 hours after decentralization) is crucial for the recovery of activity (Thoby-Brisson and Simmers, 2000). This critical time window suggests that early gene transcription is involved in recovery, which could possibly turn on late gene transcription or lead directly to translation of gene products, may produce proteins involved in the recovery of pyloric activity, such as ion channels. To determine if synthesis of new proteins are involved in the recovery of pyloric activity and the timing of these events after prolonged elimination of neuromodulators, we examined the effects of both translation and transcription inhibition on the recovery process of pyloric activity in *C. borealis*. In one set of *in vitro* experiments the STG was exposed to the protein synthesis inhibitor Cycloheximide (CHX). We have found that incubation in CHX during the first 4 hours after decentralization does not prevent recovery of pyloric activity, and had no effects on the rhythm of the non-decentralized pyloric network. These data show that inhibiting translation during a time window formerly identified as critical (for transcription) in *J. lalandii* (i.e. ~4 hrs after decentralization) has no effect on the recovery of pyloric activity in *C. borealis*. However, recovery of the pyloric rhythm is repressed when translation is blocked continuously

starting 4 hours after decentralization. A second set of similar experiments in *C. borealis* but using the transcription inhibitor Actinomycin D (ACD) showed recovery after decentralization to be blocked. However, we have not yet established if an early critical transcription window is required in this species. These results suggest that early transcription and late protein synthesis play an important role in the recovery process of rhythmic activity in the pyloric network of crabs. Late protein synthesis probably involves ion channels, given the well-known ionic conductance changes induced by decentralization, which appear to compensate for changes in the neuromodulatory environment.

Disclosures: S. Eisenbach: None. **J.P. Golowasch:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.05/LL7

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Wellcome Trust

Title: Modulation of the murine spinal locomotor network by astrocytes

Authors: D. ACTON, *G. B. MILES;
Sch. Psychology and Neurosci., Univ. St Andrews, St Andrews, United Kingdom

Abstract: Astrocytes have been shown to modulate neural activity via the Ca2+-dependent release of substances including glutamate, ATP and d-serine. However, few studies have demonstrated a link between such signalling and behaviour, and its importance for the operation of neural networks remains controversial. Given that the spinal locomotor central pattern generator (CPG) network controls a measurable behaviour, it may provide a tractable model system in which to demonstrate behaviourally relevant astrocyte-neuron communication. Recently we provided evidence that glial-derived adenosine acting on A1 receptors modulates the locomotor CPG in mice (Witts et al. 2012, J. Neurophysiol. 107, 1925-34). In the present study we have further investigated astrocyte-neuron signalling within the spinal CPG by using protease-activated receptor-1 (PAR1) to directly stimulate Ca2+ transients in astrocytes. PAR1 is an endogenous G protein-coupled receptor expressed predominantly by astrocytes, and activation by the specific agonist TFLLR has been shown to evoke astrocytic Ca2+ transients and gliotransmitter release. We first used immunohistochemistry to assess whether PAR1 expression

is restricted to astrocytes within the mouse lumbar spinal cord. PAR1 was found to co-localise with the astrocyte marker GFAP but not with the pan-neuronal marker MAP2, consistent with findings of astrocyte-specific expression elsewhere in the CNS. We next investigated the effects of astrocyte-neuron signalling on rhythmic locomotor-related activity recorded from isolated neonatal mouse spinal cord preparations. Application of TFLLR (10 μM) led to a rapid reduction in the frequency of pharmacologically-induced locomotor-related activity (10.6%±4.9%, n=7). Consistent with our previous observations, this effect was reduced by the nonselective adenosine receptor antagonist theophylline (10 μM, n=8) and by the A1 receptor subtype-specific antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 50 μM, n=8), but not by the A2A receptor antagonist SCH58261 (25 μM, n=8). We hypothesise that the modulation of network activity observed upon PAR1 stimulation is attributable to astrocytic Ca2+-dependent release of adenosine or of ATP with subsequent degradation to adenosine. We are continuing to investigate the signalling pathways by which astrocyte-derived substances modulate spinal motor control networks. These findings extend our understanding of the operation and particularly the modulation of the locomotor CPG, supporting a role for astrocyte-neuron signalling in the control of behaviour.

Disclosures: D. Acton: None. **G.B. Miles:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.06/LL8

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant DA024039

Title: Dopamine enabled, activity-dependent, bi-directional regulation of the hyperpolarization-activated current by protein kinase A (PKA) and calcineurin

Authors: A. R. PARKER, W.-D. C. KRENZ, *D. J. BARO;
Dept Biol, Georgia State Univ., ATLANTA, GA

Abstract: The output of pattern generating circuits can be continuously modulated, but certain features are invariant. We study the mechanisms that maintain stable activity features using a model pattern generator in the crustacean stomatogastric nervous system. The pyloric network contains 14 neurons in the spiny lobster, *Panulirus interruptus*, and drives the striated muscles

surrounding the pylorus to produce an ordered series of contractions. One cycle of contractions is continuously repeated to constantly filter foregut contents. The repetitive cycle of muscle contractions is underpinned by the recurrent output of the pattern generator. Each neuron in the pyloric network displays oscillations in membrane potential with bursts of spikes upon the depolarized plateaus. Pyloric neurons have specific activity phases, meaning that a given cell type fires a burst of action potentials at the same point in each reiteration of the cyclic network output. The lateral pyloric neuron (LP) is a component of the pyloric network that functions to slow increasing network cycle frequencies. The timing of the LP activity phase is critical for this function, and timing is regulated by the transient potassium current (I_A) and the hyperpolarization activated current (I_h). Population studies indicate that the timing of LP activity phase and the LP I_A:I_h ratio are invariant across individuals and lifetimes, suggesting compensatory mechanisms exist to maintain these features. We recently showed that tonic nM DA enables two homeostatic mechanisms that operate on distinct time scales (minutes vs. hours) to maintain the positive correlation between LP I_A and I_h. Our published work shows that the slow mechanism relies on transcription, translation and the RNA interference pathway. Our data suggested that DA controls translation of a microRNA(s) that co-regulates LP I_A and I_h. The fast mechanism can be observed when continuously blocking I_A with bath applied 4-AP in the presence 5nM DA. Reducing I_A alters several features of pyloric output including the timing of LP activity phase; however, phase can recover over ~10 minutes, despite the continued presence of 4AP, but only if 5nM DA is also present. The restorative mechanism involved DA-enabled, activity-dependent, bi-directional regulation of LP I_h: in the presence but not absence of DA, I_h varied linearly with change in burst duration such that increasing burst duration reduced I_h. Application of PKI (PKA inhibitor), FK-506 (calcineurin inhibitor) and Ht-31 (AKAP inhibitor) disrupted the homeostatic mechanism in distinct ways. We are currently testing the idea that an AKAP mediated signaling complex modulates H-channel trafficking.

Disclosures: **A.R. Parker:** None. **W.C. Krenz:** None. **D.J. Baro:** None.

Poster

828. Neuromodulation

Location: Halls A-C

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Program#/Poster#: 828.07/LL9

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH MH064711

Title: Voltage dependence of neuromodulator-activated current is regulated by g-proteins and myosin light chain kinase

Authors: *M. L. GRAY[1], J. GOLOWASCH[3,2];
[1]Ctr. Mol. Behavioral Neurosci., [2]Dept. of Biol., Rutgers Univ., Newark, NJ; [3]Dept. of Biol., New Jersey Inst. of Technol., Newark, NJ

Abstract: A voltage-dependent inward current activated by neuromodulators regulates the pyloric rhythm in *Cancer borealis.* Previous work has shown that the negatively sloped portion of the I_{MI} IV relation is crucial in enabling neuromodulators to transition neurons from non-oscillating to oscillating states. Since the negative slope is sensitive to both extracellular calcium and modulators of intracellular calmodulin signaling, it is important to understand how this signaling regulates I_{MI} voltage dependence to gain a mechanistic understanding of the transition from non-oscillating to oscillatory states in the target neurons. Previously, we have demonstrated that the calcium sensing-receptor (CaSR) antagonist NPS-2143 reduces I_{MI} voltage dependence. The human CaSR is known to have a calmodulin binding domain and to be sensitive to inhibitors of calmodulin signaling. As both I_{MI} and CaSR have been shown to be modulated by external calcium and by intracellular calmodulin signaling we hypothesize that I_{MI} voltage dependence is actively regulated by G-protein pathways, particularly CaSR, and that the previously demonstrated sensitivity of I_{MI} voltage-dependence to calmodulin inhibitors is mediated through calmodulin-dependent second messengers downstream of CaSR. To test this hypothesis, we measured I_{MI} in the presence or absence of modulators of calcium and G-protein signaling. As we have shown previously that the G-protein inhibitors Pertussis toxin and GDP-β-S block I_{MI} activation, obscuring our ability to quantify the voltage-dependence of I_{MI}, we tested the effects of the βγ-subunit inhibitor Gallein on I_{MI}. Gallein significantly reduced the voltage-dependence of I_{MI} (i.e. the slope increased and became positive) in a dose dependent manner without altering I_{MI} amplitude. This suggests that the βγ-subunit is involved in I_{MI} voltage-dependence. Proteins thought to work downstream of calmodulin and CaSR include myosin light chain kinase (MLCK), which requires activated calmodulin and has also been proposed to be a downstream messenger of CaSR. The MLCK blocker ML-7 significantly increased I_{MI} slope but also reduced I_{MI} amplitude. In order to determine if the ER may contribute to the calcium necessary to activate calmodulin, we tested the ryanodine receptor blocker Dantrolene. Dantrolene significantly increased I_{MI} slope, reduced I_{MI} amplitude, and negatively shifted the I_{MI} reversal potential. Our findings suggest a direct role for G-protein signaling in modulation of I_{MI} voltage-dependence with additional dependence on CaSR signaling through activation of the calmodulin-dependent MLCK, possibly through a novel βγ-subunit mechanism.

Disclosures: M.L. Gray: None. **J. Golowasch:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF IOS-1153417 (DMB)

Miami University Committee on Faculty Research Grant (DMB)

Title: Anatomical analysis of multiple inputs targeting a projection neuron

Authors: *S. E. SWALLIE, A. MONTI, D. M. BLITZ;
Biol., Miami Univ., Oxford, OH

Abstract: Projection neuron influences on central pattern generator (CPG) circuits enable CPGs to elicit multiple distinct output patterns (Marder 2012, *Neuron*). Projection neuron activity is itself influenced by various extrinsic inputs relaying different types of information (Rossignol et al 2006, *Physiol Review*). In some cases, multiple inputs act on the same projection neurons in distinct ways, enabling the same neurons to elicit different CPG output patterns (Blitz & Nusbaum 2008, *J Neurosci*). However, little is known about whether differences in the anatomical organization of multiple inputs converging onto the same projection neuron contribute to differences in their effects. We are using the stomatogastric nervous system of *Cancer borealis* to examine this issue. The projection neuron MCN1 originates in the commissural ganglion (CoG) and projects to the stomatogastric ganglion (STG) to activate and modulate CPG neurons (Coleman & Nusbaum 1994, *J Neurosci*). MCN1 activity is regulated by multiple extrinsic inputs which enter the CoG from different locations and differentially influence MCN1, resulting in distinct motor outputs from STG circuits (Blitz et al 2004, *J Neurosci*; Blitz et al 2008, *J Exp Biol*). This study examined the anatomical organization of inputs to MCN1, including the gastropyloric receptor neurons (GPR: proprioception), the post-oesophageal commissure neurons (POC: neuroendocrine), and the inferior ventricular neurons (IVN: chemosensory). Inputs were labeled using immunocytochemistry and nerve backfills while MCN1 was labeled via intracellular dye injection. Whole mount preparations were examined using confocal microscopy, including 3D analysis. We found that the POC axons entered near the ventral surface (dorsal to ventral = 0% to 100%; POC: 75 ± 6%, n=4) and terminated in the anterior region near the dorsal surface (35 ± 11%, n=5). GPR axons consistently entered the CoG near the ventral surface (82 ± 11%, n=7) and projected into the anterior region (n=14). GPR either entered the CoG and terminated in a compact bundle near the

ventral surface (n=2/7) or turned and projected dorsally (n=5/7). The MCN1 soma was consistently located near the CoG dorsal surface (10 ± 7%, n=4). The MCN1 arborization had a thickness of 186 ± 15 μm (n=3), which overlapped with the location of GPR axon terminals (n=3). We aim to determine whether there is segregation of these inputs to distinct regions of the MCN1 arborization which may contribute to their distinct effects, in order to further understand how multiple signals are integrated at the level of projection neurons to enable selection of appropriate outputs from rhythmic motor systems.

Disclosures: S.E. Swallie: None. **A. Monti:** None. **D.M. Blitz:** None.

Poster

828. Neuromodulation

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

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NSF IOS-1121973

Doherty Fund of Bowdoin College

Title: Differential distribution of two C-type allatostatins in the stomatogastric nervous system of the lobster Homarus americanus

Authors: *P. S. DICKINSON[1], M. C. YOUN[1], E. S. DICKINSON[1], J. R. KOHN[1], A. E. CHRISTIE[2];
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Abstract: The C-type allatostatin (AST-C) family is a group of pleiotropic neuropeptides originally described from holometabolous insects, but now known to be broadly conserved in arthropods. Two AST-Cs have been identified in the lobster *Homarus americanus*: pQIRYHQCYFNPISCF and SYWKQCAFNAVSCFamide. As in other members of the Arthropoda, these peptides are encoded by paralog genes. A long-standing question concerning the AST-Cs in both insects and crustaceans is whether or not the two isoforms are present in a common or distinct set of neurons. Here we have addressed this question by mapping the

distribution of the two peptides in the lobster stomatogastric nervous system (STNS) using anisera specific for either pQIRYHQCYFNPISCF or SYWKQCAFNAVSCFamide (specificity determined by antibody preadsorption controls). The STNS of *H. americanus* is composed of four ganglia: the paired commissural ganglia (CoGs; each ganglion containing approximately 500 somata), the single oesophageal ganglion (OG; containing ~12 somata) and the single stomatogastric ganglion (STG; containing approximately 25-30 somata), as well as a number of interconnecting and motor nerves. Within each CoG, pQIRYHQCYFNPISCF-like labeling was present in approximately eight somata. In contrast, approximately 30 somata in each CoG exhibited SYWKQCAFNAVSCFamide-like immunolabeling. No OG somata were labeled by either the pQIRYHQCYFNPISCF or SYWKQCAFNAVSCFamide antibody. Within the STG, pQIRYHQCYFNPISCF-like staining was restricted to immunoreactive neuropil that was distinctly floculent in appearance, whereas with the SYWKQCAFNAVSCFamide antibody, staining was seen in two somata and a neuropil composed of evenly dispersed, punctate profiles. While the overall patterns of pQIRYHQCYFNPISCF- and SYWKQCAFNAVSCFamide-like labeling in the STNS are clearly distinct, one common feature is the presence of somata in the CoGs. To determine if any of these cells possess both peptides, double immunolabeling was conducted; no overlap in immunoreactivity was found. Thus, in at least the lobster STNS, there appears to be no overlap in the distributions of pQIRYHQCYFNPISCF- and SYWKQCAFNAVSCFamide, suggesting that the two peptide likely serve distinct functions in this portion of the lobster nervous system.

Disclosures: P.S. Dickinson: None. **M.C. Youn:** None. **E.S. Dickinson:** None. **J.R. Kohn:** None. **A.E. Christie:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.10/LL12

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: F31 NS 080420-03

NS 17813

Title: Neuromodulation reduces variability in a pattern-generating neural network

Authors: *A. W. HAMOOD, E. MARDER;
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Abstract: Central pattern generating networks (CPGs) of neurons are able to produce rhythmic, patterned output that is highly similar across animals, but the degree to which the underlying structure of these networks vary is unclear. We assessed this question using the stomatogastric ganglion (STG) of the crab C. borealis, and in particular the pyloric rhythm, a triphasic motor pattern produced by neurons in the STG that is highly stereotyped and conserved throughout the animal's life. When studied *in vitro*, the STG is typically removed in a dissection that includes the intact stomatogastric nervous system (STNS), and anterior ganglia that provide modulatory inputs to neurons in the STG. These inputs are important for maintaining normal pyloric behavior under experimental conditions. We hypothesized that if the underlying network structures driving pyloric behavior are variable across animals, including variable contributions from modulatory inputs, then removal of these inputs should reveal variability in output. Thus, we performed long-term (days) continuous extracellular recordings of the pyloric rhythm following the removal of these inputs (decentralization), and compared them to recordings from intact preparations. We find that variability in all measured output features of the pyloric rhythm significantly increases following decentralization, and this increase does not recover during subsequent recordings, lasting up to 150 hours. This increase in variability was observed both within and between animals. We then asked whether these changes can be offset by acute application of a known modulatory peptide, proctolin, at various time points following decentralization. Interestingly, we find that the effects of proctolin on decentralized preparations are more variable immediately following decentralization than on subsequent days. While proctolin is often able to reduce the variability increase caused by decentralization, this effect is itself quite variable. Together, these results support the hypothesis that the stereotyped output observed across animals in the pyloric rhythm of the STG is maintained by networks which vary in their underlying structures.

Disclosures: A.W. Hamood: None. **E. Marder:** None.

Poster

828. Neuromodulation

Location: Halls A-C

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

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NIH grant NS45248

Title: Serotonin adjust actions of myelinated afferents via modulation of presynaptic inhibition by activation of predominantly 5-HT1 receptors in the mouse spinal cord

Authors: *D. GARCÍA-RAMIREZ[1], J. R. CALVO[2], S. HOCHMAN[3], J. N. QUEVEDO[2]; [1]1Physiology, Biophysics and Neurosci., [2]Physiology, Biophysics and Neurosci., CINVESTAV, Mexico City, Mexico; [3]Physiol., Emory Univ., Atlanta, GA

Abstract: Serotonin (5-HT) markedly depresses primary afferent depolarization (PAD) evoked by stimulation of myelinated afferents, as well as monosynaptic transmission in the *in vitro* mouse spinal cord (García-Ramírez et al., PLoS One, 2014). The present work applied subtype specific ligands to disclose the 5-HT receptors involved in these modulatory actions. Experiments were carried out on the P6-7 sagittally-hemisected mouse lumbar spinal cord with intact nerves for afferent stimulation. Stimulus strength was based on multiples of threshold (xT) of the most excitable fibers recorded from the incoming afferent volley. Peripheral nerves were stimulated at strengths that preferentially recruited myelinated afferents (<4xT). PAD was inferred from dorsal root potentials (DRPs) recorded at L3-L4 dorsal roots while monosynaptic responses were recorded in the deep dorsal horn as intraspinal extracellular field potentials (EFPs) or as intracellular excitatory postsynaptic currents (EPSCs). We tested several ligands (1-10 μM). The 5-HT1B-D agonist, zolmitriptam, depressed DRPs by 62±19% (n=8) of control. EFPs and EPSCs were depressed by 55±18% (n=8) and 70±16% (n=7) of control, respectively. The 5-HT1E-F agonist, BRL54443, similarly depressed DRPs and EFPs by 37±13% (n=10) and 25±17% (n=10) of control, respectively. In comparison, the 5-HT1A-7 agonist, 8-OH-DPAT only depressed DRPs (by 33±4% of control; n=6). Evoked DRPs and EFPs were not significantly altered by 5-HT2 (DOI) and 5-HT3 (SR572275) receptor agonists. As zolmitriptan most effectively depressed afferent synaptic transmission, we used a paired-pulse protocol to assess the modulatory role of 5-HT1B-D receptors in homosynaptic EPSCs depression. With a conditioning-test interval of 50 ms, zolmitriptam reduced the magnitude of homosynaptic depression (by 40±15% of control; n=4), supporting a presynaptic locus of action. We conclude that 5-HT depresses synaptic efficacy of myelinated afferents via 5-HT1B,D,E & F receptors, and this may explain comparably observed reductions in DRPs. In comparison, exclusive 5HT1A receptor-mediated depression of DRPs suggests a role in the modulation of PAD independent of incoming afferent pathways.

Disclosures: D. García-Ramirez: None. **J.R. Calvo:** None. **S. Hochman:** None. **J.N. Quevedo:** None.

Poster

828. Neuromodulation

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Support: NIH Grant 1P20GM103642-01A1

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Title: The role of adenosine receptors in the modulatory effects of ethanol on the mouse spinal network controlling locomotor output

Authors: J. ACEVEDO[1], A. SANTANA-ALMANSA[2], *M. E. DIAZ-RIOS[3];
[1]Anat. and Neurobio., [2]Biol., Univ. of Puerto Rico, San Juan, PR; [3]Dept. of Anat. & Neurobio., Univ. Puerto Rico, San Juan, PR

Abstract: Adenosine signaling has been implicated in the pathophysiology of many psychiatric disorders including alcoholism. Ethanol has been shown to increase the concentration of extracellular adenosine by decreasing its re-uptake by neurons and astrocytes, making adenosine available to bind to its receptor and reducing the release of other neurotransmitters such as dopamine and glutamate. Most of the studies assessing the effects of ethanol on locomotor behavior have been performed on freely behaving rodents using systemic administration that can activate multiple neural pathways which has yielded conflicting results regarding the specific mechanisms of action by which ethanol modulates the adenosine system and locomotor activity. Our proposed research focuses on the study of the cellular mechanisms by which the administration of ethanol modulates the motor output of the spinal central pattern generator (CPG) network using a neonatal isolated spinal cord preparation. We recorded extracellular motor activity profiles through ventral root recordings during a drug-induced locomotor pattern. A locomotor rhythm was obtained by adding a combination of serotonin (5-HT), N-methyl-D-Aspartate (NMDA; glutamate analog) and dopamine (all known to be necessary for eliciting locomotion in vertebrates) to the recording chamber and an alternating locomotor-like rhythm was confirmed by recording motor activity using suction electrodes on lumbar ventral roots. An acute (20 min) application of ethanol (100mM) to the superfusate significantly increased the cycle period of the motorneuron bursts, slowing down the ongoing locomotor-like rhythm reversibly in most preparations. The application of an A1 adenosine receptor antagonist (DPCPX; 1microMolar) increased the speed of the locomotor output by decreasing the cycle

period and duration of the motor bursts which was reversed to control levels when adding ethanol (100mM) in the presence of DPCPX. The application of an A2a antagonist (SCH58261; 1microMolar) did not have any significant effects on the locomotor output but the application of ethanol produced a significantly smaller effect than the application of ethanol alone. These results suggest that the depressive effects of ethanol on motor output when directly applied to the mouse spinal cord could be partially reduced by the blockade of A2a but not A1 adenosine receptors.

Disclosures: J. Acevedo: None. **M.E. Diaz-Rios:** None. **A. Santana-Almansa:** None.

Poster

828. Neuromodulation

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Program#/Poster#: 828.13/LL15

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NS08103

Title: Various modulatory substances differentially affect stable rhythmic output across temperature

Authors: *S. A. HADDAD, D. J. POWELL, E. MARDER;
Brandeis Univ., Waltham, MA

Abstract: Neuromodulators are substances that alter a neuron's intrinsic excitability and can result in multiple behavioral outputs from a single network of cells. Found in nervous systems in all levels of organisms, these substances have been extensively studied in the decapod crustacean, Cancer borealis. The triphasic pyloric rhythm, generated by a group of cells in the stomatogastric ganglion (STG) in the crab stomach, is continuously receiving modulatory input from descending anterior ganglia and the circulating hemolymph. The pyloric rhythm is responsible for contracting the muscles that filter the food. Such an essential behavior must be robust to external perturbation, for example, changes in temperature, something a marine invertebrate frequently encounters. This is non-trivial because the various cellular processes responsible for proper neuronal function have different temperature dependencies, or Q10's. What role do neuromodulators play in the robust rhythmic output of the pyloric network across a range of temperatures? Previous work has shown that two neuromodulators, oxotremorine and proctolin, stabilize the rhythm across temperature. We now study the effects serotonin on the

pyloric rhythm across temperature. Serotonin is different from the previously studied substances in that it activates multiple conductances on multiple cell types in the STG. To investigate the network response to serotonin across temperature, the stomatogastric nervous system (STNS) was subjected to three temperature ramp protocols (11°C-31°C in 4° increments), while simultaneously collecting extracelluar recording data from multiple pyloric motor nerves. In the first ramp, the STG received modulatory input from descending neurons (front end-on). For the second ramp, the descending inputs were severed (decentralized). In the third, the decentralized preparation was superfused with 10-5M serotonin. In contrast to oxotremorine and proctolin, serotonin destabilizes the pyloric rhythm across temperature, resulting in a premature network crash by 23°C in 100% of preparations. Upon returning to the preparations to lower temperatures, the rhythms were restored even when still in the presences of serotonin. We are now using the dynamic clamp to add specific modulatory currents to preparations that have 'crashed' at high temperatures. Preliminary data show that when modulatory inward current (IMI) is injected into the AB neuron, a previously crashed rhythm is restored. The same is not seen when IMI is injected into other cell types.

Disclosures: **S.A. Haddad:** None. **D.J. Powell:** None. **E. Marder:** None.

Poster

828. Neuromodulation

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NS 17813

Title: Spatial mapping of receptor distributions on single identified neurons

Authors: *A. G. OTOPALIK[1], M. R. BANGHART[2], B. L. SABATINI[2], E. MARDER[3]; [1]Biol., Brandeis Univ., Waltham, MA; [2]Neurobio., Howard Hughes Med. Institute, Harvard Med. Sch., Boston, MA; [3]Biol., Volen Ctr. for Complex Systems, Brandeis Univ., Waltham, MA

Abstract: Every nervous system is bathed in a variable cocktail of amino acids, amines, and neuropeptides. These local neurotransmitters and diffuse neuromodulators functionally reconfigure neuronal circuitry and allow for flexible, yet stable, behavioral output. To understand how the brain mediates this complex and dynamic chemical milieu, we must first understand how individual neurons coordinate the actions of many substances. Much work has described the

regulation of individual transmitters and their receptors at peripheral and central synapses. However, no work has comprehensively explored the spatial distributions of the full complement of transmitter and modulatory receptors across neuronal compartments on single neurons in their intact circuits. Here, receptor distributions are characterized on single identified neurons in the crustacean stomatogastric ganglion (STG). Different STG cell types are multiply modulated and express unique combinations of modulatory and transmitter receptors. STG neurons are morphologically complex, possessing large somata and highly branched primary neurites that span the surface of the ganglion. Given their complex morphology, the spatial distributions of various receptor types across neuronal compartments could be critical in determining the distinct activity profiles of individual STG cell types. We have developed techniques for focally photoactivating caged agonists across the surface of individual neurons in the intact STG in tandem with intracellular recordings. We have successfully designed novel caged variants of two peptides endogenous to the STG, CabTRP1a and TNRNFLRF-NH2. We have also coarsely mapped responses for the caged transmitters, MNI-Glutamate and DPNI-GABA, on single identified neurons using a custom microscope. Our study of glutamate responses indicates heterogeneous response types across neuronal compartments. There are minimal, and often no, responses to glutamate at the soma and initial segment of the primary neurite. Large responses are predominately seen in more distal neurites. These responses vary in amplitude and are phase-dependent relative to the neuron's activity. Taken together, this work sets a platform for investigation of the stability and dynamics of functional receptor distributions on different neuron types in the STG.

Disclosures: **A.G. Otopalik:** None. **M.R. Banghart:** None. **E. Marder:** None. **B.L. Sabatini:** None.

Poster

828. Neuromodulation

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIMH 64711

Title: Activity-dependent regulation of intrinsic properties of CPG neurons by an inhibitory peptide

Authors: *D. SALLOUM, J. GOLOWASCH;
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Abstract: Neuromodulators regulate activity of neurons within rhythmic networks by altering their firing patterns via direct regulation of ionic currents. Swensen & Marder (2000) demonstrated that multiple peptides known to modulate the pyloric rhythm all converge onto one modulator-activated inward current, I_{MI}. However, the possibility exists that neuromodulators may exert more subtle effects such as gating activity-dependent effects on voltage-gated ionic currents. Our hypothesis is that neuromodulators and activity interact to regulate the ionic currents of pyloric neurons. For the purpose of this presentation we will focus on the effects of allatostatin III (AST), the only peptidergic inhibitory modulator of the pyloric rhythm. Thus far, we have tested how ionic current levels change in response to depolarizing stimulations in the presence and absence of AST in pyloric dilator (PD) neurons under voltage-clamp control. The pyloric frequency is driven by a single anterior burster neuron coupled to two PD neurons, which make up the pacemaker kernel of this network. For this reason, we chose to test our hypothesis in this neuron type, also taking advantage of the fact that each ganglion has two PD neurons. We use one PD neuron for control measurements and the other to measure the effects of stimulation under conditions where both synaptic and neuromodulatory input are removed. Our experiments examine two potassium currents, I_A and I_{HTK}. In the absence of any neuromodulators activity (depolarizing 1Hz pulses) does not affect either of these currents. I_{HTK} did not show any activity-dependent changes but rather decreased significantly whether cells were stimulated or held at -60mV over two hours. I_A showed a spontaneous increase (not significant) in both stimulated and un-stimulated neurons. In the presence of AST, I_A increases significantly (~150%) over 2 hours in the absence of depolarizing activity (control), while it remains at control levels in cells that are stimulated. On the other hand, AST enhances the decrease in I_{HTK} in stimulated neurons. These findings suggest that the effects of AST on these ionic currents depend on the activity levels of the neurons. However, the effects are not consistent with a reduction of ongoing pyloric activity by AST as reported before. Thus, we examined the acute effects of AST on other ionic currents that may mediate this inhibitory effect on the pyloric rhythm. We found that AST increases the non-voltage gated leak current (I_{Leak}) in PD neurons after 20 minutes of application, which is consistent with the inhibitory effect of AST on the pyloric rhythm. This is the first reported modulation of a leak current in this system.

Disclosures: D. Salloum: None. **J. Golowasch:** None.

Poster

828. Neuromodulation

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Title: CSF-contacting neurons modulate slow locomotion and intersegmental coordination

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Abstract: Cerebrospinal Fluid-contacting Neurons (CSF-cNs) are GABAergic neurons located around the central canal, in the ventro-lateral part of the spinal cord. Spinal CSF-cNs have been identified in over 200 species (Agduhr, 1922; Kolmer, 1931; Vigh and Vigh-Teichmann, 1973; Djenoune et al., 2014) but their function in the spinal cord remains to be elucidated. Recent work in our lab showed that their activation using light-gated channels could lead to rebound swimming in larval zebrafish, suggesting that CSF-cNs are able to modulate the excitability of intrinsic spinal networks involved in locomotion (Wyart et al., 2009). In order to dissect how CSF-cNs regulate the activity of spinal neurons underlying locomotion, we developed an original approach, combining Channelrhodopsin-mediated activation of CSF-cNs with ventral nerve root recordings and single cell electrophysiology. We show that activation of CSF-cNs during on-going fictive slow locomotion leads to silencing of motor activity within 100 ms on average. We demonstrate that this effect is partially mediated by the activation of GABA-A receptors. In addition, we show that local activation of CSF-cNs prevents the propagation of activity along the rostro-caudal axis of the spinal cord. Anatomical and electrophysiological evidence indicates that CSF-cNs project onto spinal neurons involved in the control of slow locomotion and intersegmental coordination. Altogether, our work reveals mechanisms through which CSF-cNs tune the activity of spinal circuits controlling locomotion and uncover part of CSF-cNs connectivity.

Disclosures: K. Fidelin: None. **J. Hubbard:** None. **C. Stokes:** None. **A. Baradel:** None. **F. Del Bene:** None. **C. Wyart:** None.

Poster

828. Neuromodulation

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: BB/JO1446X/1

Title: The development and cellular mechanisms underlying dopaminergic modulation of spinal motor networks in Xenopus tadpoles

Authors: *L. D. PICTON, K. T. SILLAR;
Sch. of Psychology and Neurosci., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Dopamine (DA) is an important neuromodulator of spinal motor networks, but compared to other amines, little is known about the effects of DA on the development and modulation of amphibian swim networks. Recently, DA was shown to have opposing, dose-dependent effects on spontaneous fictive locomotion in free-swimming larval (stage 50-55) Xenopus tadpoles (Clemens et al., 2012 J Neurophysiol 107(8): 2250-9). Low DA (2μM) activates high affinity D2-like receptors to reduce spontaneous swim episodes, whereas higher concentrations (50μM) activate low affinity D1-like receptors to increase spontaneous swimming. However, neither the development of the DA system, its effects on evoked swim parameters, nor the mechanisms underlying this modulation have been explored. Here, we examined the effects of DA on evoked fictive swimming in late embryonic (stage 37/8) and early larval (stage 42) Xenopus tadpoles. At these early developmental stages, DA applied at all concentrations tested (0.5-100μM) caused a decrease in evoked episode duration and swim frequency, and an increase in the skin stimulus threshold for swimming (N=18). These purely inhibitory effects were mimicked by an agonist for the D2-like receptors (25μM quinpirole, N=3) whereas a D2-like antagonist had the reverse effects (25μM raclopride, N=3). Interestingly, blockade of D2-like receptors in stage 42, but not stage 37/8, also induced a spontaneous swimming rhythm, similar to the effects in zebrafish (Thirumalai & Cline (2008) J Neurophysiol 100(3): 1635-48), suggesting that in early larval development spontaneous swimming is suppressed by tonic D2-mediated inhibition. Whole-cell patch clamp recordings revealed that activation of D2-like receptors using DA (N=4) or quinpirole (N=7) hyperpolarized spinal neurons and decreased their input resistance, suggesting the opening of a K+ channel. We report

evidence that this is a G-protein coupled inward rectifying K+ (GIRK) channel, a common target of D2-like receptors (Neve et al (2004) J Recept Signal Transduct Res, 24(3): 165-205). The hyperpolarisation was occluded by pre-application of barium chloride (BaCl, a blocker of IR K+ channels, N=3), but not by inhibitors of other K+ channel families (TEA; glybenclamide; quinine, N=6). Therefore, early in Xenopus development, DA exerts a purely inhibitory modulation of fictive locomotion through the D2-receptor mediated opening of a K+ channel, probably a GIRK channel. During development, the introduction of excitatory D1-like receptors may then mediate the switch from a purely inhibitory influence of DA to the more complex bidirectional modulatory control observed at later free-swimming larval stages.

Disclosures: L.D. Picton: None. **K.T. Sillar:** None.

Poster

828. Neuromodulation

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APS Undergraduate Summer Research Fellowship

Paller Fund of Bowdoin College

Title: Does the ganglionic sheath serve as a passive regulator of neuromodulation in the stomatogastric nervous system of the American lobster, Homarus americanus?

Authors: *L. J. KELLER[1], R. J. DENNISON[1], A. E. CHRISTIE[2], P. S. DICKINSON[1]; [1]Bowdoin Col., Brunswick, ME; [2]Univ. of Hawaii at Manoa, Honolulu, HI

Abstract: Rhythmic movements, which are controlled by hard-wired neuronal networks, must be flexible in order to respond to environmental changes. Neuromodulators, which can either be released locally within the nervous system or hormonally from endocrine-secreting organs, provide one source of this flexibility. The pyloric motor pattern, which is generated in the lobster stomatogastric ganglion (STG) and controls the rhythmic contraction of striated muscles in the lobster foregut, is one system extensively modulated by neuropeptides. However, the STG is

encased in a connective tissue sheath, which might limit access to the ganglion, altering the extent to which hormonally-released neuromodulators can modulate the STG output. To determine whether this sheath can serve as a passive but selective barrier to neuromodulation, the pyloric pattern was recorded extracellularly from motor nerves in the isolated stomatogastric nervous system. Neuropeptides were superfused over the STG with the sheath intact and after it was removed; the threshold concentration at which each peptide modulated the motor pattern was compared between the sheathed and desheathed conditions. The modulatory threshold for the hydrophobic neuropeptide red pigment concentrating hormone (RPCH; pQLNFSPGWamide) was 10^{-9}M both with and without the sheath present. In contrast, the threshold for Val^{1}-SIFamide (VYRKPPFNGSIFamide), a hydrophilic peptide localized only to the STG, was significantly higher when the sheath was present (10^{-6}M) than after it was removed ($<10^{-7}$M). Because the thresholds for RPCH were similar with and without the sheath, this peptide could serve as an effective modulator when released either hormonally or locally. In contrast, Val^{1}-SIFamide would be less effective if released hormonally than when released locally. Interestingly, crustacean cardioactive peptide (CCAP; PFCNAFTGCamide) did not alter the pyloric pattern with or without the sheath present at hormonal concentrations (10^{-8}M), although it did modulate pyloric activity at higher concentrations (10^{-7} -10^{-6}M) even in the presence of the sheath. CCAP thus appears to have biochemical characteristics that allow it to permeate the connective tissue sheath at least at high concentrations. However, because it is localized only to neuroendocrine organs, it is likely that this peptide rarely exerts modulatory effects on the STG *in vivo*, as it would reach the ganglion only at low, hormonal concentrations. These results suggest that the connective tissue sheath encasing the STG is selectively permeable to neuromodulators and thus able to passively control hormonal neuromodulation.

Disclosures: **L.J. Keller:** None. **R.J. Dennison:** None. **P.S. Dickinson:** None. **A.E. Christie:** None.

Poster

828. Neuromodulation

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH NS083319

Title: Where temporal coding matters: the role of short-term plasticity of a neuromuscular junction in shaping postsynaptic responses

Authors: *Y. ZHANG[1,2], N. DAUR[2], D. BUCHER[2], F. NADIM[2,3];
[1]Mathematical Sci., New Jersey Inst. of Technoloy, Newark, NJ; [2]Biol. Sci., New Jersey Inst. of Technol., Newark, NJ; [3]Biol. Sci., Rutgers Univ., Newark, NJ

Abstract: Short-term plasticity (STP) allows synapses to dynamically change their strength as a function of the presynaptic activity pattern. We used theoretical approaches to investigate how STP shapes the synaptic response to bursting input and explore how changes in spike pattern modify synaptic output. This work was inspired by experimental data obtained from a crustacean neuromuscular junction (NMJ). The pyloric dilator (PD) neurons of the lobster, Homarus americanus, produce bursting activity (burst freq ~1Hz, ~19 spikes/burst) and innervate two muscles, a fast and a slow one. The PD axons are several cm long and exhibit history-dependent properties that change the temporal pattern of spikes within the bursts during propagation. The extent to which the burst pattern is affected is sensitive to dopamine (DA). Changes in burst patterns have a significant effect on the synaptic responses of both muscles. DA also has the ability to generate ectopic "extraburst" spikes in the PD axon which have a priming effect on the NMJ, particularly in the slow muscle. It is unclear whether the effects of burst pattern changes and priming are simply due to the STP properties of the synapse or whether active properties in the muscle also play a role. To address this question, we used a decoding technique to characterize the STP rules of the synapse. Briefly, the decoding results in a first order kernel K1, which is the isolated NMJ, and a second order kernel K2, which captures the facilitation and depression effects. We found that the responses and the sensitivity to pattern changes of the fast, but not the slow muscle can be described by STP rules alone. We examined the sensitivity of the response amplitude to changes in the burst duration (BD) or intra-burst spike frequency (SF). In the absence of STP, only SF had an effect, because synaptic summation saturated the amplitude after the first few spikes. With STP, either BD or SF increased the response, but SF had a stronger effect than BD because of the sensitivity of STP rules to inter-spike intervals. When BD and SF were changed in opposite directions, there was little change in response amplitude. We also found that depression was less important than facilitation in determining the sensitivity of the fast muscle NMJ to changes in the burst pattern. Finally, priming by ectopic spikes greatly increased the response amplitude and this effect was larger when the priming spikes were closer to the burst onset. The priming effect was countered by a decrease in BD. We generalize these predictions by characterizing the parameters of depression and facilitation in K2 to provide a set of rules that determine the synaptic response to any presynaptic burst pattern.

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Poster

828. Neuromodulation

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Support: Schlumberger Inc.

Title: Regulation of gene expression by the neuromodulator Proctolin

Authors: *L. LEWIS[1], C. BRAUN[3], S. KALUZIAK[2], S. VOLLMER[2], J. AYERS[2];
[1]Biol. Sci. and Marine Sci. Ctr., [2]Marine Sci. Ctr., Northeastern Univ., Nahant, MA; [3]Dept. of Cell Biol., Harvard Med. Sch., Boston, MA

Abstract: Neuromodulators act as extrinsic inputs to synaptic networks, altering cellular and synaptic permeabilities and consequently modifying the overall dynamics and connectome of the circuit. The role of neuromodulators acting over longer time courses in regulating gene expression is less well understood. The focus of this study is the pentapeptide proctolin, a neuromodulator that acts on the central pattern generating stomatogastric nervous system (STNS) in crustaceans. Previous studies have shown proctolin initiates gastric mill activity *in vivo* and increases the frequency of gastric mill and pyloric rhythms *in vitro*. Proctolin has been identified in the neurosecretory pericardial organ, the stomatogastric ganglion, and the commissural and abdominal ganglia in decapod crustaceans. This study uses quantitative mass spectrometry-based proteomic analysis to determine endogenous proctolin levels in the hemolymph of the American lobster, *Homarus americanus*. Our data reveal proctolin is present at 10^{-11} M concentrations, and show that these levels spike in the 10-minute period following a feeding event. This work also identifies longterm effects of proctolin on transcriptome-wide gene expression changes in *Homarus americanus*. We introduced exogenous proctolin at levels known to induce activity changes to the STNS (10^{-6}M) through once daily injections over a three-day period. We quantified changes to mRNA transcript type and abundance in muscle, neurosecretory, and nervous system tissues using next generation RNA sequencing and bioinformatic analysis. Compared to saline-injected controls, our results indicate the differential expression of 124 RNA transcripts. Of these transcripts, 25% annotate to known protein-coding genes. Furthermore, our results indicate that 20% of the protein-coding genes differentially expressed by proctolin treatments are differentially expressed in nervous system tissues relative to muscle tissues. Overall this study informs our understanding of the role of proctolin as a neuromodulator, and the transcriptional effects of neuromodulators on the nervous system.

Disclosures: **L. Lewis:** None. **C. Braun:** None. **S. Kaluziak:** None. **S. Vollmer:** None. **J. Ayers:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.21/LL23

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS083319

Title: High sensitivity of postsynaptic responses to neuromodulator-induced changes in presynaptic spike propagation

Authors: ***N. DAUR**, F. NADIM, D. BUCHER;
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Abstract: The axon of the pyloric dilator (PD) motor neuron in the stomatogastric nervous system of the lobster, Homarus americanus, exhibits history-dependent changes in spike propagation delay. This effect is highly sensitive to dopamine (DA) modulation of the h current (Ih). Different levels of Ih activation result in different dynamics of propagation and ultimately in different inter-spike interval (ISI) structures of bursts in the distal axon. In addition, strong activation of Ih by DA can lead to distal ectopic spike initiation in between centrally generated bursts, especially at slow cycle periods. We investigated the effect of these changes in timing on the postsynaptic responses of two target muscles of PD, cpv2a and cpv2b. Muscle fiber recordings during spontaneous PD bursting activity showed mostly graded responses with substantially faster response properties in cpv2a than cpv2b. Our initial prediction was that the slower muscle would exhibit stronger low pass filtering and therefore be less sensitive to pattern changes. However, when we stimulated the distal nerve with patterns that mimicked the different intraburst ISI structures resulting from different axonal Ih activation, the slow muscle showed substantial changes in compound EJPs in response to pattern changes. In contrast, compound EJP amplitude and integral in the fast muscle was insensitive to these pattern changes. Interestingly, application of DA directly onto the slow muscle significantly decreased its sensitivity to pattern changes. Because the changes in patterns due to axonal dynamics mainly affect initial ISIs in the burst, we tested the effect of changing the first ISI by separating the first spike from the rest (19 spikes) of the burst, which were kept at constant ISIs. The slow muscle response amplitude increased with increasing the first ISI over tens of msecs and then decreased

with intervals increasing over several hundred msecs. However, even when the first spike occurred >500ms before the rest of the burst, it increased the response amplitude compared to the 19-spike burst alone. This is a priming effect that could be relevant when DA elicits ectopic spikes during the interburst intervals. We also tested the responses of both muscles to burst patterns that were interspersed with two spikes during the interburst interval. Even though these spikes preceded the bursts by enough time to not contribute directly to the compound EJPs, they lead to an increase in responses to the following burst in both muscles. These results indicate that the subtle changes in the intraburst ISI structure due to the propagation dynamics of the presynaptic axon can have drastic effects on postsynaptic responses.

Disclosures: **N. Daur:** None. **F. Nadim:** None. **D. Bucher:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.22/LL24

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF IOS-1153417 (DMB)

Title: Projection neuron activity pattern and rate determines CPG output

Authors: ***R. M. SPENCER**, D. M. BLITZ;
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Abstract: Projection neuron inputs to central pattern generator (CPG) circuits underlying rhythmic behaviors select distinct outputs from a CPG by modulating circuit neuron cellular and synaptic properties. These projection neurons can be activated with different patterns and rates by different extrinsic inputs and distinct regulation of CPG feedback (Wood et al. 2004, JNeurosci; Blitz & Nusbaum 2008, JNeurosci; Rossignol et al 2006, Physiol Rev). However, the relationship between projection neuron activity pattern and rate and the resulting CPG output has not been fully explored. We are investigating this issue in the stomatogastric nervous system of the crab, *Cancer borealis*. The identified projection neuron MCN1 activates and modulates the gastric mill (network-driven)- and pyloric (pacemaker-driven) CPGs. Depending on mode of activation, MCN1 firing rate varies from 5-30 Hz *in vitro* and *in vivo* (Blitz et al 2004, J Neurosci; Hedrich et al 2011, J Neurophysiol). Different inputs and CPG feedback states also elicit distinct MCN1 activity patterns (Blitz et al 2008, J Exp Biol; Blitz & Nusbaum 2012, J Neurosci). Some CPG output parameters are sensitive to the MCN1 activity pattern and rate (Wood et al 2004, J Neurosci; Bartos et al 1999, J Neurosci). However, a full analysis of the

sensitivity of the gastric mill and pyloric CPGs across the physiological MCN1 activity range has not been performed. By stimulating MCN1 with different rates (5-30 Hz) and patterns (tonic and time-locked to the pyloric rhythm) we investigated the sensitivity of the gastric mill and pyloric rhythms to MCN1 activity. We found that MCN1 pattern and rate regulated the output of both CPGs. For example, the cycle period of both CPGs was negatively correlated with the rate of pyloric-timed MCN1 activation (gastric mill: $p<0.05$, n=4-14; pyloric: $p<0.01$, n=3-16). Whereas other motor pattern parameters were distinctly regulated. For instance, tonic stimulation elicited a shorter pyloric neuron burst duration ($p<0.05$, n=12), but a longer gastric mill burst duration ($p<0.01$, n=11) than pyloric-timed stimulations. Also, with tonic stimulation, pyloric rhythm activation occurred from 5 Hz (n=2/5) through 30 Hz (n=5/5), while full gastric mill rhythm activation required a minimum of 10 Hz (n=11/15) and did not occur in all preparations at higher frequencies (e.g. 30 Hz, n=5/15). These data support the hypothesis that neural circuit output depends on projection neuron activity pattern and rate, and that different CPGs can be differentially sensitive to the same projection neuron. Thus, projection neuron activity is an important control locus for enabling appropriate output from flexible CPG circuits.

Disclosures: R.M. Spencer: None. **D.M. Blitz:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.23/LL25

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSERC

CIHR

Title: A new method for detection of spontaneous rhythmic activity from ventral roots of the neonatal mouse isolated spinal cord

Authors: *S. A. SHARPLES[1,3], N. OSACHOFF[4], P. J. WHELAN[2,3];
[1]Neurosci., [2]Univ. of Calgary, Calgary, AB, Canada; [3]Hotchkiss Brain Inst., Calgary, AB, Canada; [4]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Spontaneous activity, which is widespread in the developing nervous system, contributes to the maturation of spinal circuits and axonal pathfinding. This activity is rhythmic

and regular during embryonic development and shifts to more irregular activity as intracellular chloride concentrations decrease perinatally in mice. Isolated mouse spinal cord preparations are commonly utilized to probe spinal circuits that control sensory and motor function in mammals. Such perinatal preparations can be exposed to various centrally-active drugs or electrically stimulated to evoke patterns of coordinated fictive motor activity. In the absence of electrical or neurochemical stimulation, neonatal lumbar spinal cord networks produce a rich variety of stochastic spontaneous patterns of motor activity. Compared to rhythmic episodic activity, it has been a challenge to quantify these spontaneous patterns. Here we present a custom designed MATLAB approach to quantify the nature of spontaneous ventral root activity to explore the complexity and diversity of the patterns produced. Specifically, we were interested in devising an analysis method aimed toward detecting complex spontaneous episodes that produce rhythmic bouts of activity. As a first step to evaluate this approach we manipulated basal levels of activity by manipulating the extracellular concentration of potassium. Analysis of this activity revealed that higher levels of activity induced by increasing bath potassium concentration was associated with increased number of larger amplitude and longer duration episodes that could be collapsed to a set of standard activity scores. Furthermore, we also found a larger number of complex episodes with multiple events or rhythmic bursting locomotor-like activity superimposed on top of these episodes. These rhythmic events could then be recognized and tabulated. We will be using a principle components analysis approach to further collapse the parameters within the data set.

Disclosures: **S.A. Sharples:** None. **N. Osachoff:** None. **P.J. Whelan:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.24/LL26

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R37-NS029436

Title: Distinct microcircuit response to equivalent input from a full vs. partial projection neuron population

Authors: G. F. COLTON, *M. P. NUSBAUM;
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Abstract: We aim to determine if microcircuits are differentially responsive to equivalent input from a fully- vs. partially activated projection neuron population. To this end we are using the crab (*Cancer borealis*) stomatogastric nervous system, which contains the functionally equivalent, paired projection neurons MCN1 whose activity drives the gastric mill (chewing) central pattern generator (CPG). Tonic stimulation of one or both MCN1s drives the gastric mill rhythm (Bartos et al, J Neurosci 1999). One MCN1 soma is located in each commissural ganglion, from where they project an axon that arborizes in the stomatogastric ganglion to enable activation of the gastric mill CPG. We are comparing the gastric mill circuit response to stimulating either MCN1 alone at a given firing rate (10 Hz; 20 Hz; 30 Hz) vs. costimulating both MCN1s in an alternating spike-by-spike fashion with the same cumulative firing rate (5+5 Hz; 10+10 Hz; 15+15 Hz) as the single MCN1 stimulations. Each of these MCN1 firing rate comparisons revealed distinct gastric mill rhythms during the single vs. co-MCN1 stimulation protocols. For example, for all rhythm parameters analyzed (One-way RM-ANOVA), the 20 Hz single MCN1 stimulations ($MCN1_{Right}$: n=10; $MCN1_{Left}$: n=9) were equivalent ($p>0.05$), but each was distinct from the combined 10 +10 Hz $MCN1_{L+R}$ stimulations (n=10). Specifically, the combined 10 Hz stimulations increased the cycle period by 31% ($MCN1_R$: 9.8 ± 2.8 s, $MCN1_L$: 9.5 ± 3.2 s, Combined: 12.6 ± 3.0 s, $p<0.001$), protraction phase duration by 24% ($MCN1_R$: 6.9 ± 2.3 s, $MCN1_L$: 6.7 ± 2.7 s, Combined: 8.4 ± 2.5 s, $p=0.003$), retraction phase duration by 41% ($MCN1_R$: 2.9 ± 1.5 s, $MCN1_L$: 2.9 ± 1.6 s, Combined: 4.1 ± 0.8 s, $p=0.022$), and the number of spikes/burst in the protractor CPG neuron LG by 40% ($MCN1_R$: 34.6 ± 10.3, $MCN1_L$: 34.1 ± 10.8, Combined: 48.2 ± 14.3, $p=0.005$). We next aim to determine whether cycle-by-cycle regularity of the gastric mill rhythm is also distinct during single vs. dual MCN1 stimulations. We will also test the hypothesis that there are distinct gastric mill rhythms elicited by single- vs. co-MCN1 stimulation at the same overall firing rate because the LG neuron more effectively inhibits the coactivated MCN1s due to their firing rates being half that of the singly activated MCN1. LG presynaptic inhibition of MCN1 is pivotal for gastric mill rhythm generation (Coleman et al, Nature 1995). Thus, the microcircuit response to its full complement of a projection neuron population is not necessarily equivalent to stronger activation of a subset of those inputs, an approach that might be considered in order to compensate for partial loss of an input population.

Disclosures: G.F. Colton: None. **M.P. Nusbaum:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.25/LL27

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NS066587

NS070583

MH051393

Title: Cellular basis for repetition priming and task-switch cost in an *Aplysia* CPG

Authors: *M. H. PERKINS, E. C. CROPPER, K. R. WEISS;
Friedman Brain Inst., The Icahn Sch. of Med. At Mt. Sinai, New York, NY

Abstract: Repetition priming is a process that improves the quality of repeatedly elicited responses. Since the effects of priming persist, they often give rise to a task switch cost, i.e., a reduction in performance when a new behavior is triggered. Given that organisms are often exposed to changing stimuli, we ask how priming and task switch costs interact. We study these problems in the experimentally advantageous feeding CPG in *Aplysia*. This CPG generates motor programs in response to stimulation of two inputs, CBI-2 and EN. In a rested state, stimulation of CBI-2 or EN elicits intermediate motor programs, that are neither ingestive or egestive. With repetitive stimulation, however, program definition occurs, i.e., with CBI-2 it becomes ingestive, and with EN egestive. Also, a task switch cost is observed when there is an immediate switch between egestive and ingestive activity, i.e., a cycle of activity is induced by CBI-2 immediately after EN induced egestive priming. In this situation motor programs are egestive rather than ingestive. We analyze the cellular basis of repetition priming and the egestive-ingestive task switch cost in motoneuron B48 as this neuron's activity reflects both of these phenomena. Namely, B48's firing frequency is increased during ingestive priming. During egestive-ingestive task switches, its firing frequency is relatively low - a reflection of the switch cost. Previous work suggested that repetition priming of B48 activity depends on modulation of its excitability rather than a change in synaptic inputs. To determine whether this is the case, we monitored B48 under voltage clamp conditions during ingestive priming. Phasic inputs that B48 received during motor programs were not altered. However, the average holding current was significantly modulated. This is consistent with the hypothesis that synaptic input is not changed, but that B48's excitability is regulated by CBI-2-induced modulation of intrinsic conductances. To further test this possibility we characterized inward currents induced by CBI-2 stimulation, and used dynamic clamp to 'add' these currents to B48 neurons in rested preparations. This produced increases in B48 firing frequency during motor programs that were similar to those induced by ingestive priming. The opposite was observed when currents were 'subtracted' after the induction of priming. Finally, when EN elicited conductances were injected into CBI-2 primed neurons, they reduced B48's firing to the level observed following EN stimulation.

Taken together, our data suggest that at the subcellular level, repetition priming and task switch costs are mediated by separate but additive processes.

Disclosures: **M.H. Perkins:** None. **E.C. Cropper:** None. **K.R. Weiss:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.26/LL28

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: School of Computing Science Scholarship, Newcastle University

Title: Computational modelling and analysis of the impact of dopamine on the crustacean pyloric rhythm circuit

Authors: ***J. S. STEYN**[1,2], T. ALDERSON[1], P. ANDRAS[1,2,3];
[1]Sch. of Computing Sci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Inst. of Neurosci., Newcastle upon Tyne, United Kingdom; [3]Sch. of Computing and Mathematics, Keele Univ., Keele, United Kingdom

Abstract: Neuromodulation allows nervous systems to be flexible but remain within consistent bounds without becoming pathological. For this reason, deciphering the mechanism of neuromodulatory control is vital for our understanding of neural circuit operations in general. The activity of one such circuit, the pyloric central pattern generator, found for example in the stomatogastric ganglion (STG) of the brown crab, is heavily influenced by the action of neuromodulators which are known to differentially adjust the intrinsic cellular (ionic currents) and synaptic properties of each neuron. To date, experimentalists have accumulated a considerable body of evidence detailing dopamine's effect on each kinetic parameter of the pyloric circuit but lack a comprehensive architecture for studying these effects in combination. One approach is to embody the experimental data in a detailed computational model allowing the link between low level cellular and synaptic changes and higher level systems dynamics to be established. This study uses one such model to investigate the reported changes in phase, frequency and synchronisation when high concentration dopamine (DA) is applied to the STG. We found that the LP and PY neurons experienced a phase advance relative to anterior burster neuron (AB) while the pyloric dilator neurons (PDs) showed a phase delay. DA also decreased the frequency of the circuit with early lateral pyloric neuron (LP) synaptic inhibition onto PD

decreasing the frequency and late inhibition enhancing it. DA also shifted LP synaptic inhibition into a region of the phase response curve (PD's refractory period) where it had less effect on the frequency of the circuit. These results are consistent with the experimentally observed changes in the activity of these neurons. The rhythm frequency was found to be a function of the strength of the PD to AB electrical connection with stronger connections promoting lower frequencies. The PD to AB connection is known to be moderately enhanced by dopamine and provides a possible explanation for the observed decline in circuit frequency. DA is also known to decrease synchrony between pyloric constrictor neurons (PYs) by disrupting their electrical coupling. The model circuit's spike and plateaux characteristics were found to be a direct function of PY electrical coupling and were comparable to the experimental results obtained through optical recording of the STG.

Disclosures: J.S. Steyn: None. **T. Alderson:** None. **P. Andras:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.27/MM1

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG STE 937/9-1

Title: Neuromodulator-induced rescue of temperature-elicited motor pattern breakdown

Authors: *C. STAEDELE[1,2], W. STEIN[1];
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Abstract: In most poikilotherms, body temperature follows the ambient temperature as they cannot actively maintain their body temperature. Yet, the nervous system continues to function reliably, despite the fact that various subcellular neural components show temperature-dependent dynamics. How neural systems cope with such changes is often unclear. In the crustacean stomatogastric nervous system, the motor circuits producing the pyloric (filtering) and gastric mill (chewing) rhythms are located within the same ganglion and they partly share the same neurons, but show very different responses to temperature changes. While the pyloric pattern remains functional and neuronal phase relationships are stable over a broad temperature range (5 to 25°C; *in vitro*: Tang et al. 2010, PLoS Biol 8.8; *in vivo*: Soofi et al. 2014, J Neurophys, in press), the gastric mill rhythm undergoes dramatic changes in phasing and stability. Our data

shows that a modest increase from 10 to 13°C caused significant changes in the phasing of the gastric mill neurons, a decrease in cycle period and in some animals a termination of the rhythm. In the gastric mill central pattern generating neuron LG (lateral gastric), increasing temperature hyperpolarized the membrane potential, diminished action potential amplitude and increased the latency to the first action potential. These changes were accompanied by an increase in a linear (leak) membrane conductance. A dynamic clamp-induced rescue of the LG input resistance using negative linear leak restored the temperature-induced changes in resting membrane potential and spike amplitude, indicating that counterbalancing membrane conductance may help to restore the rhythm. *In vivo*, gastric mill rhythms also occurred at temperatures beyond 13°C and temperature-induced changes of the pyloric rhythm are smaller than *in vitro* (Soofi et al. 2014, J Neurophys, in press), possibly by the actions of neuromodulators. A large subset of neuromodulators in this system is known to activate a single voltage-gated inward current I(MI), which regulates the rhythmic activity of both networks. A crucial component of I(MI) for generating oscillatory activity is a close-to-linear portion of the current-voltage relationship, which causes this conductance to effectively regulate the amount of leak current and cell input resistance (Zhao et al. 2010, Front Behav Neurosci 4). We are currently testing whether neuromodulator release, which induces I(MI), increases with temperature and whether this increase is sufficient to counterbalance the temperature-induced increase in leak conductance and hence to restore the gastric mill motor pattern.

Disclosures: C. Staedele: None. **W. Stein:** None.

Poster

828. Neuromodulation

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 828.28/MM2

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG STE 937/9-1

NIH R01DK071801

Title: Electrophysiological and mass spectral investigation of long-term *in vivo* motor activity and neuropeptide release in the crab cancer borealis

Authors: *A. M. YARGER[1], Z. LIANG[2], L. LI[3,2], W. STEIN[1];
[1]Sch. of biological sciences, Illinois State Univ., Normal, IL; [2]Sch. of Pharm., [3]Dept. of Chem., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Motor circuits must be flexible to adapt to changing internal and environmental conditions, yet stable enough to maintain function. Central pattern generators (CPGs) reliably maintain their activity patterns, and are thus ideal for studying mechanisms of stability and flexibility. The crab stomatogastric nervous system contains the well-characterized gastric mill (chewing) and pyloric (filtering of food) CPGs. *In vitro*, the pyloric rhythm is stereotyped with little variation over time. Temperature, inter-circuit interactions and neuromodulation can influence the rhythm, but most variation occurs between individuals, not within (Tang *et al.* 2012, J Neurosci 32; Bucher *et al* 2005, J Neurosci 25). *In vivo*, the range of variation of the rhythm and the extent of external and internal influences are unknown, with few exceptions (Hedrich *et al*., 2011, J Neurophys 105; Soofi *et al*., 2014, J Neurophys, in press). Using long-term recordings at constant temperature we identified the range of pyloric and gastric mill frequencies *in vivo*. There was no obvious circadian modulation of either rhythm, although light-driven changes in neuromodulators have been implicated to affect the gastric mill rhythm (Fleischer 1981, J Comp Physiol 141). Both rhythms' frequencies oscillated continuously throughout the day, interrupted by large non-rhythmic fluctuations. The gastric mill CPG neuron LG (lateral gastric) was active in 97.5% of all 30 min. data files (n=323) and pyloric frequency generally increased during these periods. Similar to *in vitro*, pyloric frequency was lower during LG bursts than during the interburst. This caused substantial variation in pyloric frequency (CV=0.22; N=4; 13 days total) but also indicated that neuromodulators that drive these rhythms *in vitro* (Nusbaum & Beenhakker 2002, Nature 417) may be present *in vivo*. To test the impact of neuromodulation, we performed mass spectral analysis of neuropeptides in the hemolymph via *in vivo* microdialysis. Initial analyses show daily patterns of release of neuropeptides known to influence both motor patterns: Orcokinins, for example, which have been indicated to play a role in regulating circadian rhythms in cockroach (Hofer & Homberg 2006, J Exp Biol 209), were elevated towards the end of the light phase, suggesting a potential role in circadian influences in crustaceans as well. The concentration of the hormone crustacean cardioactive peptide was highest before light turned on then decreased during the day. To determine whether such changes in neuropeptide content correlate with motor activity, we are currently performing simultaneous mass spectral and electrophysiological recordings in the same animal.

Disclosures: A.M. Yarger: None. **Z. Liang:** None. **L. Li:** None. **W. Stein:** None.

Poster

829. Muscle Physiology and Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.01/MM3

Topic: D.13. Motor Neurons and Muscle

Support: CONACYT Grant 178723

Title: Seasonal changes in contractile properties of the iliofibularis muscle of Sceloporus torquatus lizard: A study among sexes and color morphs

Authors: E. QUINTANA[1], T. RUBIO-BLANCO[2], P. GUEVARA-FIORE[3], D. SALINAS-VELARDE[4], J. GONZÁLEZ-MORALES[4], *V. FAJARDO[5];
[1]Ctr. Tlaxcala de Biología de la Conducta, UAT, Tlaxcala, Mexico; [2]Doctorado en Ciencias Biológicas, UAT, Tlaxcala, Mexico; [3]Escuela de Biología, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [4]Facultad de Ciencias, Univ. Autónoma del Estado de México, Toluca, Mexico; [5]Facultad de Medicina Veterinaria y Zootecnia, Univ. Autónoma Del Estado De México, Toluca, Mexico

Abstract: In vertebrates, the contractile properties of striated muscles have been mainly studied to report differences between males and females in muscles that are sexually dimorphic. Additionally, seasonal changes have also been reported, with a strong bias to study mammals as model organisms. Here we use lizards to address three neglected areas in the study of muscular physiology: 1) muscular differences due to reproductive seasons; 2) sexual dimorphism in locomotor muscles (which are not macromorphologically dimorphic); 3) muscular differences between male morphs that vary in their reproductive strategies. Our goal is to describe the force developed by the iliofibularis locomotor muscle (IF) between the sexes and between male morphs in three different seasons: before, during and after reproduction. We used Sceloporus torquatus lizard, which shows sexual dimorphism and has two male color morphs, blue and black. The females and the male morphs show differences in habitat use, behavior, limb length and locomotor performance. In pre-reproductive season, there were no differences in the IF force between sexes and between morphs, but females showed more fatigue than males and than in the other two seasons, which is probably associated to females giving birth. In the reproductive season, the IF force of females and morphs was higher than in the other two seasons, but females and the black morph were similar. Interestingly, females showed lowest IF fatigability than black and blue males, which was also lower than in the other two seasons. This might be due to increased concentrations of sex hormones. In post-reproductive season, females and morphs showed the lowest IF force, and females showed more fatigue that in the reproductive season, which seems to imply high energy expenditure. In conclusion, S. torquatus shows seasonal differences in the IF contractile properties among sexes and male morphs, probably influenced by sex hormones and differences in in foraging and territorial activity. Blue males are territorial requiring faster movement for escaping and foraging. Blue males depend on speed and not resistance for foraging and escaping, and their muscles showed greater strength but they are

more prone to fatigue. Females are also active foragers on the ground needing more resistance and less muscular force. Our results show for the first time differences in the contractile properties of a locomotor muscle between the sexes and between male morphs across different reproductive seasons, which has great implications for our understanding of the evolution of physiological differences between the sexes and why males show reproductive polymorphism.

Disclosures: **E. Quintana:** None. **T. Rubio-Blanco:** None. **P. Guevara-Fiore:** None. **D. Salinas-Velarde:** None. **J. González-Morales:** None. **V. Fajardo:** None.

Poster

829. Muscle Physiology and Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.02/MM4

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant DC008955

Burke Medical Research Institute

Title: *In vivo* metabolism of TMPyP4 generates acetycholinesterase inhibitor-like activity

Authors: ***E. CAI**[1], M. WANG[1], N. FUJIWARA[1], J. CAVE[1,2];
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Abstract: Recent studies suggest that DNA secondary structures, such as G-quadruplexes and i-motifs, are important regulators of neuronal gene transcription and translation. Small molecules that can modulate the stability of these secondary structures are potentially important pharmacological tools to manipulate neuronal gene expression. The porphyrin compound, TMPyP4 (5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)porphine), is an established agent for stabilizing both G-quadruplexes and i-motifs in both *in vitro* and cell culture experiments. Under similar conditions, however, the structural isomer, TMPyP2 (5,10,15,20-Tetrakis-(N-methyl-2-pyridyl)porphine), does not stabilize either G-quadruplexes and i-motifs. To address if either of these porphyrin compounds can modulate DNA secondary structure *in vivo*, adult wild-type C57BL6 mice were administered either TMPyP4, TMPyP2 or saline by intraperitoneal injection. Unexpectedly, exposure to TMPyP4, but not TMPyP2, induced a state of temporary paralysis consistent with acetylcholinesterase inhibition. Analysis of porphyrin metabolic pathways

suggested that heme oxygenase-dependent breakdown of TMPyP4 and TMPyP2 produces 4-methyl-pyridium and 2-methyl-pyridium, respectively. Here, we describe studies to test whether exposure to 4-methyl-pyridium is sufficient to reproduce the physiological response observed to TMPyP4 administration. We also report fluorometric cholinesterase assays to establish whether that 4-methyl-pyridium, but not 2-methyl-pyridium, is a potent acetylcholinesterase inhibitor. Together, these studies indicate that TMPyP4 is unsuitable for *in vivo* studies to modulate DNA secondary structure and neuronal gene expression due to the production of toxic metabolites.

Disclosures: E. Cai: None. **M. Wang:** None. **J. Cave:** None. **N. Fujiwara:** None.

Poster

829. Muscle Physiology and Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.03/MM5

Topic: D.13. Motor Neurons and Muscle

Support: Posdoctoral fellowship by CONACYT to Kenia López-García

PROMEP-SEP, UATLAX-PTC-109 to Francisco Castelán

PAPIIT_DGAPA-UNAM, IN206013-3 to Margarita Martínez-Gómez

CACyPI-UATx-2013 to Francisco Castelán

Title: Distribution and fractal organization of fiber types in pelvic and perineal muscles related to micturition and reproduction

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Abstract: This study was aimed to analyze the distribution and fractal organization of fiber types (fast, intermediate and slow) in the pubococcygeus (PB) and bulbospongiosus (BS)

muscles of the female rabbit (Oryctolagus cuniculus). Such muscles are essential for physiological and reproductive functions as micturition, coitus and parturition. We assume that distribution and fractal organization (random or non-random) of myofibers in the PB and BS muscles is related to their physiological role. Five virgin female rabbits (Oryctolagus cuniculus) of ≈ 10 months of age were used. Sections (10 μm thick) of the PB and BS muscles were stained with the basic ATPase histochemical technique and the fiber types were identified as fast (grey), intermediate (dark) and slow (white) and located in photomicrographs by means of the ImageJ program (NIH). To determine the distribution of fibers in muscles, we calculated the centroid values of each fiber type and of all fibers (particular and populational, respectively). In addition the distance between centroids (particular and populational) and orientation (slant) was determined and indicated as a vector. In addition the fractal organization of muscle fibers was determined by using the correlation integral fractal method (CIFM) with the R statistical program. The data obtained show that the distance vector of fast fibers in the pubococcygeus muscle (244.3 ± 73.5 μm) is larger than the vectors of slow and intermediate type fibers (117.8 ± 25.7 μm; 161.4 ± 30.8 μm) and they are oriented to the lateral side of the muscle, toward the illiococcygeus muscle. According to the CIFM, only the fast fibers have a non-random organization (D= 1.92 ± 0.01) while the others show a random distribution. Meanwhile, fast and intermediate fibers in the BS muscle have a similar vector distance (134.3 ± 33.6 μm; 136.1 ± 49.3 μm) but the intermediate fibers are slightly oriented to the dorsal region, where the muscle is attached to the vagina while fast fibers are oriented to the ventral side. In this muscle, the different fiber types show a random fractal organization (Fast, D= 2.09 ± 0.08; Intermediate, D= 1.98 ± 0.08; Slow, D= 2.02 ± 0.04). According to our results it is proposed that the PB and BS muscles showed a different fiber type distribution and only the fast fibers in PB are non-randomly distributed (structurally organized). In addition it is proposed that the structural characteristics of pelvic and perineal muscles probably are related with their physiological role during micturition and reproduction.

Disclosures: **K. López-García:** None. **S. Mariscal-Tovar:** None. **C.X. Hinojosa:** None. **E. Rodríguez-Torres:** None. **M. Martínez-Gómez:** None. **F. Castelán:** None. **I. Jiménez-Estrada:** None.

Poster

829. Muscle Physiology and Biochemistry

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Topic: D.13. Motor Neurons and Muscle

Support: Posdoctoral fellowship by CONACYT to Kenia López-García

Title: Effect of chronic undernourishment on the proportion of muscle fiber types in fascicules of the Extensor Digitorum Longus muscle in the young male rat

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Abstract: The extensor digitorum longus (EDL) muscle is considered as a fast muscle, which is constituted of four fascicules whose tendons innervate the foot toes and actively participate in their movements. The EDL is conformed of a relative proportion of slow (Type I), intermediate (Type IIA-IID) and fast (Type IIB) fibers. In a previous study we have shown that chronic undernutrition modified the relative proportion of muscle fiber types along the postnatal development of the rat (Ruiz, et al., 2014). In such study, the proportion of fiber types was obtained from basic ATP-stained transversal sections of the EDL muscle belly. However, because of the anatomical disposition of fascicules in the EDL muscle, at least one of them was not included in those sections. In this study we analyzed the effect of chronic undernutrition on the relative proportion of muscle fiber types in all the fascicules of the EDL muscle. Five control and undernourished male rat pups of 45 days of age were used. Each fasciculus of the EDL muscle was dissected separately and sectioned in 8-10 μm thick transversal slides and subsequently stained with the basic ATPase histochemical technique (pH=9.4). All the fibers in fascicules were counted and identified as fast (grey), intermediate (dark) and slow (white). The data obtained in control animals indicate that the fasciculus in EDL muscles contain different proportions of muscle fiber types. Fasciculus 2 and 3 contain a larger proportion of fast fibers than of intermediate or slow fibers while the amount of fast and intermediate fibers in the 5th fasciculus is relatively small. In the undernourished muscles, the proportion of fast muscle fiber types in fascicules changed. It increased in fascicules 2 and 5 but decreased in fascicules 3 and 4. Meanwhile the proportion of intermediate fibers decreased in the 5th fasciculus and slow fibers increased in fascicules 3, 4 and 5th. Our results may indicate that chronic undernutrition affect the relative proportion of fiber types in fascicules of the EDL muscle and that the 2nd and 5th fascicules are the most affected. It is considered that such effect may exert an influence on toes movements of the rat.

Disclosures: I. Jimenez-Estrada: None. **C.X. Hinojosa:** None. **K. López-García:** None. **J.C. Guadarrama:** None. **I. Jiménez-Estrada:** None. **B. Segura:** None.

Poster

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Topic: D.13. Motor Neurons and Muscle

Support: CONACYT S-2007-01-69989

SIP-IPN-20080283

SIP-IPN-20090304

Title: The amplitude fluctuation of the Hoffmann reflex is modified differently by a track workout depending on the fitness of the medial gastrocnemius muscle

Authors: M. E. CEBALLOS[1], J. J. SALDAÑA[2], E. HERRERA[2], A. L. GUTIÉRREZ[1], E. MANJARREZ[3], *J. LOMELI[1];
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Abstract: The Hoffmann reflex (H-r) is related to the activation sequence of the alpha motor neurons in the spinal cord. This activation sequence, which produces fluctuation in the H-r amplitude, can be assessed by the fractal dimension (FD), an index of complexity. In the event that If physical training causes plastic changes of motor activity, they can be revealed by analyzing the FD of fluctuating H-r amplitude from electromyographic recordings. Our objective was to compare the FD readings found before and after the track training of sedentary subjects and athletes. The study involved 16 healthy young men and women (19.7 ± 1.5 years, 63.09 ± 14.23 Kg and 1.66 ± 0.07m), who all gave their informed consent. The experiments were designed and carried out according the Helsinki Declaration and approved by the institutional ethics committee. Subjects were classified as sedentary (n= 8) or athletes (n= 8), and both groups were subjected training consisting of running 4 to 5 Km 3 times per week for 13 weeks. To all subjects simultaneous rectangular stimuli of constant current were applied in both popliteal fossae (between 5 and 13 mA, at 0.166 Hz), with surface electrodes connected to digital stimulators (Digitimer). The FD was determined from the variation in amplitude of 130 reflexes recorded in the right and left medial gastrocnemius muscle before and after track training by Higuchi's method. The data, expressed as the mean ± SD, were compared using the paired Student t test. In both groups, significant differences were found when comparing the FD before and after training: sedentary, 1.889 ± 0.03 and 1.944 ± 0.02 (t= 2.568, P= 0.023); athletes, 1.987 ± 0.004 and 1.938 ± 0.01 (t= 4.156, P= 0.001), respectively. After training, the mean FD was

higher in sedentary subjects and lower in athletes. It is plausible that a trained muscle does not have a wide range of possibilities for recruitment of motor units (randomness) and therefore exhibits greater complexity (high FD).

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Poster

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Topic: D.13. Motor Neurons and Muscle

Support: CONACYT (248115-IXR)

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PNPC 2014 Doctorado en Ciencias Biológicas, UATx

PAPPIT-UNAM (228110- MMG)

Title: The response of the pubococcygeus muscle during the urethrogenital reflex in the male rat

Authors: *I. XICOHTÉNCATL RUGERIO[1,2], D. CORONA-QUINTANILLA[1], L. NICOLÁS-TOLEDO[1], F. CASTELÁN[1], M. MARTÍNEZ-GÓMEZ[1,3], J. RODRÍGUEZ-ANTOLÍN[1];
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Abstract: The male sexual physiology involves complex processes as the penile erection, emission and ejaculation of seminal fluid through the urethra. These processes are regulated by supraspinal and spinal mechanisms that require coordination of sympathetic, parasympathetic and somatic nerves, which control the increased blood flow, the contraction of the smooth and striated muscle, as the pubococcygeus (Pcm) and bulbospongiosus (Bsm). The rhythmic contractions of Bsm during the urethrogenital reflex (UGR) in rats are similar to the Bsm activity in men during ejaculation. The URG is the mimic of the expulsion phase of ejaculation, since it is characterized by rhythmic contractions of the perineal muscles. But, it is necessary know the response of Pcm on male sexual function. In particular, the ways in which contractions/relaxation of Pcm are involved in the mechanisms of erection and ejaculation. The aim of this study was to determine the response of the Pcm during the UGR in the male rat. We

used male Wistar rats anesthetized with 20% urethane (n=8). In the male rats was recorded the penile occlusion, the URG and electromyograms of Bsm and Pcm before and after of the denervation the somatomotor branch of the pelvic nerve, that innervating the Pcm. The nerve transection, during the occlusion had a significant decrease the threshold urethral pressure, maximum pressure and the time of the increase of urethral pressure. In the URG increased the threshold pressure and decreased the maximum pressure and time urethral pressure. Our results suggest that the response of the Pcm regulates the URG and has a role in the erection and ejaculation process. However, some factors as the metabolic syndrome could damage the pelvic muscles and cause sexual dysfunction. A future outlook of our group is to determine whether alterations in diet, and consumption of sugar in the water, produce changes on the activity of Pcm during the URG.

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Poster

829. Muscle Physiology and Biochemistry

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Program#/Poster#: 829.07/MM9

Topic: D.13. Motor Neurons and Muscle

Title: Interaction between myostatin and the GH/IGF-1 in muscle

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Abstract: Myostatin (mstn) has been identified as a negative regulator of muscle growth, leading to research into its potential as a therapeutic agent in individuals suffering from muscle wasting disorders, as occur in old age, cancer and AIDS, for example. Mstn acts locally as an autocrine/paracrine agent to inhibit muscle hypertrophy and hyperplasia, and it is widely believed that this pathway can be silenced by the endocrine action of IGF-1 on skeletal muscle. Our present work seeks to understand the mechanisms by which mstn and the GH/IGF-1 axis potentially interact in muscle, using two animal models, the growth hormone receptor knockout (GHR-/-) and the bovine growth hormone transgenic (bGH). These mice have contrasting phenotypes. IGF-1 action is significantly reduced in the GHR-/- because of the lack of GH signaling, and results in obese and dwarf mice that are extremely insulin sensitive and live longer than their littermate controls. By contrast, the overexpression of GH and consequent

enhancement of IGF-1 action in bGH mice results in larger and leaner bodies, extreme insulin resistance, and shorter lifespan than littermate controls. We applied Western blot techniques on samples of gastrocnemius/soleus complex homogenate from each genotype, using a monoclonal antibody against the C-terminus of human mstn protein, and a purified mstn peptide as control. We found no statistically significant difference in the levels of expression of active mstn in either the GHR-/- or the bGH, compared to littermates controls, indicating that modifying the GH/IGF-1 axis does not impact mstn levels, and also that changes in body composition in those animal models are not due directly to mstn. We also analyzed muscle function in the bGH mice vs. littermate controls using a grip strength meter and the rotarod. There was no significant difference in rotarod performance, indicating that the phenotypic changes associated with these genetic mutants do not affect balance, coordination, or endurance. Surprisingly, grip strength assessment of rear limb pull force was significantly decreased in bGH mice compared to littermate controls and normalized for body weight. Although changes in mstn were not evident in these models, the decreased grip strength in bGH contributes to confirm other data from models of mstn inhibition, suggesting that a higher percentage of lean mass does not necessarily contribute to an increase in strength.

Disclosures: **N. Lozier:** None. **S. de Lacalle:** None.

Poster

829. Muscle Physiology and Biochemistry

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Topic: D.13. Motor Neurons and Muscle

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PROMEP-SEP (UATLX-PTC-109 a FC)

Infrastructure CONACYT 2014

PAPIIT-UNAM (IN206013-3 to MMG)

Title: Estrogenic sensitivity of pubococcygeus and bulbospongiosus muscles is related with aromatase expression in female rabbits

Authors: *M. D. CARRASCO RUIZ[1,2], V. GARCIA VILLAMAR[1], K. LOPEZ GARCIA[3], O. SANCHEZ GARCIA[2], P. PACHECO CABRERA[4], E. CUEVAS ROMERO[1], M. MARTINEZ GOMEZ[1,5], F. CASTELAN[1];
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Abstract: The concentration of serum estradiol (E2) declines during menopause, when most of estrogenic actions depend on peripheral synthesis of estrogens. Skeletal muscles express steroidogenic enzymes including those that synthesize E2. The pelvic and perineal skeletal muscles play important roles in the physiology of the female lower urogenital tract. Menopause is recognized as a factor that alters structure and physiology of the latter muscles, which increases the risk to develop gynaecological and urinary symptoms. Nevertheless, the use of the hormonal replacement therapy is controversial to ameliorate some of them, particularly the stress urinary incontinence. The objectives of this work were to analyze the serum estradiol levels, the estrogen sensitivity (morphometry and expression of estrogen receptors) and the expression of aromatase in pubococcygeus and bulbospongiosus muscles. Eighteen female rabbits were divided in three groups: virgins (V) and ovariectomized by 3.5 months implanted with empty (OVX) or filled estradiol benzoate (OVX+EB) for six weeks. The serum concentration E2 was quantified by ELISA. Pubococcyegus and bulbospongiosus muscles were histologically processed to evaluate the morphometry and the expression of estrogen receptors (ER) by immunohistochemistry. The expression of aromatase was assessed by Western Bloot. Present findings show a different expression of aromatase in pubococcygeus and bulbospongiosus muscles in female rabbits. This expression is affected in long-term ovariectomized rabbits exhibiting a different modulation by circulating estradiol. To analyze the expression of estrogen receptors and morphometry of both kinds of muscles, it is proposed that muscle aromatization is more relevant for pubococcygeus than for bulbospongiosus muscles.

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Poster

829. Muscle Physiology and Biochemistry

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.09/MM11

Topic: D.13. Motor Neurons and Muscle

Title: Aggravation of dystrophic phenotype with chronic exercise creates widened and more relevant therapeutic window in the MDX model of duchenne muscular dystrophy (DMD)

Authors: P. SWEENEY[1], T. AHTONIEMI[1], J. PUOLIVÄLI[1], A. NURMI[1], S. ALASTALO[1], J. OKSMAN[1], D. WELLS[2], *S. SAARIO[1];
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Abstract: Duchenne muscular dystrophy (DMD) is caused by a X linked mutation in the dystrophin gene; lack of dystrophin causes a progressive muscle necrosis which leads to progressive decrease in mobility for those suffering from the disease. The MDX mouse, a mutant mouse model which displays a frank dystrophinopathy, is currently widely employed in preclinical efficacy models for treatments and therapies aimed at DMD, in general these models have been based on invasive histopathology of muscles and serum biochemical measures like measurement of serum creatine kinase (sCK). It is established that a "critical period" between 4 and 6 weeks exists in the MDX when there is extensive muscle damage that is largely subclinical but evident with sCK measurements. However, a full characterization of the MDX model remains largely incomplete especially with respect to aggravation of the muscle damage inherent in the MDX mouse phenotype, the probably asymmetric nature of this pathology and coincidental changes in other functional, pathological and biochemical biomarkers in the MDX mouse. The purpose of this study was to attempt to aggravate the muscle damage in the MDX mouse and to create a wider, more readily translatable and discernible therapeutic window for the testing of potential therapies for DMD. The study consisted of subjecting 15 male mutant MDX mice and 15 male wild-type mice to an intense chronic exercise regime that consisted of bi-weekly (two times per week) treadmill sessions over a 12 month period. Each session was 30 minutes in duration and the treadmill speed was gradually built up to 12m/min for the entire session. Baseline plasma creatine kinase (pCK), treadmill training performance and locomotor activity were measured after the "critical period" at around 10 weeks of age and again at 14 weeks of age, 6 months, 9 months and 12 months of age. In additional a morphological and metabolic profile (including lipid profile) at the molecular level, from the muscles most severely affected, the gastrocnemius muscle and the tibialis anterior muscle, was also measured at the same time intervals. Results indicate that by aggravating or exacerbating the underlying muscle damage in the MDX mouse a more pronounced and severe phenotype in comes to light. This is displayed by a reduction in mobility in wild type mice relative to the mutant MDX mice as well as pronounced morphological and metabolic changes that can be measured by non-invasive MRI and MRS. Evidence of a progressive asymmetric pathology in imaging parameters as well as in locomotor activity was found. Taken together these show that chronic exercise is an important factor to consider when performing preclinical studies in the MDX mouse.

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Poster

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Topic: D.13. Motor Neurons and Muscle

Support: OCTOPUS project, Grant Agreement No. 231608

EC EP7 project STIFF-FLOP

Title: Characterization of the static and dynamic forces involved into the Octopus vulgaris arm muscle performance

Authors: ***L. ZULLO**[1], N. NESHER[2,3], F. BENFENATI[1,4], B. HOCHNER[2,5];
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Abstract: The octopus (Octopus vulgaris) arm is composed by a three-dimensional combination of muscle fibers and connective tissue and a complex arrangement of the nervous components. These are organized as a muscular hydrostat with constant volume that constrains the antagonistic action of muscles. In the current study we tested the hypothesis that this special organization has an important functional implication and it is constrained to create stiffening and "particles jamming". In our physiological and modeling experiments we revealed that stiffening is an important and frequently used motor primitive in the octopus arm. We further hypothesize that that the muscle cells are organized in such a way where stiffening is achieved by simultaneous co-activation of all the muscle groups. To test the stiffness hypothesis we performed an experiment where all muscles of an arm segment, held vertically in a bath, were simultaneously activated. We show that at resting length the arm segment shorten to about 85-90% of the free hanging length. Interestingly when a constant passive stretch (a holding weight)

is applied to this segment, it elongates passively and upon stimulation it contracts back to its 'attractor' default length. These results support the hypothesis that at static conditions the anatomical organization of the octopus muscular hydrostat constrains the arm to stiffen and this is achieved mainly by isometric contraction. To measure the dynamic force involved in the arm contraction we then used a Dual-Mode Lever Arm System on an in-vitro preparation of an arm sample. We used a pattern of stimulation intended to create simultaneous activation of all the muscle cells in the segment. We then measured the dynamic stiffening versus the static stiffening force on various arm lengths. We show that the initial state of the arm is indeed important for the generation of a determined force. In fact, when stimulated, arm segments under a stretch condition exert a higher force than in their resting state. We also found that the stretching force needed to elongate an arm segment to a certain length is dependent from its initial resting or contracting state. This may reflect the existence of passive elastic forces which are modulated by the level of the arm activation. Altogether these data indicate the possible involvement of 'stored energy' and 'jamming force' factors that are special for muscular hydrostats because of the close interactions among muscle layers and connective tissue and of the octopus arm intrinsic organization in counteracting muscle layers.

Disclosures: **L. Zullo:** None. **N. Nesher:** None. **B. Hochner:** None. **F. Benfenati:** None.

Poster

829. Muscle Physiology and Biochemistry

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Program#/Poster#: 829.11/MM13

Topic: D.13. Motor Neurons and Muscle

Title: A novel genetic cross to empower *in vivo* investigation of the action of titin and giant sarcomere associated proteins in neuromuscular plasticity

Authors: *P. DONES, J. L. KRANS;
Western New England Univ., Springfield, MA

Abstract: There is a long history of neuromuscular plasticity. Traditionally, events that deviated from predictive excitation-contraction coupling models were explained with electrophysiologic phenomena such as synaptic changes including receptor dynamics, channel modulation, modulated quantal content, or calcium sequestration and release kinetics both at the synapse and the sarcoplasmic reticulum. However, the most recent 20 years have ushered in an appreciation for contributions to neuromuscular physiology from unexpected sources. For example, giant

sarcomere associated proteins (gSAPs), such as titin, are non-contractile / non-actomyosin proteins that, in arthropods, are known to be modulated by neurotransmitters (e.g. 5-HT), neuromodulators (e.g. OA), and divalent cations (i.e. Ca^{2+}). We suspect many instances of neuromuscular plasticity might be explained by the physiology of gSAPs. We are specifically interested in modulatory actions of gSAPs during neuromuscular plasticity and have thus developed a novel *in vivo* system to examine their physiological roles. Our system consists of temperature sensitive [Gal80(ts)] regulation of the Gal4-UAS system driving both Dicer(2) and TRiP, to invoke RNAi against the sallimus gene (*sls*). *sls* encodes at least five gSAPs, all of which may exist in multiple isoforms. This system allows us to vary *sls* expression levels across developmental epochs, and to vary the magnitude of expression interruption by varying durations of RNAi activation. The neuromuscular physiology of animals with reduced gSAP expression were compared to wildtype and results demonstrate that gSAP expression level substantially altered sarcomere physiology. We report here upon principal components of isometric contraction: contraction amplitude, rise, and decay times. Decay times were significantly longer when gSAP expression level was reduced. Further, we devised an assay to test Nishikawa et al.'s (2012; *Proc R Soc B*) winding filament theory *in vivo*. Briefly, the theory posits upon actomyosin cycling, gSAPs (i.e. titin) are wound around actin. We propose that this wrapping should yield a tether between actin and myosin with progressively increased damping. Therefore, we hypothesized that in the absence of such a tether – RNAi against gSAP expression – force could vary more widely as motoneuron frequency changes. Animals with reduced expression of gSAPs showed greater range of forces resulting from a 30 Hz range of motoneuron spike rates than in wildtype animals. These early findings support that gSAPs are a critical group of proteins in understanding the multitude of historical deviations from excitation-contraction coupling models.

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Poster

829. Muscle Physiology and Biochemistry

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Title: Sensory encoding by muscle receptors can be affected by epimuscular myofascial loads

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Abstract: Contrasting traditional beliefs, recent results indicated that skeletal muscles are not exclusively connected to bone via origin and insertion, but also via neighboring structures. Such epimuscular myofascial connections can cause local force differences within a muscle. In addition, finite element modeling has predicted that myofascial loads will result also in local length changes. Our aim was to investigate the effects of epimuscular myofascial connections on feedback from muscle receptors. We hypothesized that output from muscle spindles and tendon organs can be affected by length changes of adjacent muscles. In fully anesthetized male Wistar rats (n=16), the distal tendon of soleus muscle (SO) and the distal tendons of gastrocnemius and plantaris muscles (GAS+PL) were dissected, cut and tied to servo motors, which controlled muscle-tendon unit (MTU) length and measured tendon force. Connective tissues at the muscle belly level were left intact. Action potentials from single afferents were recorded intra-axonally by penetrating dorsal roots. Axons from muscle spindles and tendon organs were identified using their characteristic response properties. Afferent firing was measured in response to 3 mm ramp stretches of the corresponding muscle at different MTU lengths of its synergistic muscle(s). Firing responses of an afferent located within one muscle was measured also during ramp stretches of its neighboring muscle(s). For all recorded muscle spindles and tendon organs of SO, lengthening GAS+PL distally resulted in a lower instantaneous firing rate at the peak of the ramp, less action potentials during the SO ramp, an increased length threshold and a decreased force threshold. 11 out of 14 SO afferents responded to ramp stretches of GAS+PL. Firing responses of most GAS muscle afferents (16 out of 23) to GAS+PL ramp stretches were affected significantly by changing SO length. In approximately 25% of GAS afferents firing could be evoked by ramp stretches of SO. These results indicate that the output of muscle spindles and tendon organs is not only a function of the mechanics of the muscle in which they are located, but also of the conditions of neighboring muscles. We conclude that the central nervous system can sense effects of epimuscular myofascial loads. This suggests that muscle receptors not only encode local muscle state variables, but also provide the CNS with information about global hind limb state.

Disclosures: H.A. Smilde: None. **T.C. Cope:** None. **G.C. Baan:** None. **P. Nardelli:** None. **H. Maas:** None.

Poster

829. Muscle Physiology and Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.13/MM15

Topic: D.13. Motor Neurons and Muscle

Title: MRI/MRS analysis of lower hindlimb and hip muscles of dysferlin deficient mice reveal muscle specific differences in disease progression

Authors: H. WINDISH[1], *T. T. AHTONIEMI[2], J. OKSMAN[2], J. PUOLIVÄLI[2], K. LEHTIMÄKI[2], A. NURMI[2], D. ALBRECHT[1];
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Abstract: Mutations in the dysferlin gene cause Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi Myopathy. In order to understand the disease better and to develop and test new therapeutic approaches, new translatable biomarkers need to be characterized and validated for dysferlinopathy animal models. Dysferlin deficient B6.A-Dysf^{prmd}/GeneJ (Bla/J) mice were longitudinally evaluated at 3, 6, 9 and 12 months of age using magnetic resonance imaging (MRI) to compare the volumes of 2 lower limb muscles (TA, gastroc) and two hip muscles (psoas, gluteus maximus) with C57/Bl6 control animals. The composition (fat/water content) and metabolite levels of the gastroc and gluteus was also monitored by magnetic resonance spectroscopy (MRS). Beginning at 9 months of age, Bla/J mice showed gross abnormalities in their hip muscles, compared to control (C57BL/6). Bla/J gluteus maximus and psoas muscles were reduced in volume, and areas of tissue atrophy and fat infiltration were detected. These findings are supported by increased lipid concentration detected by 1H-MRS in gluteus maximus. In contrast, the lower limb muscles of the BlaJ animals did not show a detectable decline in volume or a change in composition. We conclude that hip muscles of dysferlin deficient mice are dramatically more affected than the commonly studied lower limb muscles, and that MRI and MRS can be used to monitor disease progression and assess the potential of possible therapies in these muscles. In continuing studies, we are evaluating the efficacy of rationally selected FDA-approved drugs, such as those that interfere with calcium channels or components of the immune system.

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Poster

829. Muscle Physiology and Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.14/MM16

Topic: D.13. Motor Neurons and Muscle

Title: Temporal profile of T2 MRI and 1H-MRS in the MDX mouse model of Duchenne muscular dystrophy (DMD)

Authors: *P. SWEENEY[1], T. AHTONIEMI[1], J. PUOLIVÄLI[1], T. LAITINEN[1], K. LEHTIMÄKI[1], A. NURMI[1], D. WELLS[2];
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Abstract: Duchenne muscular dystrophy (DMD) is an X-linked, lethal muscle wasting disease for which there are currently no treatment that effectively prevents the muscle necrosis and progressive muscle loss. The MDX mutant mouse model has been extensively studied as a model for DMD but to-date an extensive temporal, non-invasive imaging profile that utilizes MRI and 1H-MRS has not been extensively applied to this model. In addition, longitudinal non-invasive imaging characterization has not coincided with attempts to exacerbate the progressive muscle damage that is part of the phenotype in this rodent model. In this study we employed an 11.7 T small animal MRI in order to characterize the MRI and MRS profile of MDX mice longitudinally during a 12 month period. Male mutant MDX mice and male wild-type mice were profiled with baseline MRI and plasma creatine kinase (pCk) starting at 6 weeks of age and again at 3 months, 6 months, 9 months and 12 months of age. In addition, due to the fact that the MDX mouse shows no clear outward clinical signs of muscular dystrophy, although it shows clear histopathology and biochemical changes, the mice were subjected to a chronic exercise regime of treadmill walking 2 times per week for 30 minutes for the entire period of the study in order to exacerbate muscle damage associated with the underlying disease pathology. The longitudinal development of this pathology was examined using MRI and 1H-MRS in order to obtain a morphological and metabolic profile from the gastrocnemius muscle and the tibialis anterior muscle at various ages. The results indicate that by utilizing non-invasive imaging such as MRI/MRS, as well as exacerbation of the dystrophic phenotype and muscle damage associated with the clinical symptoms of the disease, a temporal morphological and a temporal metabolic profile of progressive muscle damage can be built up and that this temporal profile can be used to more effectively design preclinical therapeutic efficacy trials. This information can also be used along with standard functional tests like gait analysis, locomotor activity, strength tests, serum biomarkers and detailed histopathology to assess the effects of future treatments for DMD and similar neuromuscular diseases.

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Poster

829. Muscle Physiology and Biochemistry

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 829.15/MM17

Topic: D.13. Motor Neurons and Muscle

Title: Phantom weight sensation induced by application of constrictive force on the forearm accompanies increased muscle activity

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Abstract: Applying constrictive force on the forearm using an air pressure cuff creates a weight sensation when the operator lifts a virtual object on a screen. The phantom weight felt from the constrictive force is proportional to the air pressure inside the cuff. This phenomenon can be applied to the virtual reality system. Conventional force displays comprise robot arms, which are not suitable for wearable systems because their weight and rigidity compromise the user's comfort. In contrast, a force display using phantom weight sensation requires only wearing a lightweight and soft air pressure cuff around the forearm. To identify the source of phantom weight sensation, we compared between the surface electromyogram (EMG) when constrictive force was applied to the forearm and that when real force was applied. Eight male students aged 22-25 years participated in the experiments. In the first experiment, the phantom weight felt when constrictive force was applied on the forearm was measured using a psychophysical experiment where the participants pulled up a wire attached to a weight. The sensation was compared with the phantom weight felt when constrictive force was applied on the forearm. The result shows that the phantom weight was proportional to the air pressure inside the cuff, which is consistent with a previous report (T. Mitsuda, Presence, Vol. 22, No. 3, 2013). In the second experiment, the EMGs of the flexor carpi radialis, palmaris longus, flexor carpi ulnaris, biceps brachii, and triceps brachii were recorded when the same participants pulled up a wire attached to a weight (0, 50, 100, or 150 g) and when the participants pulled up a wire without any weight but with constrictive force applied to the forearm using an air pressure cuff (0, 4, 6, or 8 kPa). A significant increase in the activity of the flexor carpi ulnaris was observed when a real weight was lifted (Friedman's two-way analysis of variance, $p < .05$) and when constrictive force was applied to the forearm ($p < .05$). The activity of the biceps brachii also increased in both conditions, although significantly only in the phantom weight condition ($p < .05$). Additionally,

the recorded EMGs of the flexor carpi ulnaris and biceps brachii when the participants felt a phantom sensation were the same as those when the participants lifted real weights. The activities of the other muscles significantly increased in neither condition ($p > .05$). This shows that when constrictive force is applied on the forearm, the increased muscle activity is accompanied with a phantom weight sensation.

Disclosures: **T. Mitsuda:** None. **S. Tanaka:** None.

Poster

829. Muscle Physiology and Biochemistry

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Program#/Poster#: 829.16/MM18

Topic: D.13. Motor Neurons and Muscle

Support: NIH/NIAMS R00AR054695

Title: The muscle cell line C2C12 transfected with mutant valosin-containing protein (VCP) exhibits resolution deficiencies upon cellular insults

Authors: ***C. J. RODRIGUEZ-ORTIZ**, J. C. FLORES, J. VALENZUELA, M. KITAZAWA; Sch. of Natural Sci., UC Merced, Merced, CA

Abstract: Mutations in the valosin-containing protein (VCP) causes a rare disease called inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD). Inclusion body myopathy is the most common clinical manifestation that is present in 80-90% of patients with VCP mutations. However, the pathogenic mechanisms by which mutant VCP triggers skeletal muscle degeneration remain largely unknown. One of the physiological functions of VCP is alleviating cellular stress. We hypothesize that disease-relevant VCP mutations attenuate tolerance to cellular stress, triggering a degenerative process. In this study, we transiently transfected the murine myoblast C2C12 cells with human wildtype or mutant VCP and exposed to the oxidative stressor arsenite. We observed that C2C12 cells with mutant VCP led to deficits in the resolution phase of the stress response, reflected by a slower clearance of stress granules. Similarly, the presence of mutant VCP significantly delayed the resolution of NF-kB activation in C2C12 cells following an acute exposure to the inflammatory stressor LPS. These results indicate that mutant VCP impairs cellular stress responses, leading to dysregulation of stress granules resolution and sustained activation of NF-

kB. Impaired tolerance and slower recovery from exogenous cellular stressors may result in a chronic overactive response and skeletal muscle degeneration.

Disclosures: C.J. Rodriguez-Ortiz: None. **J.C. Flores:** None. **J. Valenzuela:** None. **M. Kitazawa:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.01/MM19

Topic: D.16. Posture and Gait

Support: I-CORE grant

Title: Single unit representations of lower and upper extremity movements in the subthalamic nucleus and thalamus

Authors: *A. TANKUS[1,2], A. MIRELMAN[1], N. GILADI[3,4,5], I. FRIED[2,6,4], J. M. HAUSDORFF[1,4,5,7];
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Abstract: A large percentage of the patients with Parkinson's disease (PD) suffer from gait disorders which markedly reduce the quality of life, often cause functional dependence, and may lead to falls. A surgical symptomatic treatment is implantation of deep brain stimulation (DBS) electrodes in two brain areas undergoing substantial dopamine loss in PD, whose stimulation was shown to improve gait and postural symptoms: the subthalamic nucleus (STN) and the thalamic ventral intermediate nucleus (Vim). However, whereas neurodegenerative processes in PD were widely investigated, less is known about the underlying gait control circuits or the mechanisms affected by DBS. The goal of this research is to investigate the encoding of gait in the activity of single units in the human STN and Vim. Seven PD patients with movement disorders (5 Parkinson's disease and 2 essential tremor patients) undergoing DBS implantation performed various lower and upper limb movements while the activity of single STN and Vim neurons has been recorded intra-operatively, in synchrony with the kinematics of their limbs: acceleration,

angular velocity and orientation. Movements included feet tapping against a surface, which was performed bipedally (alternating) and unipedally with each foot and with and without the addition of a cognitive, dual task (serial 7 subtractions). Each type of tapping was repeated at 3 paces: patient self-selected "normal" pace, slow pace, and fast pace. For comparison purposes, patients performed these tasks also using the upper limbs. We recorded a total of 51 units, 36 in the STN and 15 in the thalamus (Vim). A high percentage (>40%) of the variability of the raw firing rate function can be explained by kinematic models in 22.2% (8/36) of the recorded STN units and in 80.0% (12/15) of recorded thalamic units. In the vast majority of the neurons, the average firing rate (over all repetitions) was highly correlated (even: $r > 95\%$) with average kinematic parameters. The representation, in many neurons, depends on the pace (slow/normal/fast) of the movements and on the performing limb. The analytical models thus reveal direct representation of the kinematics of both feet movements and hand movements in both STN and Vim. Our findings suggest an important role for the STN and Vim in the gait control cycle in the human brain, expand our understanding of the physiological roles of the two brain areas, and explains, to a certain extent, why gait and posture are affected by DBS to the STN and Vim.

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Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: D.16. Posture and Gait

Support: NSF Grant BCS-0957920

Title: The muscle torque coordination to stabilize upright standing posture

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Abstract: Introduction: Control strategy of upright standing posture is often explained by a single-inverted pendulum model (ankle strategy), or double inverted pendulum model

(combination of ankle and hip strategy). Kinematic analysis using the Uncontrolled Manifold (UCM) approach, however, has suggested that stability during upright standing is maintained by the multi-joint coordination. To investigate multi-joint coordination for the standing posture control, UCM approach has been applied in the space of muscle modes (i.e., groups of muscles) and joint angles. In this study, we applied the UCM approach at the level of muscle torques of joints to determine if the coordination is originated from the neural control. Methods: Subjects were asked to standing on floor (QS) and narrow base (NB). Based on the kinematic data, Lagrangian mechanics was applied to estimate net torques at the ankle, knee and hip joints and to decompose them into gravitational, motion dependent and generalized muscle torques (MUS). MUS at the four joints were applied to the UCM analysis as elemental variables to investigate whether MUS torque variance were coordinated to stabilize the COM anterior-posterior (COM_AP) position. We further tested this hypothesis by removing co-variation among the four joints' MUS and repeating the analysis. We hypothesized that evidence of flexible combinations of MUS related to COM stability would be reduced significantly after removing covariance between the elemental variables. Results: Data currently is available on 12 subjects. The variance of MUS associated with the stabilization of the COM_AP position (V_UCM) was higher than the variance related to the motion of the COM_AP (V_ORT) for all conditions (QS: $F = 13.97$, $p = 0.001$; NB: $F = 10.03$, $p = 0.004$),. The relative difference between two variance components (ΔV) was decreased after removing co-variation for all conditions (QS: $p < 0.001$; NB: $p < 0.001$). Conclusion: The muscle torques of ankle, knee and hip are coordinated to stabilize upright standing posture. This result supports the hypothesis that the multi-segment coordination is originated from the neural control.

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Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

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Topic: D.16. Posture and Gait

Support: European-Canadian Stroke Network Partnership Grant

Canadian Institutes of Health Research

Title: Ankle plantarflexor activity is more strongly related to centre of pressure velocity than displacement with external perturbation in people with stroke and healthy controls

Authors: *C. L. POLLOCK[1], T. D. IVANOVA[1], A. GALLINA[1,2], T. M. VIEIRA[2,3], S. J. GARLAND[1];
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Abstract: Sensorimotor deficits after stroke contribute to increased risk of falls. Sensory input related to the derivative of ankle sways shows a strong relationship with plantarflexor electromyography (EMG) modulation in healthy controls, suggesting that information regarding speed of movement facilitates the prediction of movement outcome (Masani et al. 2003). The purpose of this study was to examine the relationship of ankle plantarflexor EMG with the anterior-posterior centre of pressure (APCOP) velocity and displacement of people with stroke compared to controls using high density surface EMG (HDsEMG). Methods: Ten people with chronic stroke (mean ± sd 66.2 ± 9 yrs, 8 males) and ten age-matched controls (7 males) participated. Participants stood with each foot on a separate force platform. Anteriorly-directed loads were dropped by a pulley system and cable attached to a belt around the participants' pelvis. Loads of 1% body mass were added every 25-30s until a total of 5% body mass was reached. The static maintenance of standing balance was determined for 15s between loads. The APCOP displacement was measured simultaneously with HDsEMG from the soleus (SOL) (24 electrode grid, 2 cm interelectrode distance), medial (MG) and lateral gastrocnemius (LG) (20 electrode grids each, 1.5 cm interelectrode distance) of both legs. APCOP velocity was derived from APCOP displacement. For each channel, cross-correlation values between rectified, 4 Hz low-pass filtered EMGs (EMG envelopes; 18 bipolar for SOL, 16 for MG and LG) and APCOP displacement and velocity during the static maintenance of standing balance were computed; for each muscle, the median correlation value was considered as representative. Results: The median correlation of each plantarflexor EMG envelope with the APCOP velocity was stronger than with the APCOP displacement in all groups (paretic, non-paretic and control; $p<0.01$). Furthermore, correlations were significantly higher in MG than LG and SOL muscles in controls ($p<0.01$), and both MG and LG were significantly higher than SOL in the non-paretic leg ($p<0.01$), but the correlations were not significantly different amongst the paretic plantarflexors. Conclusions: These data suggest that sensory information reflected in APCOP velocity interacts more strongly than APCOP displacement with plantarflexor EMG activity in plantarflexor muscles in people with stroke and controls. However, the gastrocnemii EMG activity demonstrates a greater relationship with APCOP velocity than the SOL muscle in non-paretic and control groups but not in paretic muscles. This may be related to remodeling of the plantarflexors following stroke, moreso in the gastrocnemii.

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Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.04/MM22

Topic: D.16. Posture and Gait

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Title: The effect of simulated microgravity on the motor control of landing from a jump

Authors: C. N. GAMBELLI[1], D. THEISEN[2], P. A. WILLEMS[1], *B. SCHEPENS[1]; [1]IoNS/LOCO, Univ. catholique de Louvain/ Inst. of Neurosci. / LOCO, Louvain-La-Neuve, Belgium; [2]Lab. de Recherche en Médecine du Sport, Ctr. de Recherche Publ. de la Santé, Luxembourg, Luxembourg

Abstract: The pre-programmed muscular activity is thought to play a key role in preparing the human body to the forthcoming impact forces in landing movements (Santello, 2005). The aim of this study was to assess the adaptability of the motor control of landing from a jump in simulated microgravity conditions. Experiments were carried out in the A300 0g during ESA parabolic flight campaigns. Participants were equipped with a loading system (Gosseye et al., 2010) creating 4 simulated gravity conditions (1g, 0.6g, 0.4g and 0.2g) during the 0g phases. Eight subjects were instructed to perform several consecutive counter-movement jumps (CMJs) and to land without re-bounding. Kinetics, kinematics, and muscular activity of the lower limbs were recorded. The first 3 trials per gravity condition were rejected to avoid a learning effect. Subjects were able to perform CMJs in 0g and to land without re-bounding in the 4 gravity conditions. In the 1g condition, aerial time was 267 ± 35 ms (n=184); at landing, the peak vertical ground reaction force was 3.0 ± 0.9 times body weight with a whole body stiffness of 429 ± 386 s^{-2}. Muscular activity was present before touchdown for most of the recordings in the 1g condition. With decreased gravity conditions, jump height and aerial time increased. Peak

vertical ground reaction force decreased proportionally to the gravity condition, and whole body stiffness and amplitude of pre-landing muscular activity were reduced. These results suggest that the human body adapts whole body compliance and muscular activity with respect to the gravity condition. The presence of a pre-landing muscular activation suggests that the instant of touchdown can be predicted in different simulated gravity conditions. The fact that pre-landing muscular activity tends to disappear in 0.2g condition could be part of the specific landing control. Indeed, whole body stiffness has to be sufficiently low to avoid re-bounding on the ground in 0.2g condition.

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Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

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VA R&D Service Career Development Award (E7108M)

Title: Recruitment of subcortical muscle synergies during balance and walking in individuals with Parkinson's disease can be improved through rehabilitation

Authors: *J. L. ALLEN[1], J. L. MCKAY[1,2], M. E. HACKNEY[1,3], L. H. TING[1,2]; [1]Emory Univ., Atlanta, GA; [2]Georgia Inst. of Technol., Atlanta, GA; [3]Atlanta VA Med. Ctr., Atlanta, GA

Abstract: There is a growing body of evidence that muscle synergies represent neurally-encoded motor patterns that can produce consistent motor functions and as such are recruited in multiple motor tasks. For example, in healthy, young adults (HYA) we previously found that a common pool of muscle synergies is recruited during reactive balance, mainly a subcortically

driven process, and walking, which involves a complex interplay between subcortical and cortical mechanisms (Chvatal 2013). We concluded that the identified muscle synergies were subcortically encoded and recruited by multiple descending pathways for balance and walking. In individuals with Parkinson's disease (PD), cortical mechanisms may compensate for basal ganglia dysfunction in maintaining ongoing movements. Specifically we hypothesize that walking in PD is mediated by increased cortical recruitment of muscles. Thus we predict that muscle synergies for walking will not be shared with those used for reactive balance in PD. We also hypothesize that successful rehabilitation improves automaticity of walking via subcortical circuits and predict an increase in the number of shared synergies across walking and reactive balance after successful rehabilitation. We collected data from five individuals with PD (1 female, age = 63.2 ± 18.4, Hoehn & Yahr stage 2 [n=3], 2.5 [n=1], 3 [n=1]) who participated in an adapted tango rehabilitation intervention demonstrated to improve clinical scores of walking and balance. All assessments were conducted while participants were ON antiparkinsonian medications. Before and after rehabilitation, electromyography (EMG) from 13 muscles on the ride side leg and lower back were collected while 1) responding to multidirectional support-surface perturbations and 2) walking at self-selected speed. Muscle synergies were identified from EMG using nonnegative matrix factorization. Consistent with our hypothesis, the number of shared muscle synergies was lower in PD (1.0 ± 0.7) than in HYA (3.4 ± 1.1) before rehabilitation. Clinical scores of both gait and balance were improved after rehabilitation in all participants and of the five PD participants, three increased the number of shared muscle synergies (increases of 1, 2 and 3) and two had no change after rehabilitation. Interestingly, changes in the number of shared muscle synergies were primarily due to changes in muscle synergy structure during walking. These results provide preliminary evidence that clinical improvements in gait and balance may be due to increased automaticity of gait resulting from an increased generalization of subcortically encoded muscle synergies across tasks.

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Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

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Topic: D.16. Posture and Gait

Support: NIH Grant HD059844

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Title: A virtual reality obstacle course to improve lateral balance control in lower limb trauma patients

Authors: R. C. SHEEHAN[1], J. H. RYLANDER[1], J. M. WILKEN[2], *J. B. DINGWELL[3]; [1]Univ. of Texas at Austin, Austin, TX; [2]Brook Army Med. Ctr., San Antonio, TX; [3]Kinesiology, Univ. of Texas At Austin, AUSTIN, TX

Abstract: Lower limb trauma (LLT) leads to significant locomotor impairments resulting in a high risk of falling. Many falls occur during dynamic activities like walking which is least stable in the mediolateral direction. Thus, it is important to develop rehabilitation programs to improve mobility and lateral stability in individuals with LLT. However, little research exists to guide creation of such programs. It is imperative to investigate how patients respond to perturbations, not just steady state walking, since this is when falls are most likely to occur. Several balance and training studies physically perturbed the support surface or participant in the mediolateral direction either while standing or walking. However, such perturbations are not fully ecologically relevant and are not the only influences on lateral stability. Rapid lateral movements made during walking create internally derived, self-induced perturbations to normal walking dynamics. Such movements are required in many real-world contexts like walking down a crowded grocery store aisle or sidewalk. Virtual reality is becoming more prevalent and is being used during rehabilitation because it can simulate real-world destabilizing conditions, like navigating a crowded walkway, in a safe, controlled, and repeatable manner. VR also gives therapists more control of the training environment and allows them to quantitatively monitor patient progress in real time. Our goal was to develop a VR program that provides ecologically relevant perturbations through rapid lateral movements while navigating a virtual obstacle course. Patients walk in a "Computer Assisted Rehabilitation ENvironment" (CAREN) which consists of a 2 m wide treadmill surrounded by a semi-spherical screen. The position of an avatar on the screen is controlled by the tracked position of makers on the patient's pelvis. The goal is to navigate the avatar through a series of arches/gates placed in one of four lanes. Locations of the sets of arches are randomized to require patients to make transitions of 1 or 2 lanes to the right or left. A single trial consists of 16 transitions over ~3 minutes. The program also varies the distance from which patients see each new set of arches coming, so transitions can be more "anticipatory" or more "reactive". Ultimately, this program allows us to: (1) ASSESS impairments in lateral balance control during walking and (2) TRAIN people in a controlled environment to improve lateral stepping movement control and lateral balance. Initial findings suggest that patients both learn the task and improve their ability as they decrease the number of arch collision across the trials.

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Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.07/MM25

Topic: D.16. Posture and Gait

Support: This research was funded by the European Commission through MOVE-AGE, an Erasmus Mundus Joint Doctorate program (2011-0015).

Title: Timing of fast online corrections after tripping

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Abstract: Objectives: To successfully land after tripping may depend on how well one can make online corrections. For example, when one needs to avoid stepping on a dangerous surface (slippery patch, uneven ground) after tripping one needs to be able to modify the initiated recovery foot path. Can online adjustments during tripping be achieved and how fast can the nervous system intervene? Methods: Sixteen young adults (25.1 years, 6 females) walked at their comfortable speed over a walkway equipped with a hidden obstacle that could appear in mid-swing (eliciting an elevating strategy; Pijnappels et al. 2001). Participants were tripped 10 times in between a random number of normal walking trials; 5 trips included a projection of a forbidden zone (FZ, 30x50 cm) at the subject's preferred landing position. The FZ was triggered to appear 100 ms prior to obstacle release. Participants were instructed to land their recovery foot outside the FZ, if it was presented. To investigate how fast the nervous system achieves these adjustments, the EMG activity of the first active muscle (biceps femoris; BF) of the tripped leg was analyzed. This was done for five subjects (21-30 years, 1 female) who successfully used step shortening to avoid the FZ in all trials. After subtracting average normal walking activity from

the trips, activity onsets and EMG amplitudes normalized to normal walking and averaged over 20 ms bins were compared for the first 3 trips (without the FZ) and 5 trips with the FZ (T-FZ). Results: Overall, subjects succeeded in 80% of trials, by shortening their recovery steps (84%) or stepping to the side of the FZ (16%). The adjustments had no major negative effects on angular momentum. In all subjects, we observed an early BF activity burst just after obstacle contact in all tripping trials (20-60 ms). In the T-FZ trials this burst was followed by a second burst of a much higher amplitude than when no FZ was shown (related to the shortening of the step; BF is a hip extensor). This second burst occurred in an interval of 160-320 ms after obstacle contact. In addition, in T-FZ there were small but consistent very early changes in BF EMG activity (20-60 ms after contact), presumably related to the appearance of the FZ. Discussion: The high success rate shows that young adults are indeed able to adjust their foot trajectory in a balance challenging task as tripping. It is suggested that the success relies on two processes: one process responsible for the early changes, possibly in response to the appearance of FZ, and one slow process that occurs much later and clearly can rely on cortical processing. Further studies on elderly need to show whether there are age related changes in these processes.

Disclosures: Z. Potocanac: None. **S. Verschueren:** None. **J. van Dieën:** None. **M. Pijnappels:** None. **J.E. Duysens:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.08/MM26

Topic: D.16. Posture and Gait

Support: Jerome Lejeune Foundation

Georgia State University

Title: Walking dynamics in preadolescents with and without Down syndrome

Authors: *J. WU, M. BEERSE, T. AJISAFE, H. LIANG;
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Abstract: Children with Down syndrome (DS) display poorer kinematic and kinetic patterns of walking than age-matched typically developing children. It is generally suggested that low

muscle tone and joint laxity contribute to motor deficits in children with DS. However, there is limited information on neuromuscular control in children with DS during locomotion. A force-driven harmonic oscillator (FDHO) model sheds light on general muscular activation with respect to gravitational load of the thigh-shank-foot system. This model demonstrates its clinical values by revealing different muscular function in typically developing infant walkers and children with cerebral palsy. This study aimed to investigate the K/G ratio derived from the FDHO model between children with and without DS, i.e., a scaling between the elastic restoring torque from muscles and soft tissues and the gravitational torque from the weight of the leg during walking. Twenty six children with and without DS aged 7-10 years completed overground walking, and 20 children with and without DS completed treadmill walking in a lab setting. Overground and treadmill walking trials were collected on two lab visits. During overground walking, participants walked at two speeds: normal and their fastest speed. During treadmill walking, participants walked at 75% and 100% of the preferred overground speed without holding the handrails. Two load conditions were manipulated at both lab visits: no load and ankle load that was equal to 2% of body mass on each side. Children with DS showed a similar K/G ratio compared to their healthy peers during overground walking regardless of walking speed and ankle load. Children with DS produced a lower K/G ratio at the fast speed of treadmill walking without ankle load, but the inclusion of ankle load helped children with DS produce a similar K/G ratio as their healthy peers. Although no specific muscle was identified, the FDHO model demonstrates that children with DS are able to produce a similar general muscular activation like their healthy peers during overground walking, an important daily motor activity. However, performing a novel task such as walking on a treadmill at a fast speed exposes the neuromuscular limitations in children with DS. The inclusion of external ankle load appears to improve general muscle activation in children with DS, and may be a promising training component when designing an intervention protocol for children with DS to improve their motor function and bone health.

Disclosures: **J. Wu:** None. **M. Beerse:** None. **T. Ajisafe:** None. **H. Liang:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.09/MM27

Topic: D.16. Posture and Gait

Support: NASA EPSCoR Grant NNX11AM06A

Title: Locomotor adaptation to support surface perturbations is characterized by environmental decoupling

Authors: *D.-J. A. EIKEMA[1], J. H. CHIEN[1], N. STERGIOU[1], M. SCOTT-PANDORF[2], B. PETERS[3], J. BLOOMBERG[3], M. MUKHERJEE[1];
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Abstract: Human locomotor adaptation requires control processes driven by sensory feedback to maintain dynamic stability in response to environmental perturbations. Effective postural adaptation is characterized by a decoupling of the environmental perturbation and concurrent multisegmental movements of the trunk. Maladaptive adaptation in conditions of inaccurate sensory input may lead to postural instability. Whereas adaptation to support surface motion on static postural stability in standing is a much investigated topic, characteristics of dynamic stability in locomotion in similar environmental conditions are largely unknown. In this study we investigated locomotor adaptation to a continuously rolling treadmill. While walking on the treadmill, 20 participants performed a traditional motor learning and adaptation paradigm, consisting of baseline, adaptation (sinusoidal roll: ± 10°), catch, retention and transfer phases. Learning was quantified as a systematic decoupling of mediolateral treadmill supra-pelvic segment roll, computed as the cross-correlation function (XCF) between treadmill - trunk and treadmill - pelvis roll motion. Paired samples t-tests were performed to compare correlation coefficients between trials, within subjects. The maximum cross correlation is reported (lag range of 0-10s). The results revealed that adaptive decoupling primarily occured the learning phase, indicated by a systematic reduction in XCF in treadmill - trunk ($p<0.01$) and treadmill - pelvis coupling ($p<0.01$). The learned sinusoidal roll dynamics transfer to a random treadmill roll perturbation ($p<0.05$). The results indicate that as learning environmental dynamics occurs in locomotion, over time a stable decoupled state is achieved and maintained. Significant transfer of decoupling behavior to the random perturbation, suggests sensory reorganization as the driving phenomenon. Increased reliance on visual and vestibular information to encode physical orientation, as opposed to lower-limb proprioceptive signals allows supra-pelvic body segments to be controlled using sensory feedback from more stable sources in the environment. Over time this allows for the minimization of mechanical environmental influences. Subsequently, training and rehabilitation of sensorimotor organization abnormalities may benefit from the application of traditional learning paradigms and the inclusion of repeated exposure to varied sensory conditions to learn environmental dynamics and reorganize sensory systems accordingly.

Disclosures: D.A. Eikema: None. **J.H. Chien:** None. **N. Stergiou:** None. **M. Scott-Pandorf:** None. **J. Bloomberg:** None. **M. Mukherjee:** None. **B. Peters:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.10/MM28

Topic: D.16. Posture and Gait

Support: CFI New Opportunities Fund

University of Ottawa (UROP)

Title: Use of spike-triggered averaging (STA) to determine the contribution of a verbal reaction time task to center of pressure (CoP) movements during a dual-task

Authors: *M. BILODEAU[1,4,2], M. TAYLOR[3], V. MÉNARD[3], D. MCEWEN[1,4];
[1]Sch. of Rehabil. Sci., [2]Sch. of Human Kinetics, [3]Univ. of Ottawa, Ottawa, ON, Canada; [4]Aging & Movement Lab., Bruyère Res. Inst., Ottawa, ON, Canada

Abstract: INTRODUCTION : The dual task paradigm is commonly used to assess attentional demands associated with the control of posture, and often involves a secondary verbal cognitive task competing with a primary postural task for the same resources. It has been suggested that the simple production of words (articulating), regardless of cognitive load, may induce alterations in postural control variables. However, this has yet to be confirmed, particularly for discrete verbal responses used in reaction time tasks. PURPOSE: This study aims at documenting the magnitude of movement in the center of pressure (CoP) induced by a simple verbal reaction time task using the spike-triggered averaging technique. METHODS: Eleven healthy young adults consented to participate. Each of them were asked to perform a simple reaction time task consisting of verbally responding “top” as fast as possible following a computer-generated tone presented at random intervals, about 6 seconds apart. They were asked to perform the reaction time task under two postural conditions: 1) standing with feet together on a force platform (firm surface) with their eyes closed and 2) standing with feet together on a compliant surface (foam) placed on a force platform with their eyes opened. A given trial lasted 90 seconds and there were 8 trials per condition, for a total of about 120 verbal responses for averaging. CoP movements in both the antero-posterior and medio-lateral directions were obtained from the force platform and collected on a computer along with the auditory tones and verbal responses. Spike-triggered averaging was used to extract the movements of the CoP associated with the verbal responses in both postural conditions. RESULTS: A significant movement in the antero-posterior direction due to the verbal response was extracted from CoP

variations, and was particularly evident for the firm surface/eyes closed condition. The amplitude of this movement amounted to about 10% of peak-to-peak CoP variations. DISCUSSION: Even in the context of a discrete verbal reaction time task, the simple action of verbalizing a word intermittently can affect movements of the CoP. However, the magnitude of this effect may not be sufficient to lead to significant changes in global CoP variables typically used to quantify postural control.

Disclosures: **M. Bilodeau:** None. **M. Taylor:** None. **V. Ménard:** None. **D. McEwen:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.11/MM29

Topic: D.16. Posture and Gait

Title: Work potential of *Drosophila* larval bodywall muscles before and after actomyosin activation: implications of the winding filament theory

Authors: *J. L. KRANS[1], A. S. MACKIE[2], A. E. SCIBELLI[2];
[1]Neurosci., WNE, Springfield, MA; [2]Neurosci., Western New England Univ., Springfield, MA

Abstract: Titin is a giant sarcomere associated protein (gSAP) found in the muscle of a broad phylogeny that is positioned between the sarcomere's z-disc and myosin. This protein has been called elastic for its ability to recapitulate energy during lengthening and may be paramount to many forms of neuromuscular plasticity. Nishikawa et al. recently posed the winding filament theory, which suggests that upon actomyosin cycling, actin rotates about α-actinin and because titin's N2A domain can bind actin, the gSAP becomes wound around actin (2012, *Proc R Soc B*, v279). An implication of the hypothesized winding is a tethering effect between actin and myosin (by titin / the gSAP), effectively damping sarcomere length change as the tether length of titin progressively reduces with actin rotation. This is compatible with the multitude of examples that demonstrate active lengthening energy recapitulation; the effective spring constant of titin should increase as its length decreases concurrent with winding. We have examined this hypothesis by comparing mechanical tissue properties of muscle with or without prior actomyosin activation. We examined stress, strain, and work capacity of larval fruit fly muscles with a custom force ergometer capable of μN resolution. Body wall muscles of third instar D. melanogaster larvae were activated via electrical stimulation / direct depolarization. We based

activation parameters on en passant recordings of EMG patterns during locomotion: stimulus frequency was modulated from 5 to 30 Hz, and train duration, from 0.125 to 2 s. Net work over a +/- 10% body length range changed as a function of prior stimulus rate and duration, consistent with the tethering effect of a winding filament. Electrical activation prior to length muscle change increased basal tonus, significantly increased positive work during 60% of the duty cycle centered about the minimal tissue length, and marginally decreased negative work during 40% of the duty cycle centered about the maximal tissue length. These results suggest that as myosin slides past the N2A binding site on actin (approaching the Z-disc), titin and other gSAPs act as shortening tethers that have progressively increasing spring constants, damping length change and increasing energy recapitulation. Moreover, our data further support that a diversity of examples of neuromuscular plasticity - from molluscan catch to residual force enhancement - may arise from this winding mechanism.

Disclosures: J.L. Krans: None. **A.S. Mackie:** None. **A.E. Scibelli:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.12/MM30

Topic: D.16. Posture and Gait

Support: Univ. of Texas Research Grant (to NKB & JBD)

Title: Adaptability of stride-to-stride control in humans at a predicted walk-to-run transition speed

Authors: *N. K. BOHNSACK[1], J. B. DINGWELL[1], J. CUSUMANO[2];
[1]UT Austin, Austin, TX; [2]Engin. Sci. and Mechanics, Pennslyvania State Univ., University Park, PA

Abstract: Walking and running are essential tasks people take for granted every day. However, these are highly complex task that require significant neural control. This is complicated both by the inherent redundancy of the system and by physiological noise. Walking and running are thought of as two different tasks that usually occur at independent speeds, with potentially different goals. The walk-to-run transition (W-R) indicates that these are in fact two separate forms of locomotion. This study investigated the (W-R) transition dynamics during both walking

and running, based upon the assumption that these conditions would be more difficult or more "destabilizing" for subjects than their preferred walking and running conditions. Subsequently, Humans may adopt distinct control strategies to achieve these "uncomfortable" or in-between speeds. Of the many possible strategies that can achieve both walking and running, previous research has shown that humans prefer to try to maintain approximately constant speed from each stride to the next [Dingwell JB et al. PLoS Comput. Biol., 2010]. However, how humans alter the stride-to-stride regulation of their gait when the task goals may change (e.g., walking/running at the walk-to-run transition) has not been demonstrated. Here, 10 healthy adults (age 18-35) were asked to either walk or run on a motorized treadmill during the following 4 conditions: 1) preferred walking speed, 2) walking at the subjects predicted walk-to-run transition, 3) running at the subjects predicted walk-to-run transition, and 4) preferred running speed. Goal functions derived from the task specifications yielded new variables relative to each goal that define fluctuations relevant and irrelevant to achieving each goal. We calculated both the magnitude of the variability, as well as the stride-to-stride temporal fluctuations in these variables. Young healthy adults exploited different redundancy relationships in different ways when asked to walk/run at the same speed. While either walking or running at the predicted theoretical W-R transition, subjects were able to utilize a larger portion of the GEM solution space and operate in very different locations along the GEM to achieve the same goal. These subjects were able to largely exploit the redundancy within task goal (more so than in Dingwell, John et al. 2010), and to effectively operate at these "uncomfortable" speeds. Additionally, these results suggest that stride speed control is robust even with additional novel tasks and uncomfortable, abnormal speeds of locomotion.

Disclosures: N.K. Bohnsack: None. **J.B. Dingwell:** None. **J. Cusumano:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.13/MM31

Topic: D.16. Posture and Gait

Title: The effects of vibrotactile cuing on recovery kinetics after treadmill-induced trip in healthy young adults

Authors: *B.-C. LEE, M. STEFAN, T. A. THRASHER, C. S. LAYNE;
Univ. of Houston, Houston, TX

Abstract: It has been demonstrated that vibrotactile biofeedback decreases body sway during standing and gait. However, applications aimed at reducing and preventing falls with the use of vibrotactile cuing have not yet been fully investigated. The purpose of this study is to determine the effects of gait phase-dependent vibrotactile stimulus as a predictive cue to augment dynamic stability during a treadmill-induced trip perturbation. Currently five healthy young adults (25 ± 3.4 yrs) were instrumented with a safety harness and three miniature vibrating actuators (i.e., Tactors) placed on the skin over the left lateral head of triceps brachii, left external oblique, and left fibularis longus muscles. The participants walked on a split-belt treadmill equipped with two force plates at a self-selected walking speed. A trip perturbation was applied to the participant's non-dominant foot (left foot) by stopping the left belt at a loading phase coinciding with the period of initial double-limb support. During cuing trials, participants were instructed to take their first response step (non-trip foot) as quickly as possible after the trip perturbation. The stopped belt returned to the previous speed when the first heel strike of their non-trip foot (right foot) occurred. Participants walked a minimum of 10 steps prior to the first and all subsequent trip perturbations and baseline gait kinetic parameters were obtained. Vibrotactile cuing was provided to a single body location during each trial, either 250 or 500 ms prior to the time of the perturbation (lead time). The participants performed 1 trial without cuing, followed by 6 trials with cuing (2 lead time X 3 cuing locations), and followed by 1 trial without cuing. The cuing was stopped with the first response step. In each trial, the cuing locations were randomized. The number of steps for each perturbation (min. 10 steps) was randomized. Response time (defined as the time from trip perturbation to first response step) and recovery time (defined as the time from trip perturbation to return to baseline gait) were utilized to quantify recovery from the trip perturbation. When vibrotactile cuing was provided, participants significantly reduced their response time and recovery time. There was no effect of lead time or location of warning cue. The response step and recovery times were not significant across the non-cuing conditions, suggesting participants did not adapt their recovery strategy to trip perturbation over multiple exposures. These findings reveal that real-time vibrotactile cuing can be used to improve trip recovery by minimizing the response and recovery time by the use of vibrotactile cuing.

Disclosures: B. Lee: None. **M. Stefan:** None. **T.A. Thrasher:** None. **C.S. Layne:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.14/MM32

Topic: D.16. Posture and Gait

Title: Effects of adiposity on postural stability in overweight and obese adults

Authors: *H. MENG, B.-C. LEE, C. LAYNE, S. GORNIAK;
health and human performance, Univ. of Houston, Houston, TX

Abstract: In the U.S. it is estimated that over one-third of adults are obese (body mass index (BMI) greater than 30 kg/m2). It is well known that the incidence of obesity increases the risk of cardiovascular diseases and metabolic alternations. Recent studies that highlight correlations between neural activity and obesity have drawn increasing attention. These studies have suggested that obesity is associated with memory deficits and changes in motor function. The objective of the current study was to examine sensorimotor and cognitive responses of overweight and obese adults during basic standing tasks. Ten normal weight (BMI = 18 -24.9 kg/m2), ten overweight (BMI = 25 - 29.9 kg/m2), and ten obese (BMI = 30 - 40 kg/m2) adults were recruited (age: 24 ± 4). Study participants performed (1) a battery of sensorimotor evaluations via the Sensory Organization Test (via NeuroCom, Clackamas, OR) and (2) a working memory task (3 levels; 0-back, 1-back, and 2-back) while maintaining upright stance. Center of pressure (COP) area, COP path length, COP trajectory time to boundary values, and working memory reaction times were calculated to evaluate motor performance. BMI values were calculated from anthropometric data. Whole body and limb specific adiposity values were measured via dual-energy X-ray absorptiometry (DEXA) scans. Our results indicate that whole body adiposity percentage, rather than BMI, is significantly correlated with postural instability in overweight and obese individuals in all postural tasks evaluated.

Disclosures: H. Meng: None. **B. Lee:** None. **C. Layne:** None. **S. Gorniak:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.15/MM33

Topic: D.16. Posture and Gait

Title: How humans use visual optic flow to regulate stepping movements during walking

Authors: *M. SALINAS, J. B. DINGWELL;
Dept. of Kinesiology and Hlth. Education, Col. of Educ., The Univ. of Texas At Austin, Austin, TX

Abstract: When performing specific tasks, the motor system can often use many strategies to achieve the same task goal. For unperturbed walking, analyses that quantify averages across multiple strides cannot capture how each stride affects subsequent strides. Such methods provide little insight to the stride-to-stride control strategies humans use to regulate stepping. Conversely, healthy humans walking on a motorized treadmill try to maintain an approximately constant stride speed (*Sn*) at each successive stride, *n* (Dingwell et al., 2010). They do this by explicitly exploiting the inherent redundancy between stride length (*Ln*) and stride time (*Tn*). Analyses of stride-to-stride fluctuations revealed that subjects allowed deviations in *Ln* and *Tn* to persist across multiple consecutive strides, whereas deviations in Sn were rapidly corrected on subsequent strides. These results were obtained on a treadmill where subjects walked with *no optic flow*. However, during overground walking, healthy people allowed fluctuations in *Ln*, *Tn*, and Sn to persist, without correcting deviations in *Sn* (Terrier et al., 2005). Since visual optic flow is used to regulate walking, it is possible these different experimental results arose from differences in optic flow in each context. This experiment determined how humans alter stride-to-stride control of their stepping movements when optic flow is removed and/or systematically altered in a virtual reality (VR) environment. Twenty healthy subjects walked on a motorized treadmill at fixed speed and were presented with five different optic flow conditions: static VR scene (STA), blank screen (BLK), and VR scene with optic flow speed either matched to (MAT), slower than (SLO), or faster than (FAS) walking speed. We calculated magnitudes of variability, and the statistical persistence of stride-to-stride temporal fluctuations of *Ln*, *Tn*, and *Sn*. In general, subjects took shorter and faster strides during the BLK condition compared to the other four conditions. Thus, when visual information was minimized, individuals walked more cautiously. Likewise, during BLK, subjects exhibited greater stepping variability compared to the other conditions. Subjects exhibited the strongest covariance between *Ln* and *Tn* during STA. However, people adopted mostly similar fluctuation dynamics across all five conditions. These findings suggest that although manipulations of visual optic flow did significantly affect various measures of stepping performance, subjects generally tried to achieve the same stride-to-stride control strategy during each condition tested. These results are relevant to understand how VR systems may influence how people walk.

Disclosures: M. Salinas: None. **J.B. Dingwell:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.16/MM34

Topic: D.16. Posture and Gait

Support: Large scale project LSP4, Center of Excellence (EXC 277)

Title: Force and torque profiles of stick insects walking on compliant surfaces

Authors: *J. SCHMITZ, C. J. DALLMANN;
Dept. Biol. Cybernetics, Univ. of Bielefeld, Bielefeld, Germany

Abstract: Coordinated movements of multi-jointed limbs demand a considerable control effort for biological and technical walking systems alike. Feedback mechanisms that control forces for body weight support and propulsion have to be integrated with processes that establish and maintain adequate substrate adhesion in a context-dependent manner. Studies on insects have provided important insights into neuromechanical strategies underlying single joint control. However, surprisingly little is known about how joint controllers operate during unrestrained locomotion and manage to adapt to external disturbances such as unstable ground. Here, we study the functions of single legs and joints of stick insects (Carausius morosus) during free walking in different scenarios. We combined high-resolution motion capture with 3D ground reaction force (GRF) measurements to calculate net joint torques of single legs in stance. Force patterns support a functional division into accelerating hind legs that propel the animal forward, middle legs that support the body weight, and front legs that act either as 'feelers' or as rigid struts over large portions of their stance phases. Locomotor functions of single legs thus shared many characteristics of a climbing system, quite different from fast runners. On the level of single joints, locomotor functions varied depending on the leg and the progression of its stance phase. Important functional aspects include that torques at different joints can contribute to an overall leg function such as acceleration, and similar joint torques can produce functionally different force profiles depending on the operating point of the leg within its working range. Changes in horizontal substrate stiffness in the range of 0.3-400 N/m did not impair coordinated walking. When stepping on the softest substrate, the leg compensated for most of the substrate yielding (~6 mm) by increasing its extension, thus limiting the lateral shift of the body to less than 2 mm. Interestingly, the leg extension did not reduce the GRF components despite an increased lever arm. Full body weight support and acceleration was ensured by appropriate torque adjustments at the coxa-trochanter and femur-tibia joint. These findings indicate that the

integration of force feedback into the control scheme, in addition to movement control, is crucial for generating adaptive motor patterns.

Disclosures: J. Schmitz: None. **C.J. Dallmann:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.17/MM35

Topic: D.16. Posture and Gait

Support: Grant-in-Aid for Scientific Research (B) (23300238)

Title: Event-related brain potential and postural muscle activity during standing on oscillation floor with the joints of knee, hip and trunk fixed

Authors: *K. FUJIWARA[1], M. IREI[2], N. KIYOTA[2];
[1]Grad. Sch. of Med. Sci., Kanazawa Univ., Kanazawa, Japan; [2]Osaka Hlth. Sci. Univ., Osaka, Japan

Abstract: Dynamic postural control has been investigated during floor perturbation task. When the task is relatively novel, postural adaptation is clearly recognized. In the adaptation process, with the automatization of postural control, voluntary attention to the control target would change. If the degree of freedom on the postural control is restricted, the attention would be clearly focused on the target information, which could be measured by event-related brain potential (ERP). Thus in this study, with fixing the joints of knee, hip and trunk using the cast brace, postural control and ERP were investigated during standing on oscillation floor. Subjects were 12 healthy young adults. Cast brace was made for each subject in order to fix joints of the legs and trunk, except for the ankle. The subject maintained standing posture for 1 min (1 trial) on the force platform oscillated in the anteroposterior direction at 0.5Hz frequency and 2.5cm amplitude. The trial was repeated until the adaptation was recognized (5-10 trials), in the condition of no-joint fixation followed by the fixation. ERP from Cz electrode, postural muscle activity and joint movement angle were analyzed with divided into the 1st and 2nd trials (before adaptation) and the last two trials (after adaptation). With the fixation, movement angles of hip and knee joints decreased remarkably and postural control mode turned to pivoting on the ankle. Regardless of the conditions, relative muscle activity was large in the triceps surae (TS) and

erector spinae (ES), especially in gastrocnemius (GcM). Only GcM activity increased just after the fixation, and significantly decreased according to the adaptation. After the adaptation, a significant correlation was recognized between the activity peak latencies of the following muscles: ES-biceps femoris (BF) and BF-GcM in the no-fixation, and ES-TS in the fixation. Negative ERP gradually increased in the period from the posterior reversal point to the anterior point and peaked 80 ms after the anterior reversal point. In the fixation, the ERP peak latency showed the highest correlation with peak latencies of ES and TS after the adaptation. With the joint fixation, the knee strongly fixed and the ankle showed the highest mobility. It was possible that advancing the postural control adaptation under this condition, attention would be strongly directed to the muscle sensory information processing of TS and ES, and ERP peak near the anterior reversal point was closely correlated with these muscle activity peak latencies. In addition, a new synergy would be generated between TS and ES activities, relating to some mobility in the trunk.

Disclosures: K. Fujiwara: None. **M. Irei:** None. **N. Kiyota:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

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Program#/Poster#: 830.18/MM36

Topic: D.16. Posture and Gait

Support: ORF RE 04-47

NSERC Discovery Grant 227920-2010

Title: Postural adjustments are associated with rapid corrections to the upper limb and are dependent on the task goal

Authors: *C. R. LOWREY, J. Y. NASHED, S. H. SCOTT;
Ctr. for Neurosci., Queens Univ., Kingston, ON, Canada

Abstract: *Background:* Previous work has shown that standing postural adjustments precede corrections to arm trajectory by ~80-85ms following unexpected visuomotor perturbations. Such anticipatory postural adjustments, where postural corrections consistently lead arm corrections, seem implausible following mechanical perturbations given the rapid speed of arm corrective

responses (muscle activity changes within 60ms). We therefore hypothesized that the posture system is updated with feedback from the perturbed arm and that rapid corrective responses are initiated at the same time for both the postural system and the upper limb. We further hypothesized that postural responses would be modulated by the behavioural goal as previously observed for the upper limb. *Method:* Standing subjects performed rightward reaches to a target while grasping a robotic handle. Step torque perturbations were applied at the handle, orthogonal (anterior or posterior) to the direction of movement at a fixed onset distance of 3cm from the start target. Trials with perturbations (~20% of trials) were randomly interleaved with unperturbed reaches. In order to probe the flexibility of corrective responses, targets were presented as a circular dot or a rectangular bar. *Perturbation Response:* Arm muscle activity in the lengthened muscle increased at approximately 25-45ms following the mechanical perturbation to the handle. We observed that leg muscle activity also increased, but at 75-100ms post-perturbation. This led to corresponding deviations in center of pressure (COP) ~25-50ms later that opposed the direction of perturbation. *Responses are dependent on the goal:* As previously shown (in a seated paradigm) subjects corrected the hand back to the dot, but when reaching to the bar they utilized the redundancy along the end target and corrected to off-center locations. Both hand and COP trajectory were increased for corrections to the dot vs the bar which was observable at ~150-200ms following the perturbation. Correspondingly, ~75-120ms after perturbation arm and leg muscle activity was increased for corrections to the dot as compared to the bar. *Conclusion:* These findings suggest that the postural system is rapidly updated with feedback from the arm to elicit appropriate postural responses that are dependent on the demands of the task.

Disclosures: C.R. Lowrey: None. **J.Y. Nashed:** None. **S.H. Scott:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.19/NN1

Topic: D.16. Posture and Gait

Support: PHS Grant UL1 RR025008

NIH R21 HD075612-01

Department of VA R&D Service Career Development Award (E7108M)

Title: Does successful adapted tango rehabilitation improve postural response scaling in individuals with Parkinson's disease?

Authors: *K. C. LANG[1], J. L. MCKAY[3], H. COMPTON[2], M. HARRIS[2], J. PERRY[2], C. ROBERTS[2], M. E. HACKNEY[4,5], L. H. TING[3];
[1]Neurosci. Grad. Program, [2]Doctor of Physical Therapy Program, Emory Univ., Atlanta, GA; [3]Wallace H. Coulter Dept. of Biomed. Engin., Georgia Tech. and Emory Univ., Atlanta, GA; [4]Div. of Gen. Med. and Geriatrics, Emory Sch. of Med., Atlanta, GA; [5]Rehab R&D Ctr. for Visual and Neurocognitive Rehabil., Atlanta VA Med. Ctr., Atlanta, GA

Abstract: Rehabilitation programs have successfully ameliorated balance deficits in individuals with Parkinson's disease (PD), but the neural mechanisms underlying these improvements remain unclear. In postural responses to support-surface perturbations, individuals with PD demonstrate an impairment in the scaling of muscle response magnitudes across different biomechanical contexts. While participants without PD use lower muscle response magnitudes when standing at wide stance compared to a narrow stance, individuals with PD use similar muscle response magnitudes at both stance widths. We hypothesize that the impaired modulation of central set, or sensitivity to perturbations, across biomechanical contexts leads to postural instability in individuals with PD. Here, we predicted that central set modulation would be improved after successful balance rehabilitation, as reflected by larger differences in leg muscle response magnitude between wide and narrow stances. We studied 9 individuals with PD (Unified Parkinson's Disease Rating Scale mean ± SD: 30 ± 4.7; Hoehn & Yahr range: 2-3) who successfully completed a high-volume adapted Argentine tango rehabilitation program (90 minute sessions, 5 days/week for 3 weeks). Participants improved on the Berg Balance Scale and Fullerton Advanced Balance Scale ($p<0.05$). Surface electromyography (EMG) was recorded bilaterally from tibialis anterior and medial gastrocnemius and from the right tensor fasciae lata and soleus. Lateral support surface perturbations were administered while participants stood at either narrow stance (10 cm) or wide stance (>30 cm). Average EMG amplitude of each muscle in each trial was calculated over a time window of 80-450 ms after perturbation onset, to capture the long-latency postural response. Three of the 6 muscles demonstrated trends toward a greater difference in muscle response magnitude between wide stance and narrow stance after AT ($0.1 \leq p \leq 0.21$). These results suggest that successful balance rehabilitation may improve muscle response magnitude scaling across biomechanical contexts. Therefore neurorehabilitation interventions may improve basal ganglia deficits in modulation of central set, alleviating deficit in postural instability in PD.

Disclosures: K.C. Lang: None. **J.L. McKay:** None. **H. Compton:** None. **M. Harris:** None. **J. Perry:** None. **C. Roberts:** None. **M.E. Hackney:** None. **L.H. Ting:** None.

Poster

830. Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 830.20/NN2

Topic: D.16. Posture and Gait

Title: Tactile information from special underwear improves sitting posture of upper body both at rest and computer work

Authors: *Y. ATOMI[1], T. ATOMI[2], K. TANAKA[2], N. HIROSE[2], M. SHIMIZU[1], Y. KOYAMA[3], H. SUZUKI[4];
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Abstract: BACKGROUND AND AIM: Recently working time to use personal computer (PC) is extraordinary increasing. Long sitting using PC is apt to bring posture round-shouldered, induce eyestrain, and neck shoulder region (NSR) ache, resulting in the onset of "Repetitive Stress Injury (RSI)". We have previously developed and reported tactile information via closing special wear is effective to improve standing and walking posture. Our aim is to examine the effect of new inner for PC work (PC-inner, with attached patch on the back) , based on the hypothesis to help the function of tholacolumber fascia and to decrease COG change of upper body, and the balance of NSR muscle activities, to avoid NSRA and RSI. METHODS: Kinematic, kinetic and electromyography analyses were performed to determine effects of posture-support underwear at quiet rest and during PC-work by using 3D motion analysis system (VICON-MX, Oxford Matrics Inc.) , force plate (OR6-WP, AMTI Inc.) and surface electromyography (Noraxon Inc.). Quiet sitting posture with wearing PC-wear or control wear (with special patches and normal underwear) was evaluated using Vicon. The effect of PC wear during typing for continuous 5 minutes PC work was evaluated by the difference of right and left electromyography (EMG) of NSR (muscles of scalene, sternocleidomastoid, splenius cervicis, and trapezius). Subjects are 10 healthy adults students ages between 20-25 years old. RESULTS: PC-inner significantly brought quiet sitting posture to decrease trunk flexion angle (more extended position of trunk). PC-inner wearing influenced the gradual decrease of laterality difference of EMG of muscles of scalene, sternocleidomastoid, splenius cervicis during 5 min typing. Interestingly, EMG trapezius with control wear linearly increased during 5 min, while laterality ratio of trapezius EMG wearing PC-inner conversely decreased and attained to 1.0 (no

difference of left and right) . CONCLUSIONS: Our new developed PC-inner wear is effective underwear to help promote a more extended or straight position of the trunk, and to possibly avoid NSR ache in sitting positions.

Disclosures: **Y. Atomi:** None. **T. Atomi:** None. **K. Tanaka:** None. **N. Hirose:** None. **M. Shimizu:** None. **Y. Koyama:** None. **H. Suzuki:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.01/NN3

Topic: D.16. Posture and Gait

Support: NIH Grant NS058659

Title: Strategies for avoidance of small obstacles on the walking path

Authors: *V. MARLINSKI, K. M. I. CHU, S. H. SETO, M. D. FOE, J. H. TRAN, M. H. IZADY, I. N. BELOOZEROVA;
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Abstract: Avoidance of obstacles is essential for successful navigation through complex environments. Animals have the capacity to modify locomotor movements in different ways. The goal of this study was to clarify what are the preferred strategies for avoidance of small obstacles during quadrupedal locomotion. Three cats were trained to walk along 2.5 m corridor 30 or 15 cm wide. Small objects, white round pellets of 2.5 cm in diameter and 1 cm tall, were placed on the floor in various locations. Movements of the right forelimb were monitored with a motion capture system (Visualeyez) using LEDs attached to skin projections of forelimb joints. During unobstructed walking the stride length varied between cats and was 45±2, 47±3, or 50±1 cm. When a single pellet was placed in the wide corridor, cats avoided the obstacle by threading the walking trajectory around it, while keeping stride lengths similar to those during unobstructed walking. When a single pellet was placed in the narrow corridor, which limited lateral deviations of walking trajectory, cats avoided the obstacle by changing the stride length. When the pellet occupied an area where a cat normally steps during a passage, cats placed the foot before the pellet, shortening the stride. When the pellet was positioned in a way that shortened walking distance to the obstacle, foot placement became ambiguous: cats preferred to shorten the stride, but occasionally lengthened it by stepping over the pellet. When this distance was shortened to ~10% of normal stride length, cats always stepped over the obstacle. When the wide corridor was cluttered with 64 pellets, locations of which did not coincide with preferred foot placements

seen during unobstructed walking, cats did not change walking trajectory or stride length when passing through the corridor. When one of pellets did occupy an area of a usual foot placement, cats avoided the obstacle by stepping laterally to it, while maintaining the stride length. During walking along a pathway cluttered with pellets, cats elevated paws ~1 cm higher than during unobstructed walking and increased wrist plantar flexion during swing. Results show that avoidance of small obstacles on the walking path during quadrupedal locomotion can be achieved using two strategies. During walking in an environment that does not restrict lateral movements of the body, the preferable strategy is to make a lateral deviation of walking trajectory while maintaining a constant stride length. During walking in an environment that restricts lateral movements of the body, the strategy of obstacle avoidance is to change the stride length, preferably by shortening the stride, while maintaining the walking trajectory.

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Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.02/NN4

Topic: D.16. Posture and Gait

Support: Northeastern University Undergraduate Research Award

Title: A reinforcement approach to gait rehabilitation

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Abstract: In existing gait training programs, therapists usually act as “supervisors” who guide patients towards a predetermined, normal gait pattern. Such supervised learning (SL) is associated with rapid adaptation, but improvements quickly disappear once the supervisor is removed. In this study, we removed the supervisor and used a reinforcement learning-based approach (RL). The training “goal” was not specified to the learner. Instead, the learner needed to explore different gait patterns and was provided with reward signals to guide exploration. We hypothesized that a RL-based gait training approach would result in slower adaptation compared to SL, but improved retention and transfer of learned gait patterns. To test the hypothesis, we asked healthy young subjects to learn a new gait pattern on a treadmill. Subjects were randomly

assigned into either SL or RL groups. Both groups had the same goal: achieve 10° of ankle eversion 200 ms after toe-off. In the SL condition, subjects received visual feedback showing the goal ankle position, their actual ankle position, and the error between the two positions. In the RL condition, the goal was not revealed and subjects received categorical visual feedback showing "very close," "close," "fair," "away," or "far away," based on the proximity of the actual ankle position to the goal ankle position. RL subjects were asked to achieve "very close" as much as possible. Preliminary results show that both groups were able to consistently achieve the goal ankle position by the end of the training. Compared to the SL group, the RL group had a slower learning rate but was better able to reproduce the goal ankle position immediately and 24 hr after training in both treadmill and overground contexts. These preliminary results suggest that a gait training approach based on reinforcement learning principles may slow adaptation, but improve retention and transfer of learned gait patterns. Enhanced exploration associated with reward-based feedback may have contributed to these improvements.

Disclosures: C. Hoyt: None.

Poster

831. Posture and Gait

Location: Halls A-C

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Program#/Poster#: 831.03/NN5

Topic: D.16. Posture and Gait

Support: NIH Grant HD46922

NIH Grant R90 DA033462

Title: Hip and ankle responses for reactive balance emerge from task-level control of trunk and center-of-mass kinematics: A simulation study

Authors: *C. VERSTEEG[1], J. L. ALLEN[2,1], L. TING[2,1];
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Abstract: Why is one movement strategy selected over another to achieve a motor goal? For example, in reactive balance so-called ankle and hip strategies can be used to stabilize the center of mass (CoM) in the face of perturbations. Moreover, our prior research suggests that muscle activity in both hip and ankle "strategies" is governed by task-level feedback of CoM kinematics. Here, our goal was to test whether CoM feedback is sufficient to determine multi-joint strategies

for reactive balance control. We hypothesized that recruitment of muscles based on stabilizing the CoM would be insufficient to generate appropriate multi-joint strategies for reactive balance. To test this, we generated forward dynamic simulations of a human musculoskeletal model with 23 degrees of freedom controlled through 46 Hill-type muscles per leg in response to a backwards support-surface translation. We used a simulated annealing optimization algorithm to find muscle excitations that minimized muscle stress and the CoM deviation, as measured through anterior-posterior (AP) CoM movement. Including AP CoM as the only task-level variable in the cost function resulted in a "back-bend" response in which CoM movement was minimized by shifting the pelvis forward and leaning the trunk backwards, which is not typically observed in humans. We then added vertical trunk orientation as an additional task-level goal in the cost function. Interestingly, both hip and ankle response emerged depending on the weighting between the two task-level goals. Higher weights for trunk orientation produced simulations utilizing an ankle strategy, and lower weights for trunk orientation produced simulations utilizing a hip strategy. These results suggest that multi-joint strategies emerge from an interplay between control of the trunk and CoM, and that flexible and context-dependent weighting of task-level variables results in a continuum of multi-joint strategies for movement.

Disclosures: C. Versteeg: None. **J.L. Allen:** None. **L. Ting:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.04/NN6

Topic: D.16. Posture and Gait

Support: NSF Grant 0932263

Title: An FES controller for arm stability predicts human behavior in interaction tasks

Authors: *Y.-W. LIAO[1], E. M. SCHEARER[1], E. J. PERREAULT[2,3], M. C. TRESCH[2,3], K. M. LYNCH[1,4];
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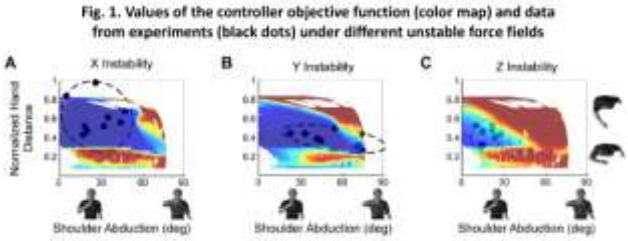
Abstract: Many daily interaction tasks are inherently unstable. For example, exerting forces on a tool such as a screwdriver can cause a destabilizing load on the arm that compromises postural

stability. The nervous system accounts for instability when producing movements and forces in healthy subjects, and a functional electrical stimulation (FES) controller must do so when attempting to restore movements in paralyzed subjects. The arm can be stabilized by generating a limb stiffness that sufficiently counters the destabilizing load. The endpoint stiffness of the arm can be modulated by co-contracting antagonist muscles or by adjusting posture while achieving the same endpoint location. Recently we developed an FES controller that considers these issues for tasks involving interaction forces. This controller chooses muscle activations and limb configuration in order to ensure limb stability while minimizing control effort. This study investigates whether our controller is consistent with human motor control strategies, examining whether it can predict the arm postures used by subjects in similar tasks. We use simulations replicating prior experiments (Trumbower et al. 2009), which showed that unimpaired subjects use the freedom to select arm posture to achieve better endpoint tracking in unstable force fields. Arm postures were chosen to increase the stiffness of the arm in the direction of unstable force fields. Our controller predicts the arm postures selected by the subjects in the experiment (Fig. 1). Moreover, the values of our objective function in different force fields are capable of explaining the variability of arm postures observed in the experiments (Fig. 1). These results indicate that our FES controller can predict humans' self-selected stabilizing postures during interaction tasks, and holds promise for restoring interactive movements to paralyzed subjects. Furthermore, these results suggest that our controller may be consistent with human motor control strategies during interaction tasks, though more rigorous testing will be required to draw such a conclusion.



Disclosures: **Y. Liao:** None. **E.M. Schearer:** None. **E.J. Perreault:** None. **M.C. Tresch:** None. **K.M. Lynch:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.05/NN7

Topic: D.16. Posture and Gait

Title: Intracortical inhibition is reduced during the control of upright standing in both young and old adults

Authors: *S. PAPEGAAIJ[1], W. TAUBE[2], S. BAUDRY[3], J. NÉGYESI[4], T. HORTOBÁGYI[1,5]; [1]Univ. of Groningen, Groningen, Netherlands; [2]Univ. of Fribourg, Fribourg, Switzerland; [3]Univ. Libre de Bruxelles, Brussels, Belgium; [4]Semmelweis Univ., Budapest, Hungary; [5]Northumbria Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Introduction Previously, we demonstrated an age by task difficulty interaction, showing lower short-intracortical inhibition (SICI) recorded from the tibialis anterior while standing on unstable vs. stable surface in old but not in young adults (Papegaaij et al. 2014). Here, we compared SICI recorded from the soleus while young and old adults stood with or without support on a stable surface, in order to determine whether there is also an age-related difference in the modulation of SICI during relatively simple postural tasks. Methods Fourteen young (age 23 ± 2.8 years, range 18-29, 9 men) and fourteen old adults (age 65 ± 4.1 years, range 60-76, 8 men) received single and paired pulse transcranial magnetic brain stimulation while they stood with or without support on a force platform. In the unsupported standing condition, participants stood naturally upright. In the supported standing condition, participants stood upright while lightly touching a wooden board at chest level to remove the need for the nervous system to control sway. Motor evoked potentials (MEPs) were recorded from the soleus muscle. Interstimulus intervals of 2.5 and 13 ms were used to assess SICI and intracortical inhibition (ICF), respectively. Results Center of pressure (CoP) velocity increased by 23% (SD = 12%) from supported to unsupported standing ($p < 0.001$), but was similar in young and old adults ($p = 0.397$). The amplitude of the test MEP was about 50% higher in old compared with young adults ($p = 0.041$), and was larger in unsupported compared with supported standing ($p = 0.032$). SICI was less during unsupported standing (35% inhibition) than during supported standing (50% inhibition) ($p = 0.016$), but was not affected by age ($p = 0.281$). Including background EMG as a covariate in the analysis shows that differences in background EMG can explain the age and condition effects in test MEP amplitude, but not the condition effect in SICI. Neither age nor condition affected ICF ($p = 0.694$, $p = 0.944$) and there was no age by condition interaction (CoP velocity: $p = 0.492$, SICI: $p = 0.816$, test MEP: $p = 0.477$, ICF: $p = 0.247$). Discussion In contrast to our previous study (Papegaaij et al. 2014) the present results indicate a posture-related modulation of SICI that is independent of age. Overall the results suggest that an age-related difference in modulation of SICI occurs most likely when postural demands are high. References Papegaaij, S., Taube, W., Hoogenhout, M., Baudry, S., Hortobágyi, T., 2014. Age-related decrease in motor cortical inhibition during different postural tasks. Neural Control of Movement 24th Annual Meeting, Amsterdam.

Disclosures: **S. Papegaaij:** None. **W. Taube:** None. **S. Baudry:** None. **J. Négyesi:** None. **T. Hortobágyi:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.06/NN8

Topic: D.16. Posture and Gait

Support: Fapesp

CNPq

Title: Coherence analysis of body segments during quiet stance over inclined surfaces

Authors: C. R. SILVA[1], A. P. PICON[2], *A. F. KOHN[1];
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Abstract: Human bipedal stance is often modeled as a single inverted pendulum that pivots at the ankle joint in the sagittal plane. However, recent studies have used coherence analysis to show that there are two, frequency-dependent, different patterns of coordination between the leg and trunk segments of the body during quiet stance on a horizontal surface. The present investigation had the objective of investigating whether standing on inclined surfaces (14º) changes the coherence functions of angles between leg and trunk segments. Eighteen healthy barefoot subjects were requested to keep a quiet stance for 60 s on three different surfaces: (1) toes-up (ankle dorsi-flexion, TU), (2) toes-level (horizontal, TL), and (3) toes-down (ankle plantar-flexion, TD) with eyes closed, hands lightly clasped behind the body and with feet shoulder-width apart. The subjects were asked to stand comfortably and no special instruction was given in any of the surface inclinations, leaving the subject to find his/her most comfortable orientation with respect to the gravity. Body segment angles (leg and trunk) were measured using an Optotrack motion tracking system and the root mean square of the center of pressure (COPAP RMS) was computed by a force platform. Coherence between leg and trunk angles with respect to vertical were significantly different from 0 in a frequency range from approximately 0.1 to 0.7 Hz for TU, TD and TL, with a phase of approximately 0º (in-phase). After this frequency range the coherence dropped and the phase could not be reliably estimated. Coherence increased again for frequency ranges from approximately 1.8 to 5 Hz, 3.0 to 3.5 Hz and 2.4 to 3.6 Hz for TU, TD and TL, respectively, with an anti-phase relationship between the leg and trunk segments in all conditions. The COPAP RMS value for TU was higher than for TL ($p<0.05$) but for TD there was no difference from TL. This suggests that TU standing (at 14o) poses a much greater challenge to the postural control system than a TD standing. The resulting changes in coherence were supportive of this point of view, since the frequency range of anti-phase movement

between the leg and trunk segments increased for TU compared with TL, but decreased to a very small frequency range for TD. This suggests that during TU there is an increase in non-pedular movement as compared with standing on a horizontal surface, but during TD the body's movement is more pendular, with very little anti-phase movement. Further analyses of other variables such as muscle EMGs are under way to provide a more global understanding of the possible mechanisms behind postural control of humans standing on ramps.

Disclosures: C.R. Silva: None. **A.F. Kohn:** None. **A.P. Picon:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.07/NN9

Topic: D.16. Posture and Gait

Title: Locomotor sequence learning in visually guided walking

Authors: *J. T. CHOI[1,2], P. JENSEN[2], J. B. NIELSEN[2];
[1]Univ. of Massachusetts Amherst, Amherst, MA; [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: We tested whether spatial sequence learning can be integrated with a highly automatic task like walking. Based on the serial reaction response task, introduced by Nissen and Bullemer (1987), we developed a visuo-locomotor task where subjects controlled step length to hit visual targets on a monitor while walking on a treadmill (Fig 1a). Subject performed a total of 7 blocks that each consisted of 100 steps (Fig 1b), without explicit knowledge of the sequence. In random blocks, the targets appeared randomly at locations that required different step lengths (i.e., short, normal, long). In sequence blocks, subjects were presented with a repeating sequence (i.e., short-long-normal-long-short-normal). The first random block (R1) was used to familiarize the subject to the task. The second random block (R2) provided a measure of final baseline performance. In subsequent training blocks (S1-3), subjects were presented with a repeating sequence. The last random block (R3) was used to measure overall improvement achieved. None of the subjects gave accurate descriptions of the repeating sequence when asked at the end. Implicit sequence-specific learning was calculated as the difference in performance between the last training block (S3) and the last random block (R3); non-specific learning was calculated as the difference between blocks R2 and R3. We tested 6 healthy control subjects walking at 2.3 ± 0.2 km/h. Group averaged number of hits increased over the three training blocks, but decreased again in the last random block (*$P < 0.05$). Performance changes were also measured using

endpoint error. We analyzed step frequency, and found that cadence was maintained across 7 blocks. The difference in performance on re-introduction of the random sequence indicates sequence-specific effects rather than non-specific effects. In other words, subjects used implicit knowledge about the step length sequence to plan and execute the movement (rather than simply reacting to the visual stimuli). The results suggest that step-by-step gait modifications can be optimized through visuo-locomotor training.

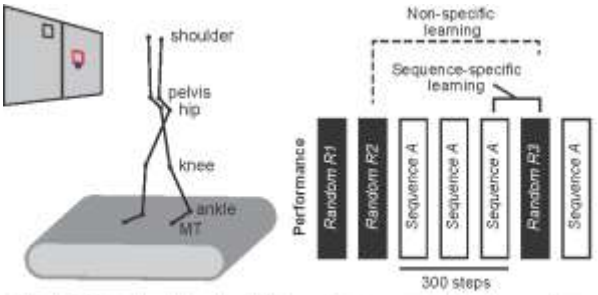

Fig 1. a) Experimental setup. b) Locomotor sequence learning paradigm.

Disclosures: **J.T. Choi:** None. **P. Jensen:** None. **J.B. Nielsen:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.08/NN10

Topic: D.16. Posture and Gait

Support: NSERC

Title: Revealing the cortical control of balance reactions during spontaneously occurring instability

Authors: *J. PAROKARAN VARGHESE[1], K. B. BEYER[1], V. MIYASIKE-DASILVA[1,2], L. WILLIAMS[1], W. E. MCILROY[1,2];
[1]Univ. of Waterloo, Waterloo, ON, Canada; [2]Canadian Partnership for Stroke Recovery, Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Balance reactions are critical to maintain upright stability and reveal highly complex responses evoked by moments of whole body instability. Even the control of static stability (standing still) is highlighted by continuous regulation of balance control involving multiple body segments over a small base of support. Despite the complexity of control, the task of

standing still appears to be very automatic leading to a historical view that balance control may be largely subcortical. However, the control of balance is likely dependent on a distributed neural network involving many regions of the CNS including a potentially important role for the cortex. The current study aims to reveal the specific involvement of cortical activity linked to the 'automatic' balance reactions that occur continuously when one is standing still. This work is focused on exploring the cortical events that temporally align with the occurrence of center-of-pressure (COP) reactions. The rationale is that COP excursions, specifically rapid reversal in direction, reflect corrective reactions to maintain stability of the center-of-mass and thus would yield a more focused view of the time-locked cortical activity linked to the control of reactive balance control. It was hypothesized that a fronto-central cortical activity is linked to the occurrence of naturally occurring transient COP corrections during quiet stance. Twelve young healthy adults (6 females) performed 30 sec of quiet standing (Standard Romberg and Tandem Romberg stance) on a force plate. Cortical activity during quiet standing was recorded using 36-channel EEG and analyzed by time-locking to peak COP excursions. Initial results revealed a fronto-central N1 response with mean (SD) peak amplitude and latency of -2.89 (2.34) μV and 84.3 (30.6) ms for Standard and -4.8 (2.31) μV and 100 ms (27.9) ms) for Tandem Romberg stance respectively. This study revealed a well pronounced negativity at approximately 100±10 ms before the peak COP excursions with a significant increase ($p<0.05$) in amplitude during Tandem Romberg stance. This finding parallels the N1 that arises within 100-200ms over the fronto-central cortical regions after any unpredictable postural perturbation suggesting that supplementary motor area is involved in maintaining postural stability. The present results suggest that this N1 also arises for naturally occurring perturbations thus revealing the cortical control in maintaining upright stability. This work is an important next step in evolving the methods to explore the cortical control of balance and will have potential application in the assessment of individuals with balance disorders.

Disclosures: **J. Parokaran Varghese:** None. **K.B. Beyer:** None. **V. Miyasike-daSilva:** None. **L. Williams:** None. **W.E. McIlroy:** None.

Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.09/NN11

Topic: D.16. Posture and Gait

Support: Award Number P20 RR016435 from the National Center for Research Resources

Title: Neural mechanisms and functional correlates of altered postural responses to perturbed standing balance with chronic low back pain

Authors: *J. V. JACOBS, C. L. ROY, J. R. HITT, R. E. POPOV, S. M. HENRY;
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Abstract: Background: Chronic low back pain (LBP) associates with modified postural responses to an induced loss of standing balance, but the neural mechanisms and functional relevance of these modified responses remain unclear. **Objective:** We sought to determine the effects of chronic LBP on cortical evoked potentials, muscle activation, and kinematic profiles of postural responses to a loss of standing balance, as well as to examine how response characteristics relate to pain, disability, and psychological coping. **Methods:** Participants included 13 subjects with chronic, recurrent, non-specific LBP (8 females; mean age = 37 yr) and 13 subjects without LBP (9 females; mean age = 35 yr). The subjects responded to an induced loss of balance while standing on a platform that randomly rotated either "toes up" or "toes down" to five degrees with a peak velocity of 23 degrees per second. Passive-marker motion capture was used to calculate joint displacements and center-of-mass (CoM) displacements. Surface electromyography was used to record muscle onset latencies. Scalp electroencephalography (EEG) was used to calculate the peak negative and subsequent peak positive potentials of the EEG voltage signal in response to the perturbations (the N1 and P2 perturbation evoked potentials, respectively). The Brief Pain Inventory (BPI) was used to assess pain and disability. The Fear Avoidance Beliefs Questionnaire (FABQ) and Coping Strategies Questionnaire (CSQ) were used to assess fear of activity and pain catastrophizing. Mixed-model ANOVA determined differences in outcome measures between groups and perturbation conditions. Pearson's correlation coefficients were used to correlate response characteristics to questionnaire scores. **Results:** Subjects with LBP exhibited significantly delayed erector spinae, rectus abdominae, and external oblique onset latencies, as well as smaller hip extension but larger hip flexion, knee flexion, and ankle dorsiflexion displacements compared to the subjects without LBP. Subjects with LBP also exhibited larger P2 cortical potentials. Smaller CoM displacements, as well as lower BPI disability subscores, CSQ catastrophizing subscores, and FABQ scores significantly correlated with larger P2 potentials at the CPz and Cz electrodes. **Conclusions:** Chronic LBP associates with higher cortical responsiveness over the sensory-motor cortex to perturbations of standing balance, and this enhanced cortical response associates with less postural instability, disability, fear of activity, and pain catastrophizing.

Disclosures: J.V. Jacobs: None. **C.L. Roy:** None. **J.R. Hitt:** None. **R.E. Popov:** None. **S.M. Henry:** None.

Poster

831. Posture and Gait

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.10/NN12

Topic: D.16. Posture and Gait

Support: NIA grant P30-AG028747

Veterans Administration Career Development Award

Title: Modeling differences in neuromotor balance control in response to falls-prevention training

Authors: J. E. BARTON[1,3], D. N. SAVIN[2], M. W. ROGERS[2], R. MACKO[1,3];
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Abstract: Falls account for 2/3 of all unintentional injuries leading to death in older adults. A variety of clinical tools have been developed to assess fall risk but most provide insufficient objectivity & sensitivity to assess risk, & are of limited use in quantitative analyses of balance. To objectively & quantitatively measure whole-body performance we developed a Balanced Reach Test (BRT). We hypothesize that this can discriminate clinically relevant performance differences between high fall risk (HFR) populations vs benchmark young healthy subjects (YHS) The BRT consists of a series of 90 s tracking tasks of a disk projected onto a screen that moves according to a 14-term sum of sines. Subjects stand in front of the screen & point with dominant index finger to the moving disk center. Overall excursion amplitude takes 1 of 5 values from 0.5 - 1.125 arm lengths. We also developed a 3D, 13 segment biomechanical model. We used it to compute joint forces, torques, & frequency response characteristics from the BRT results We tested 27 YHS, & 6 older HFR subjects before & after a 2 x 60 min/week, 8 week balance intervention. Our goal was to characterize benchmark performance in the BRT & identify how HFR subjects differed before & after training In YHS joints perform different but highly coordinated tasks to track disk & maintain balance. Lower extremity (LE) joints provide platform for upper extremity, conducting low frequency (< 0.15 Hz) weight shifts in response to broad left-right motion of disk. L5/S1 acts in mid-high frequency range of disk motion (> 0.55 Hz), positioning trunk to bring pointing finger near disk. Pointing shoulder & elbow also act in mid-high frequency range to bring finger into contact with disk. C7/T1 acts over entire frequency range to position head & keep disk in eyes' field of view Heterogeneity of HFR group required case by case analysis. One HFR leaned heavily to right side throughout task. Frequency response of LE, L5/S1 & C7/T1 joint activations were nearly identical & concentrated at the low end of range, indicating subject moved as a rigid inverted pendulum. Post-training performance dramatically improved. Weight distribution & shifting were symmetrical. L5/S1 & C7/T1

activated independently & frequency response shifted to mid-high range indicating independent motion of LE, trunk & head YHS BRT & model results highlight the high degree of multi-segmental control & coordination involved in task. This was clearly diminished for HFR pre-training but became similar to YHS post-training. Joint force/torque activations & frequency response in BRT are sensitive performance metrics & further study is underway to quantify their relationship to balance & fall propensity

Disclosures: J.E. Barton: None. **D.N. Savin:** None. **M.W. Rogers:** None. **R. Macko:** None.

Poster

831. Posture and Gait

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The views expressed herein are those of the authors and do not reflect the official policy or position of Brooke Army Medical Center, U.S. Army Medical Department, Department of the Army, Department of Defense or the U.S. Government.

Title: Strategies for controlling lateral stepping movements in human walking

Authors: *J. RYLANDER[1,2], J. M. WILKEN[2], J. P. CUSUMANO[3], J. B. DINGWELL[1]; [1]Dept. of Kinesiology and Hlth. Educ., The Univ. of Texas at Austin, Austin, TX; [2]Dept. of Orthopedics and Rehabilitation, Ctr. for the Intrepid, Brooke Army Med. Ctr., San Antonio, TX; [3]Engin. Sci. and Mechanics, Penn State Univ., University Park, PA

Abstract: Variability in human walking arises from musculoskeletal redundancy, physiological and external noise, and from the neurophysiological control actions that help regulate movement. Understanding how humans control the interplay between variability, redundancy, and task performance is important, especially in the frontal plane where people are less stable. This study sought to determine how humans regulate lateral (frontal plane) stepping movements during treadmill walking. Computational models operating on a step-to-step basis were developed based on pre-defined goal functions. Three candidate controllers tried to maintain either (a) constant absolute lateral position, (b) constant heading (forward motion), or (c) constant step width. A 4th multi-objective (MO) controller was developed to combine aspects of both step width and

position control. For each candidate control strategy, walking data were simulated for twenty trials of 1000 steps each. Experimental data were collected from 13 able bodied individuals (age 22-40). Each participant walked in a "CAREN" virtual reality environment on a wide (1.8m) treadmill for five 3-minute walking trials. Stepping parameters of interest included time series of right and left foot placements, change in lateral position (dLP), net lateral position (LP), and step width (SW). Means, standard deviations, and Detrended Fluctuation Analysis alpha values were calculated for each time series and used to compare controller predictions to experimental results. Each controller satisfied the goal of staying on the treadmill, but used different step-to-step strategies. No single controller fully captured all aspects of the behavior exhibited in the experimental data. Qualitative observation of right and left foot placements as well as quantitative evidence from the variability and DFA alpha values for LP, dLP, and SW time series indicated the SW controller exhibited the best fit compared to the pure LP or dLP controllers. The MO controller that was primarily weighted towards SW control with some additional contribution from the LP controller yielded predictions that more closely represented the experimental results. Therefore, evidence from computational controller predictions supports the idea that humans walk with a multi-objective control structure that prioritizes step width control, but also takes into account some degree of lateral position control. The prioritization of SW control is likely directly related to maintaining lateral balance during walking and is thus highly relevant for those who are prone to falling.

Disclosures: **J. Rylander:** None. **J.M. Wilken:** None. **J.P. Cusumano:** None. **J.B. Dingwell:** None.

Poster

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Topic: D.16. Posture and Gait

Support: US Army Medical Research and Materiel Command Grant W81XWH-13-C-0189

Title: Feasibility of an affordable custom-designed device with immersive virtual environment for postural assessment

Authors: A. P. DUMONT, K. O. APPIAH-KUBI, M. DUMONT, *W. WRIGHT;
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Abstract: Postural stability is a complex, multisensory process, which can reflect the overall health of the sensorimotor system. Clinical assessment of balance using equipment such as the Neurocom Sensory Organization Test (SOT) focus on sensory integration of visual, vestibular, and somatosensory inputs and there are established norms for healthy and compromised performance on these tests. We propose an alternate approach using affordable commercially available equipment such as the Nintendo Wii Balance Board (WiiBB), large flat-screen television, and custom-designed software. The purpose of this study was to compare the results in a series of objective postural assessments obtained using this novel device, which emulates the conditions that have been traditionally used in the SOT and Clinical Test of Sensory Interaction and Balance (CTSIB). Eleven healthy adults (21-45 years) without musculoskeletal injury or known sensorimotor pathology performed postural tasks standing directly on the WiiBB and on foam placed on the WiiBB while facing a virtual environment (VE) scene displayed on a 60" television. Three visual conditions were eyes closed/dark screen, eyes open/static image and eyes open/dynamic image (60 deg/s). The WiiBB recorded the COP at 30Hz for 60 sec each trial. The data was analyzed using traditional quantitative measures (COP velocity, RMS, sway area) and non-linear entropy measures (ApEn, SampEn, and Multi-scale). The results showed a significant effect of surface ($p<0.0001$) with foam conditions predictably being more destabilizing than firm conditions, and visual condition affecting postural stability ($p<0.0001$), with the most to least stable condition being eyes-open viewing a static scene, eyes closed in the dark, and eyes-open viewing a dynamically rotating scene. This was true for all traditional metrics: COP-velocity, RMS of the anterior-posterior and mediolateral COP, and sway area. Multi-scale entropy showed significant effects ($p<0.05$) due to changing visual and surface conditions across temporal scales, which were not evident using other metrics. In conclusion, a custom-designed device which employs affordable commercially available equipment demonstrated results that replicate previous findings using research-grade medical devices but was sensitive to changes in postural behavior that current clinical tests cannot detect. Future studies will determine concurrent validity and test-retest reliability of this device relative to criterion measures and determine its sensitivity/specificity for detecting sensorimotor deficits in injured populations.

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Poster

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Topic: D.16. Posture and Gait

Support: CIHR grant (MOP- 77548)

Richard and Edith Strauss Fellowship

Title: Influence of a simultaneous cognitive task on obstacle avoidance abilities in post-stroke individuals with and without visuospatial neglect

Authors: G. ARAVIND[1,3], M. VILLENEUVE[1,3], T. OGOURTSOVA[1,3], *A. LAMONTAGNE[2,3];
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Abstract: Dual-tasking that involves the performance of a cognitive task while walking can be challenging for post-stroke individuals. The presence of an attentional disorder such as visuospatial neglect (VSN) may further compromise the performance on the cognitive task, locomotor task or both. We observed the differences between participants with and without VSN on the ability to avoid moving obstacles while walking and performing a cognitive task. Methods: Three post-stroke participants- two with VSN (V1, V2) and one without VSN (S1) were assessed in a virtual environment consisting of a target and three obstacles located head-on (HO) and 30° contralesionally (CL) and ipsilesionally (IL). Participants were instructed to walk towards the target while avoiding the obstacle that randomly approached from one of the three directions. A simple pitch-identification task was used as a cognitive task. 3 tasks were performed a) a seated- cognitive task b) walking task alone and c) walking with cognitive task. We compared rates of cognitive error, the collision rates (CR), walking speeds and minimum distances from the obstacle between the cognitive alone /walking alone- single task (ST), and walking with cognitive task- dual task (DT) conditions. Results: No participant showed errors on the cognitive alone task. During the walking task, participant S1 showed collisions with HO obstacle during both the ST (CR= 40%) and DT condition (CR=40%) with no errors on the cognitive task. During the DT condition, however, participant S1 maintained larger minimum distances from obstacles as well as faster walking speeds for all three obstacle conditions, compared to the ST. Participant V1 showed no collisions during the ST but collided with CL (CR= 50%) and HO (CR= 20%) obstacles during the DT. Participant V2 showed collisions with CL obstacle during the ST and DT condition (CR=50%). Both V1 and V2 showed errors in the cognitive task during the DT condition for all three obstacle approaches (range= 20-100% of trials). V1 and V2 also maintained slower walking speeds and smaller minimum distances for all three obstacle approaches during the DT compared to the ST. Discussion: In the participant without VSN, addition of a simple dual task did not result in worsening of collision rates. Participants with VSN, however, demonstrated deterioration in cognitive or both locomotor &

cognitive performance during the DT condition. These preliminary results suggest that the addition of a simple cognitive task may burden the attentional resources in VSN resulting in worsening of task performance. Deterioration in obstacle avoidance performance may compromise safety while walking in the presence of moving objects.

Disclosures: G. Aravind: None. **M. Villeneuve:** None. **T. Ogourtsova:** None. **A. Lamontagne:** None.

Poster

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Program#/Poster#: 831.14/NN16

Topic: D.16. Posture and Gait

Support: NASA Grant

Title: Vestibular and somatosensory convergence in postural equilibrium control: Insights from spaceflight and bedrest studies

Authors: A. P. MULAVARA[1], C. D. BATSON[2], R. E. BUXTON[3], A. H. FEIVESON,[4], I. S. KOFMAN[5], S. M. C. LEE[5], C. A. MILLER[5], B. T. PETERS[5], T. PHILLIPS[5], S. H. PLATTS[4], L. L. PLOUTZ-SNYDER[1], M. F. RESCHKE[4], J. W. RYDER[1], M. B. STENGER[5], L. C. TAYLOR[5], *H. S. COHEN[6], J. J. BLOOMBERG[4];
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Abstract: The goal of the Functional Task Test study is to determine the effects of spaceflight on functional tests that are representative of high-priority exploration-mission tasks and to identify the key underlying physiologic factors that contribute to decrements in performance. We are currently conducting studies on both International Space Station (ISS) astronauts experiencing up to 6 months of microgravity and subjects experiencing 70 days of 6 deg head-down bed rest as an analog for spaceflight. Bed rest provides the opportunity for us to investigate the role of prolonged axial body unloading in isolation from the other physiologic effects produced by exposure to the microgravity environment of spaceflight. This allows us to parse out the contribution of the body unloading somatosensory component on functional performance. Both ISS crewmembers and bed rest subjects were tested using a protocol that evaluated

functional performance along with tests of postural and locomotor control before and after spaceflight and bed rest, respectively. Functional tests included ladder climbing, hatch opening, jump down, manual manipulation of objects and tool use, seat egress and obstacle avoidance, recovery from a fall, and object translation tasks. Astronauts were tested 3 times before flight, and on 1, 6, and 30 days after landing. Bed rest subjects were tested 3 times before bed rest and immediately after getting up from bed rest as well as 1, 6, and 12 days after re-ambulation. A comparison of bed rest and spaceflight data showed a significant concordance in performance changes across all functional tests. Tasks (i.e., fall recovery, seat egress/obstacle avoidance during walking, object translation, jump down) that require a greater demand for dynamic control of postural equilibrium showed the greatest decrement in performance. Functional tests with reduced requirements for postural stability showed less reduction in performance. Results indicate that body unloading resulting from prolonged bed rest impacts functional performance particularly for tests with a greater requirement for postural equilibrium control. These changes in functional performance were paralleled by similar decrement in tests designed to specifically assess postural equilibrium and dynamic gait control. These results indicate that body support unloading experienced during spaceflight plays a central role in postflight alteration of functional task performance. These data also support the concept that spaceflight may cause central adaptation of converging body-load somatosensory and vestibular input during gravitational transitions.

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Poster

831. Posture and Gait

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 831.15/NN17

Topic: D.16. Posture and Gait

Title: Effects of increased attentional demand on postural control vary across healthy individuals

Authors: *K. TERRY, J. TO-ALEMANJI, S. LYDICK;
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Abstract: There remains a critical need to reduce injurious falls by identifying diminished balance in individuals with underdiagnosed neurological and neuromuscular deficits. The use of multitasking to detect abnormal postural control has been studied for some time, but it is still unclear how tasks that increase attentional demand can be reliably applied in a clinical setting. Recently, postural sway in individuals with Parkinson's disease was found to be significantly higher than that for healthy controls as quantified by the jerk (rate of acceleration change) of the body's center of mass (COM). However, these studies have largely compared results between groups without examining individual responses. Additionally, most studies have measured sway either in terms of COM displacement and velocity or by using the combined centers of pressure (COP) of both feet. This study examined both COM and COP jerk for each foot under various visual and support conditions, and under different attentional demands. Five (of 20 planned) young, healthy individuals (2 male, 3 female) were screened for balance and mobility deficits using the Activity-specific Balance Confidence scale and High-level Mobility Assessment Tool. After screening, each individual stood quietly with one of two visual conditions (eyes open and closed) on one of two surfaces (firm and compliant) under three levels of attentional demand (no counting, counting backward by 10, counting backward by 7). Each of the 12 combinations was repeated 3 times for a total of 36 trials. A three-way ANOVA was then used to assess the effects of vision, surface type, and attentional demand on COM and COP jerk. As indicated by COM jerk, all participants were sensitive to surface conditions in both anterior-posterior (A-P) and medio-lateral (M-L) directions, and 4 participants were sensitive to visual condition in at least one direction. Only 3 subjects were sensitive to attentional demand in either direction. Alternately, COP jerk did not change significantly with visual condition, but 4 participants were significantly sensitive to different surface conditions and attentional demand. Notably, COP jerk for one participant was not sensitive to changes in any condition. Finally, COP jerk generally decreased with increasing attentional demand, whereas COM jerk increased with demand. These trends indicate that increased COM sway may be due to reduced COP control of the dominant foot as attentional demand is increased. Therefore, COM and COP jerk sensitivities to different sensory and cognitive demands may be more reliable indicators of individual fall susceptibility than the normative group means typically used in current assessments.

Disclosures: K. Terry: None. **J. To-Alemanji:** None. **S. Lydick:** None.

Poster

831. Posture and Gait

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Topic: D.16. Posture and Gait

Support: DFG Grant SCHO 336/7-1

Title: Stabilizing CoM velocity instead of position explains the slow drift in quiet stance

Authors: *H. REIMANN, G. SCHOENER;
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Abstract: The human body in quiet, upright stance is a mechanically unstable system. It has to be stabilized by an active feedback process generating appropriate muscle activation responses to counteract sensed deviations from a stable state. A combination of noise in the sensory estimation, neural processing and motor activation processes continuously perturbs the system, generating postural sway. Spectral analysis of the sway trajectories revealed two different sway components corresponding to distinct 'bumps' in the spectrogram (Zatsiorsky and Duarte, Motor Control 3:28-38, 1999). A fast, component oscillating around a reference point at high frequencies, superposed with a movement of that reference point at low frequencies. It is generally accepted that the high-frequency component is due to properties of the musculoskeletal periphery like viscoelasticity of muscles and tendons and reflex arcs in the spinal cord, similar to an underdamped PD-controller. The origins of the the low-frequency component are less well understood. It has been hypothesized that it comes from drift of the neural representation of the reference point due to integration of neural noise. This view has been disputed by Kiemel, Oie and Jeka, who showed that the origin of the low-frequency component is inside the feedback loop (J Neurophysiol 95:1410-8, 2006). They argued for misestimations of the state variables as the origin for the low-frequency component. As a step towards a mechanistic explanation of the low-frequency component of sway, we developed a physiologically detailed model of the body in upright stance using Hill-type muscles and monosynaptic stretch reflexes. We assume that the reference point of the stretch reflex is modulated based on sensory estimates of the body movement in space. A relatively simple feedback rule shifting the reference point backward when forward motion is sensed, and vice versa, successfully stabilizes the body against sensory and neural processing noise. This rule only uses estimates of velocity and acceleration, not position, in agreement with results from systems identification studies (Jeka et al., J Neurophysiol 92:2368-79, 2004). Our model successfully explains the high-frequency component as a result of low-level stretch reflexes and viscoelastic properties of the muscle-tendon complex. The reference point of that reflex loop is shifted based on high-level feedback from sensory signals. These signals are subject to noise, which is integrated over time by the system, resulting in the low-frequency sway component.

Disclosures: H. Reimann: None. **G. Schoener:** None.

Poster

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Topic: D.16. Posture and Gait

Support: Award Number P20 RR016435 from the National Center for Research Resources

Title: Evoked cortical potentials associate with center of mass displacement in response to an induced loss of standing balance

Authors: *R. E. POPOV[1], C. L. ROY[2], J. R. HITT[2], S. M. HENRY[2], J. V. JACOBS[2];
[1]Neurosci. Grad. Program, [2]Rehabilition and Movement Sci., Univ. of Vermont, Burlington, VT

Abstract: Background: Induced loss of standing balance evokes cortical potentials that can be recorded by electroencephalography (EEG). The functional relevance of these cortical potentials to balance recovery remains unclear because they have never been correlated to the balance recovery response. Objective: We sought to determine if the evoked N1 cortical potential relates to the magnitude of induced body displacement. Methods: Twelve healthy subjects (eight females and four males, ages 22-50 yr, mean = 35 yr) responded to an induced loss of balance while standing on a platform that randomly rotated either "toes up" or "toes down" to five degrees with peak velocity of 23 degrees per second and a duration of 490 ms. Passive-marker motion capture was used to estimate center-of-mass (CoM) displacement from the initial point of equilibrium. Scalp EEG was used to derive the peak negative amplitude of the EEG voltage signal following the perturbation (the N1 potential). Pearson's correlation coefficients were used to correlate CoM displacements with N1 potential amplitudes. Results: The N1 potential significantly correlated with CoM displacement in the "toes down" condition only at the Pz electrode ($r = -0.583$, $p = 0.047$), with near-significant correlations at the CPz ($r = -0.536$, $p = 0.072$) and Cz electrodes ($r = -0.533$, $p = 0.074$). Conclusions: The N1 potential appears related to the extent of induced body displacement, which is most readily detected over midline sensory cortex when responding to a forward loss of balance.

Disclosures: R.E. Popov: None. **C.L. Roy:** None. **J.R. Hitt:** None. **S.M. Henry:** None. **J.V. Jacobs:** None.

Poster

831. Posture and Gait

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Program#/Poster#: 831.18/NN20

Topic: D.16. Posture and Gait

Support: NSF Grant BCS-1230311

Title: The use of mechanical and visual perturbations to probe the control of human walking

Authors: *D. LOGAN[1], T. KIEMEL[1], J. JEKA[2];
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Abstract: To probe how the neural control of human locomotion ensures upright stability and the maintenance of position on a treadmill, we applied simultaneous independent visual and mechanical perturbations. Weak and continuous sagittal plane mechanical perturbations were applied to subjects (n=19) at the trunk via a harness attached to a translating linear motor while subjects walked on a treadmill placed within a three-panel virtual reality cave. A rotating virtual scene was projected consisting of randomly oriented triangles rotating around the ankle. Treadmill speed was 5 km h-1 in 10 trials (4 min) per subject. Phase-dependent impulse response functions (PD-IRFs) between the stimuli and trunk orientation, anterior-posterior (A-P) hip displacement and estimated gait cycle phase were computed across the gait cycle in normalized gait cycle time. The PD-IRF of trunk orientation relative to the mechanical stimulus showed responses in the same direction of the stimulus with a similar delay of 9% (±.25% s.e.m) of a gait cycle across stimulus phases. PD-IRF of trunk orientation relative to the visual stimulus also showed responses in the same direction of the stimulus with a similar delay across stimulus phases, yet this delay was on average 35% (±1.2% s.e.m) of a gait cycle across stimulus phases. The PD-IRF for A-P hip displacement also showed similar changes in the same direction of the stimulus across stimulus phases for both perturbations with responses to the mechanical stimulus starting earlier. The PD-IRF from the mechanical perturbation also showed A-P hip displacement in the opposite direction of the mechanical stimulus across the majority of stimulus phases. These counteracting changes began later (3.5-4 cycles from stimulus onset), and were not observed in the PD-IRF from the visual stimulus. These late, counteracting motions of hip displacement to the mechanical stimulus are likely why a phase lag, or elongation of gait cycle period, is observed in the PD-IRF of estimated phase approximately 9 cycles from stimulus onset. The results of the PD-IRF from mechanical perturbation suggest that counteracting changes in whole body position and modulation of gait period can be used to counteract continuous mechanical perturbations of the trunk across stimulus phases. This finding combined

with results of a simultaneous visual perturbation demonstrate that mechanical perturbations can be used during treadmill walking to gain insight about the control of human locomotion.

Disclosures: D. Logan: None. **T. Kiemel:** None. **J. Jeka:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

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Program#/Poster#: 832.01/NN21

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS-0923301

Title: Sex differences in aggression in response to serotonin (5HT) 1a receptor activation in the anterior hypothalamus (AH)

Authors: *J. I. TERRANOVA[1,2], Z. E. SONG[1,2], T. E. LARKIN[1,2], A. NORVELLE[1,2], H. E. ALBERS[1,2];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Ctr. for Behavioral Neurosci., Atlanta, GA

Abstract: Serotonin profoundly inhibits male aggression in species ranging from invertebrates to humans. In contrast, little is known about the effects of serotonin on aggression in females. Aggression is regulated by arginine vasopressin (AVP) and 5HT within the AH. Microinjection of AVP into the AH facilitates aggression in males and inhibits aggression in females, while a V1a receptor antagonist injected into the AH inhibits aggression in males and facilitates aggression in females. In male Syrian hamsters 8-OH-DPAT, a serotonin receptor 1a (5HT-1a) agonist, microinjected into the AH reduces aggression. However, the contribution of 5HT-1a receptors in the AH to the control of aggressive behavior in female Syrian hamsters is unknown. The goal of this study is to explore how 5-HT1a receptors in the AH contribute to the neural control of aggression in female hamsters. We tested the hypothesis that 5HT-1a receptors in the AH regulate aggression in males and females differently. Hamsters were singly housed for two weeks and were implanted with a unilateral cannula aimed at the AH. Following surgery, hamsters were handled and females were cycled daily. On testing days, hamsters were microinjected with either 8-OH-DPAT (100 μM) or saline. Five minutes after microinjection, hamsters were subjected to a five minute agonistic encounter with a non-aggressive intruder (NAI). Females were only tested during the diestrus stage of the estrous cycle and female NAIs were only used during proestrus. Duration of aggressive behavior was assessed for all

microinjected animals. There was a significant main effect of sex, with female hamsters (66.65 seconds ± 57.17 seconds) being more aggressive than male animals (16.46 seconds ± 28.32 seconds). There was no main effect of drug treatment but there was a significant interaction for sex and drug treatment on aggression (8-OH-DPAT males: 5.54 ± 11.06 seconds; saline males: 53.59 seconds ± 38.61 seconds; 8-OH-DPAT females: 74.42 seconds ± 63.78 seconds; saline females: 56.29 seconds ± 47.67 seconds). These data indicate that there is a substantial sex difference in the effects of activation of 5HT-1a receptors within the AH on aggression. This work was supported by NSF IOS-0923301

Disclosures: J.I. Terranova: None. **Z.E. Song:** None. **T.E. Larkin:** None. **A. Norvelle:** None. **H.E. Albers:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.02/NN22

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF-IOS-0923391

Title: Sex differences in the regulation of arginine-vasopressin (AVP) V1a receptors by social experience: Role in the expression of aggression

Authors: *A. P. ROSS, E. Z. SONG, H. E. ALBERS;
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Abstract: Social isolation increases aggression in both male and female Syrian hamsters. In males, AVP increases aggression by acting on V1a AVP receptors in the anterior hypothalamus (AH), and social isolation increases the number of V1a receptors in the AH. In females, however, AVP decreases aggression by acting on V1a AVP receptors in the AH, but the effects of social isolation on the number of V1a receptors in the AH are not known. The following experiment investigated whether there are sex differences in the effects of social isolation on the number of V1a receptors in the AH. Male and female Syrian hamsters were housed individually or with 2 other hamsters for 4 weeks. During the 4th week, all hamsters were handled, and estrous cycle was determined in female hamsters. To measure aggressive behavior, each hamster was paired with a same-sex nonaggressive intruder in a neutral arena for 5 min. Brains were collected immediately after testing and later processed for V1a receptor binding using

autoradiography. Social isolation increased aggression in both males and females. V1a receptor binding was greater in the AH of socially isolated males compared to those housed in groups. In contrast, there was no difference in V1a receptor binding between females that were socially isolated and those that were housed in groups. In conclusion, although sex differences were observed in the effects of social isolation on V1a receptor binding in the AH, no sex differences were observed in the effects of social isolation on aggression. The data support the hypothesis that social isolation increases aggression in males by increasing the number of V1a receptors in males. In contrast, the effects of social isolation on aggression in females are not mediated by the effects of social isolation on the number of V1a receptors in the AH. Importantly, these data suggest that there can be substantial sex differences in how the AVP system regulates social behavior.

Disclosures: **A.P. Ross:** None. **E.Z. Song:** None. **H.E. Albers:** None.

Poster

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Program#/Poster#: 832.03/NN23

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS-0923301

Title: Microinjection of arginine-vasopressin (AVP) in the ventral tegmental area (VTA) enhances conditioned place preference for social interaction

Authors: *Z. E. SONG, T. E. LARKIN, II, H. E. ALBERS;
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Abstract: AVP plays an important role in regulating social behaviors including social approach/avoidance and aggression, by its actions in brain regions that form part of the social behavior neural network. Little is known, however, about how AVP interacts with the mesolimbic reward system to affect social behavior. Previous studies in our lab have shown that social interactions between male Syrian hamsters have rewarding properties. Here we report microinjections of AVP into the VTA enhances the rewarding properties of social interactions (measured in a conditioned place preference (CPP) paradigm). Male hamsters were first tested for their initial preference for the chambers of a three-chamber CPP apparatus (pre-test). AVP or saline was then injected into the VTA prior to each conditioning session. Hamsters were

conditioned in the non-preferred chamber with another male hamster and in the empty preferred chamber alone in a counterbalanced manner each day for five days. After conditioning, hamsters were tested for their chamber preference in the post-test in the same way as in the pre-test. Our previous finding that social interactions induce CPP in saline control animals was replicated; hamsters spent more time in the non-preferred chamber in the post-test than in the pre-test (326.0±29.2s in the post-test versus 269.4±17.6s in the pre-test). In addition, injection of AVP into the VTA enhanced the rewarding properties of social interaction; there was a larger increase of time spent in the non-preferred chamber after conditioning in hamsters injected with AVP than in hamsters injected with saline (increase of time spent in non-preferred chamber: 128.7±24.5s in AVP animals versus 56.6±22.1s in saline animals). Interestingly, hamsters injected with AVP versus saline did not differ in their expression of aggression (attacking, pushing, and pursuing), social behavior (time spent interacting with the other conspecific) or non-social behavior (time spent alone) during the 5 days of conditioning. Hamsters injected with AVP, however, displayed more flank marks than controls during conditioning (16.3±4.4 in AVP animals versus 6.3±2.1 in saline animals). Our data demonstrate that AVP enhances the rewarding properties of social interactions by acting in the VTA and suggests that AVP might be important component in the mechanisms regulating social reward.

Disclosures: Z.E. Song: None. **T.E. Larkin:** None. **H.E. Albers:** None.

Poster

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Program#/Poster#: 832.04/NN24

Topic: E.03. Behavioral Neuroendocrinology

Title: A peripherally administered positive allosteric modulator of the oxytocin receptor enhances partner preference formation in prairie voles (Microtus ochrogaster)

Authors: *K. A. KITTELBERGER[1], H. SALAH-UDDIN[2], C. WAHLESTEDT[2], L. J. YOUNG[1];
[1]Emory Univ., Atlanta, GA; [2]Ctr. for Therapeut. Innovation and Dept. of Psychiatry and Behavioral Sci., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Oxytocin (OT) modulates several aspects of social cognition and behavior in mammals, including humans. The ability of OT to enhance social cognition makes the OT system a viable target for improving social functioning in psychiatric disorders, including

Autism Spectrum Disorders (ASD). Indeed, intranasal OT enhances some facets of social cognition in individuals with ASD. However, the efficacy of intranasal OT is likely limited by its poor penetration of the blood brain barrier and unknown diffusion properties within the CNS upon entry. The development of small molecule, brain penetrant compounds able to affect OT signaling represents an alternative, potentially highly effective strategy for enhancing social cognition. CTI-007 is a component of natural product that was recently discovered to be the first CNS-penetrant and highly selective positive allosteric modulator of the OT receptor. Partner preference formation in the socially monogamous prairie vole (Microtus ochrogaster) is an OT-dependent social behavior that has face, construct and predictive validity for functional human social cognition. In this study, adult ovariectomized female prairie voles received peripheral i.p. injections of CTI-007 (1mg/kg; n=11) or saline (n=11) prior to being cohabitated with a novel male (partner) for 6 hrs in the absence of mating. This paradigm does not typically result in a partner preference. Immediately following cohabitation, subjects were tested for partner preference, and time huddling with the partner or a novel stranger male was scored. In a two-way Friedman's ANOVA, there was no main effect of treatment ($F(1,20)=0.27$, $p=0.6$). There was a significant effect of stimulus animal (partner vs. stranger; $F(1,20)=16.2$, $p=0.0007$) as well as a significant treatment by stimulus animal interaction effect ($F(1,20)=4.34$, $p=0.05$). Post-hoc analysis confirmed that only females receiving CTI-007 spent significantly more time with the partner than the stranger (Student's T-test, $p=0.01$). A partner preference is defined as an animal spending twice as much time with the partner compared to the stranger. A one-tailed Chi square suggests that significantly more subjects receiving CTI-007 displayed partner preference compared to controls ($p=0.0381$). Thus, CTI-007 represents a novel approach to enhancing OT-dependent processes with potential therapeutic value for improving social cognition in psychiatric disorders characterized by social deficits.

Disclosures: K.A. Kittelberger: None. **H. Salah-Uddin:** None. **C. Wahlestedt:** None. **L.J. Young:** None.

Poster

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Support: NIMH R01MH095894

DOD W81XWH-11-1-0584

Title: Inhaling oxytocin alters social dominance behavior in rhesus macaques

Authors: *D. L. XIE[1,2], J.-F. GARIEPY[1,2,3], E. DU[1], M. L. PLATT[1,2,3];
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Abstract: In many species, animals emit signals that are interpreted by others to guide ongoing social interactions. In species with social hierarchies, such signals are critical for establishing social dominance. A key component in the brain mechanisms underlying these behaviors is oxytocin (OT), a peptide hormone that modulates social vigilance, social exploration, and prosocial behavior. Exogenous OT therapy has also been evaluated as a potential treatment for social dysfunction in neuropsychiatric and developmental disorders such as autism. Here we evaluated the effects of exogenous OT on social dominance behavior in rhesus macaques. Pairs of male monkeys were studied during controlled confrontations, in which one monkey (M1) was treated intranasally with either OT or saline and a second male (M2) was untreated. 64 pairs of 8 total monkeys from the same colony were studied. Each confrontation was video recorded and coded by two condition-blind experimenters. Monkeys were classified as dominant or subordinate based on the relative frequency of turning away from the opponent and averting gaze. OT reduced both turning away and gaze aversion in subordinate monkeys, but increased these behaviors in dominant monkeys. These effects are consistent with a "flattening" of the dominance hierarchy. Thus, exogenous OT can alter group dynamics during social interactions in nonhuman primates, with important implications for understanding the potential impact of OT therapy in humans.

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Poster

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Topic: E.03. Behavioral Neuroendocrinology

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UNO GRACA

Title: Treatment with centrally available oxytocin alters maintenance responsibilities in well-established adult sociosexual bonds

Authors: *J. CAVANAUGH[1], M. HUFFMAN[1], A. HARNISCH[1], J. A. FRENCH[1,2];
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Abstract: Maintaining long-lasting social relationships is a fundamental feature of living in a social group, and requires each individual to contribute equally. Behavioral strategies, such as maintaining close proximity and engaging in high levels of affiliative and sociosexual behavior with a long-term partner, are critical for the preservation of high-quality, adult sociosexual bonds. Oxytocin (OT), which has well-known functions in the mammalian birthing process, has been implicated in sociosexual bond formation and the modulation of social behavior in newly established adult sociosexual bonds (Lim & Young, 2006), yet there is still relatively little known about the mechanistic underpinnings of sociosexual bond maintenance. Thus, the goal of the study was to examine the effects of pharmacological manipulation of the OT system on the behavioral maintenance of well-established marmoset (Callithrix jacchus) pairs. We found that under control conditions, male and female marmosets engaged in equal levels of grooming behavior. However, when female marmosets were treated with an OT agonist, their male pair-mate undertook a greater proportion of the responsibility for maintaining grooming behavior. Additionally, under control conditions, male marmosets were more responsible for maintaining close proximity with their partner. However, when males were treated with an OT agonist, their female pair-mate undertook a greater proportion of the responsibility for maintaining proximity. Thus, treatment with centrally available OT resulted in a shift in responsibilities for maintaining close proximity and grooming behavior with a partner, away from the treated individual and toward a long-term pair-mate. Adult sociosexual bonds function to facilitate reproduction, and lessen detrimental health outcomes due to stress and anxiety (Carter, 1998). Therefore, behavioral strategies that help maintain long-term sociosexual relationships are essential, and it appears that central OT activity plays an important neuromodulatory role in the behavioral maintenance of long-lasting sociosexual bonds.

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Poster

832. Social Behavior: Oxytocin

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QUIP-RS

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P51OD11132

Title: Early social environment interacts with oxytocin receptor gene (Oxtr) variants to influence adult partner preference in monogamous prairie voles (Microtus ochrogaster)

Authors: *T. H. AHERN[1], L. B. KING[3], L. J. YOUNG[3], K. MORSE[2], S. REED[2], K. LUCYK[2], V. HEGEDUS[2];
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Abstract: Prairie voles are socially monogamous and biparental, and are ideal for modeling the interaction of early social environment and genetics on adult social behavior and brain. Prairie voles exhibit diversity in the 3' UTR of the Oxtr gene, and allelic SNP differences (e.g., C/C vs C/T) are associated with density differences in nucleus accumbens OXTR, a system that regulates social bonding. Here, we reared prairie voles under biparental (BP) or single-mother (SM) conditions. Homecage observations revealed differences in the amount of licking and grooming ($P = 0.006$) and nest exposure ($P < 0.001$) pups experienced in early life. Preliminary partner preference testing and genotyping of adult BP- and SM-reared offspring revealed a near significant trend toward a gene by environment interaction on adult social behavior ($P = 0.054$): BP-reared C/C carriers exhibited a significant partner preference while SM-reared C/C males did not, whereas C/T carriers exhibited robust partner preferences regardless of rearing condition. These data are consistent with findings in humans demonstrating a moderating effect of OXTR polymorphisms on the influence of early social experience on adult outcomes.

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Poster

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P51-OD011132

Title: Ontogenesis of oxytocin peptide expression in the prairie vole

Authors: *C. E. BARRETT, R. TRIANA DEL RIO, S. E. ARAMBULA, L. J. YOUNG; Cntr. Translational Social Neurosci, Emory Univ., Atlanta, GA

Abstract: The neuropeptides oxytocin (OT) and vasopressin (AVP) have been tightly linked to the control of social behaviors including pair bonding, parental care, social recognition, and aggression. The socially monogamous prairie vole (Microtus ochrogaster) is an excellent model to study the neural basis of complex sociality. Early postnatal experiences are salient predictors of adult social behavior and neurobiology, and recent work from our lab has suggested that early neuropeptide signaling may mediate the long-term effects of parental care in voles. However, the ontogenesis of OT and AVP in prairie voles is yet unknown. In rats, only the OT precursor (OT-neurophysin) and C-terminal extended forms of OT are detectable embryonically, suggesting OT is not functionally active around the time of birth. Here, we investigated perinatal neuropeptide expression in the prairie vole between embryonic day (E) 16 to postnatal day (P) 21 and the mouse between E18-P2, with P0 defined as the day of birth. Immunohistochemistry was performed using an antibody targeting the cleaved OT or AVP peptides (VA10, VA4; generously donated by H. Gainer, NIH). The number of OT-positive neurons in the paraventricular nucleus (PVN) of the hypothalamus increased between E16 to P2 and decreased to a final count at P21 (weaning; $F(6,30)=6.49$, $p<0.001$). OT was detectable in both mouse and prairie vole embryonically, but the pattern and level of expression was qualitatively and quantitatively distinct between the species. Prairie voles displayed significantly more OT neurons in the PVN at E18 ($p=0.011$), P0 ($p<0.001$), and, P2 ($p=0.001$). Oxytocin expression was more caudal in the mouse at E18, resembling earlier prairie vole E16 expression. This early species-specific expression may suggest that neonatal oxytocin signaling plays a distinct role in prairie voles. Sex differences in expression, analysis of fibers, and results of vasopressin ontogenesis will also be presented. An understanding of the normative progression of gene expression may shed light on the functional importance of neuropeptides during particular developmental windows. Neuropeptides may be critical in the development of social neural circuits and processing relevant social information from parent-offspring and sibling-sibling interactions.

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Poster

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Title: An oxytocin-induced facilitation of neural and emotional responses to social touch correlates inversely with autism traits

Authors: *D. SCHEELE[1], K. M. KENDRICK[3], C. KHOURI[1], E. KRETZER[1], T. E. SCHLÄPFER[1], B. STOFFEL-WAGNER[2], O. GÜNTÜRKÜN[4], W. MAIER[1], R. HURLEMANN[1];
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Abstract: Social communication through touch and mutual grooming can convey highly salient socio-emotional signals and has been shown to involve the neuropeptide oxytocin (OXT) in several species. Less is known about the modulatory influence of OXT on the neural and emotional responses to human interpersonal touch. The present randomized placebo (PLC)-controlled within-subject pharmaco-functional magnetic resonance imaging (fMRI) study was designed to test the hypothesis that a single intranasal dose of synthetic OXT (24 IU) would facilitate both neural and emotional responses to interpersonal touch in a context (female vs male touch) and trait (autistic trait load) specific manner. Specifically, the experimental rationale was to manipulate the reward value of interpersonal touch independent of the intensity and type of actual cutaneous stimulation administered. Thus, forty heterosexual males believed they were touched by either a man or a woman, although in fact an identical pattern of touch was always given by the same female experimenter blind to condition type. Our results show that OXT increased the perceived pleasantness of female, but not male touch, and associated neural

responses in the insula, precuneus, orbitofrontal and pregenual anterior cingulate cortex. Moreover, the behavioral and neural effects of OXT were negatively correlated with autistic-like traits. Taken together, this is the first study to show that the perceived hedonic value of human heterosexual interpersonal touch is facilitated by OXT in men, but that its behavioral and neural effects in this context are blunted in individuals with autistic traits.

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Poster

832. Social Behavior: Oxytocin

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Topic: E.03. Behavioral Neuroendocrinology

Title: Different oxytocin receptor polymorphisms are associated with independent effects on gray and white matter volume in the human brain

Authors: *T.-V. NGUYEN[1], G. HANSEN[2], J. KIPPENHAN[2], B. KOLACHANA[1], P. J. SCHMIDT[3], K. F. BERMAN[2];
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Abstract: Background: Oxytocin is increasingly understood to be a central modulator of human social behavior. Among the oxytocin receptor gene (*OXTR*) single nucleotide polymorphisms(SNP), the minor (A) alleles of both rs2254298 (G/A) and rs53576 (G/A) have been linked to socio-behavioral impairment and to sexually dimorphic structural changes in social processing regions, such as the hypothalamus, amygdala and anterior cingulate cortex. These two SNPs are located at different points along the largest *OXTR* intron, which separates the 7th transmembrane domain and 3' untranslated region from all other coding regions. Despite evidence linking genetic variation in these two polymorphisms to specific brain and behavioral phenotypes, the extent to which each SNP carries independent effects on brain structure remains unclear. **Methods:** We examined associations between SNPs rs53576 and rs2254298 and Jacobian-modulated gray matter volume (GMV) and white matter volume (WMV) in 289 subjects (1.5T T1-weighted MRIs, 18-55 years old, 157 females). We tested for main effects of genotype and haplotype, and sex-by-genotype and sex-by-haplotype interactions, controlling for

age, total brain volume and multiple comparisons across the whole brain (cluster-level $p<0.05$). **Results:** Each SNP was associated with a distinct brain phenotype. While the rs53576 A/A genotype was associated with decreased WMV in the internal capsule, the rs2254298 A/A genotype was associated with increased WMV in parts of the internal capsule and geniculocalcarine fibers, increased GMV in the right caudate and brainstem, and decreased GMV in the right temporal pole. Sex-by-genotype analyses showed similarly distinct effects: the rs53576 A/A genotype was associated with decreased prefrontal and cerebellar GMV in males relative to females and the rs2254298 A/A genotype was associated with increased cerebellar WMV and thalamic GMV in males relative to females. The two SNPS showed weak linkage disequilibrium ($r^2=0.011$) and no consistent haplotype dosage effect in any brain regions. **Conclusions:** Findings support distinct effects of each *OXTR* SNP on brain structure, possibly related to different regulatory function of rs53576 (closer to the 5' coding section) and rs2254298 (closer to the 3' non-coding section of the gene). Findings in diverse brain regions suggest that these SNPs may play a role in both primary sensory and motor function and higher-order cognitive processes.

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Poster

832. Social Behavior: Oxytocin

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Topic: E.03. Behavioral Neuroendocrinology

Support: VIEP-BUAP Salud 2014

PROFOCIE-BUAP 2014

Title: Central administration of oxytocin produced a different behavioral syndrome in the high-respect to low-yawning subline of Sprague-Dawley rats

Authors: *J. EGUIBAR[1], M. CORTES[2], A. UGARTE[3], O. ISIDRO[3];
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Abstract: Central administration of low doses of oxytocin induced more yawning and penile erections in the high-yawning rats and more scratches episodes in the low-yawning (LY). These differences were maintained after systemic administration of D2-like dopaminergic agonists or after intracerebroventricular (i.c.v.) injection of adrenocorticotrophic hormone being more responsive the HY rats. The aim of this study is to analyze the behavioral effects induced by i.c.v. injection of oxytocin (OXY) in a range of 15 to 480 ng/ 2 microliters (microL) of isotonic saline solution (SS). All subjects were maintained under standard conditions with a light-dark cycle 12/12 with lights on at 0700 with free access to rodent pellets and water. At 100 days old, the male rats were implanted with a stainless steel cannula in the right lateral ventricle using stereotaxic frame and Paxinos and Watson atlas. One week after recovery, all subjects were acclimated to experimental conditions and the fourth day each receive only one dose of OXY or SS using a Hamilton syringe and Microdrive to inject 2 microL in a 3 min period. The entire behavioral repertoires were recorded using video camera by 120 min and analyzed later by a blind observer using The Observer XT software. Data were analyzed through ANOVA followed by Tukey test. The results showed that male HY rats OXY-induced a larger increase on yawning and penile erections frequencies than in the LY subline ($P<0.05$). However, the number of scratching bouts were higher in the LY respect to that obtained in the HY rats ($P<0.05$). The number of grooming bouts did not differ between sublines. In conclusion, yawning and penile erection behaviors had higher responses to OXY with low-doses in the HY rats, in a quite similar pattern as already demonstrated with D2-like agonists, which imply differences in the connectivity or in the responses of the paraventricular nucleus of the hypothalamus, a key hypothalamic area for the induction of these behaviors. On the other hand, LY rats showed an increased in scratching episodes indicating that supraspinal or the central pattern generator in the spinal cord is more sensible in this subline.

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Title: Mechanisms underlying sex-specific regulation of social play by vasopressin: An *in vivo* microdialysis study

Authors: *R. BREDEWOLD, J. K. SCHIAVO, M. VERREIJ, A. H. VEENEMA;
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Abstract: We recently showed that arginine vasopressin (AVP) within the lateral septum (LS) regulates social play behavior in sex-specific ways in juvenile rats. Specifically, blockade of the AVP V1a receptor enhances social play in males, but reduces social play in females. However, the mechanisms by which AVP regulates social play are unknown. Because *in vitro* studies suggest that AVP modulates GABA and glutamate responses in the LS, we first determined whether AVP modulates the release of GABA and glutamate in the LS of males and females. Using *in vivo* microdialysis combined with retrodialysis in freely moving single-housed juvenile rats, we found that exogenous AVP increased LS-GABA release in males while no change was seen in females. Moreover, AVP decreased LS-glutamate release in males while increasing it in females. These findings suggest that the sex-specific regulation of social play by AVP may involve differential GABA and glutamate signaling in the LS. We next determined whether social play modulates GABA and glutamate release in the LS of males and females. By exposing rats to social play during ongoing *in vivo* microdialysis, we found that social play induced similar release patterns of GABA and glutamate in males and females. Because males have denser AVP fibers in the LS than females, males may release more AVP than females. Such a sex difference in AVP may have induced the similar release patterns of GABA and glutamate observed during social play behavior. We are currently testing this hypothesis by measuring AVP release in the LS of males and females exposed to social play. Finally, we will determine whether blockade of AVP V1a receptors prior to social play induces sex-specific release patterns of GABA and glutamate. Together, these findings will reveal for the first time the involvement of GABA and glutamate within the LS in the sex-specific regulation of social play by AVP.

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Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.13/NN33

Topic: E.03. Behavioral Neuroendocrinology

Support: NRSA Predoctoral Fellowship 1F31MH100891-01A1 to KMD

Title: Oxytocin administration induces sex-specific alterations in brain activation in awake rodents using fMRI

Authors: *K. M. DUMAIS[1], P. KULKARNI[2], C. F. FERRIS[2], A. H. VEENEMA[1]; [1]Psychology Dept., Boston Col., Chestnut Hill, MA; [2]Ctr. for Translational Neuroimaging, Northeastern Univ., Boston, MA

Abstract: Oxytocin is a sexually dimorphic neuropeptide involved in the regulation of social behavior in both rodents and humans. Importantly, oxytocin has been shown to have promising effects on improving social dysfunction in patients suffering from sex-biased psychiatric disorders, such as autism. However, little is known about potential sexually dimorphic effects of oxytocin on brain function. Our aim was to investigate sex-specific neural changes after oxytocin administration using functional magnetic resonance imaging (fMRI) in awake male and female rats. Our experiments were conducted in a Bruker Biospec 4.7-T/40-cm horizontal magnet (Oxford Instrument, Oxford, U.K.). Functional images were acquired using a multi-slice fast spin echo sequence. A single data acquisition included 22, 1.1mm slices which covered the entire brain from brain stem to olfactory bulb. The functional imaging session consisted of 25 min (5 min of baseline data followed by 20 min of experimental data). Rats were habituated daily for 4 consecutive days to the fMRI procedure. Subjects were then tested on two separate days, receiving intraperitoneal injections of either vehicle or oxytocin (0.1mg/kg), in counterbalanced order. We found that systemic injections of oxytocin induced greater positive BOLD activation in a variety of cortical areas, including the insular cortex and somatosensory cortex in females compared to males. In addition, systemic oxytocin induced greater negative BOLD in hippocampal areas, ventral tegmental area, and ventral pallidum in females compared to males. To compare differences in brain activation due to the type of route of administration of oxytocin, we are also currently investigating sex differences in brain activation after intracerebroventricular injections of oxytocin in rats. Results will be informative for how oxytocin may be acting differently in the brains of males and females, which is imperative to consider if oxytocin-based drugs are to become a major treatment option for psychiatric disorders of social dysfunction.

Disclosures: K.M. Dumais: None. **P. Kulkarni:** None. **C.F. Ferris:** Other; Animals Imaging Research. **A.H. Veenema:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.14/NN34

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Graduate Research Fellowship 2012138127 to CJWS

Brain and Behavior Research Foundation grant 17382 to AHV

Title: Age differences in the brain oxytocin system: Implications for juvenile social motivation

Authors: *C. J. SMITH, M. L. POEHLMANN, K. B. WILKINS, R. BREDEWOLD, A. H. VEENEMA;
Boston Col., Chestnut Hill, MA

Abstract: Juvenile animals engage in peer interactions more frequently than their adult counterparts and appear to find these social interactions to be more rewarding. These differences may reflect age-specific alterations in brain systems implicated in social behavior. One candidate is the oxytocin (OT) system, as it is a well-established regulator of a wide variety of social behaviors. While OT has been shown to act in concert with components of the brain reward circuitry to promote socially rewarding behaviors in adults (such as mother-infant attachment and pair-bonding), its role in the regulation of social reward in juvenile animals is less well understood. To address this issue, we compared OT receptor (OTR) binding densities in the brains of juvenile and adult rats. We hypothesized that OTR binding differences between juveniles and adults might mediate the age difference in peer interactions. Our results reveal a multitude of age differences spanning a wide range of brain regions. We find higher OTR binding densities in juveniles as compared to adults in the medial and ventral anterior olfactory nucleus, nucleus accumbens core, dorsal and medial caudate putamen, intermediate and ventral lateral septum, and ventral hippocampus. In contrast, OTR binding is higher in adults than juveniles in the bed nucleus of the stria terminalis, posteroventral medial amygdala, ventromedial hypothalamus, agranular insula, and perirhinal cortex. No age differences were found in the dorsal peduncular nucleus, nucleus accumbens shell, dorsal lateral septum, central amygdala, or dorsoventral medial amygdala. The observed age differences in OTR binding in the nucleus accumbens core and dorsal caudate putamen are of particular interest as these regions are involved in motivation and reward. We are currently testing the hypothesis that higher OTR binding in these areas may underlie the heightened propensity of juvenile animals to engage in behaviors such as social novelty-seeking and social play. Elucidating the role of OT in socially rewarding behaviors during the juvenile period may be critical to understanding its potential as a treatment option for development disorders such as autism spectrum disorders.

Disclosures: C.J. Smith: None. **M.L. Poehlmann:** None. **K.B. Wilkins:** None. **R. Bredewold:** None. **A.H. Veenema:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.15/NN35

Topic: E.03. Behavioral Neuroendocrinology

Title: Mechanisms underlying sex differences in the brain oxytocin system

Authors: *N. B. WORLEY, L. E. NEWMAN, A. H. VEENEMA;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: The neuropeptide oxytocin (OT) has been shown to modulate social behaviors, often in sex-specific ways. This may be due to sex differences in the brain OT system. In support, our lab has recently shown sex differences in OT receptor (OTR) binding densities in various forebrain regions of adult rats. Some of these differences correlated with social behavior in sex-specific ways. The most robust sex difference was found in the posterior part of the bed nucleus of the stria terminalis (BNSTp), in which males showed higher OTR binding density than females. However, the molecular mechanisms underlying these sex differences in OTR binding remain unknown. We therefore investigated the potential molecular mechanisms that may lead to differential OTR binding density in the BNSTp. We hypothesized that the sex difference in OTR binding density may be mediated by transcriptional and/or epigenetic modifications of the OTR gene resulting in a sex difference in OTR mRNA expression. However, our preliminary results do not show a significant difference in OTR mRNA expression in the BNSTp between male and female rats. If we can verify these findings, this suggests that other mechanisms, perhaps translational modifications, may underlie the sex difference in OTR binding density. A next step would then be to compare OTR protein levels in the BNSTp between males and females. Furthermore, we will determine whether similar mechanisms mediate sex differences in OTR binding densities in other brain regions. Identifying the mechanisms mediating sex differences in OTR binding densities may help understand OT-mediated sex differences in social behavior.

Disclosures: N.B. Worley: None. **A.H. Veenema:** None. **L.E. Newman:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.16/NN36

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS – 1310908

Title: Vasotocin and V1aR exert organizational influences on courtship and pair maintenance behaviors in the zebra finch

Authors: *N. M. BARAN[1], M. L. TOMASZYCKI[3], E. ADKINS-REGAN[2];
[1]Dept. of Psychology, [2]Dept. of Psychology, Neurobio. & Behavior, Cornell Univ., Ithaca, NY;
[3]Psychology, Wayne State Univ., Detroit, MI

Abstract: Zebra finches demonstrate long-term pairing (selective affiliation) which is characterized by proximity, vocal communication and contact behaviors. This experiment tested the hypothesis that the nonapeptide arginine vasotocin (AVT, avian homologue of vasopressin) and the V1a receptor subtype (V1aR) play an organizational role prior to fledging in courtship, affiliative behavior, and pair bonding in adults. Zebra finch hatchling males received daily intracranial injections (posthatch days 2-8) of either AVT, Manning Compound (MC, a V1aR antagonist) or a saline control. We assessed social development through a series of behavioral assays. On posthatch day 90, males were introduced to an unmanipulated female and allowed to pair for seven days. We measured courtship behaviors (directed song, beak wipes), attempted copulations, and pair maintenance behaviors (time spent perching in contact and allopreening) with the cage mate. To induce the expression of pair maintenance behaviors, the birds were separated from their cage mate for one hour seven days after introduction and then reunited. Ninety minutes following reunion, the subjects were sacrificed, and their brains removed, sectioned, and processed using double-label fluorescence in-situ hybridization to measure the co-localization of the protein ZENK and V1aR mRNA. We then tested whether the early exogenous administration of AVT or MC altered the adult distribution of neurons expressing V1aR in the extended medial amygdala and the lateral septum. In addition, we tested whether the neurons expressing V1aR were active during courtship, pair bonding and affiliation. These results assess whether AVT and V1aR play organizational roles in social development, perhaps modifying early attentiveness to social stimuli and attachment leading to downstream differences in courtship and pair bonding behaviors.

Disclosures: N.M. Baran: None. **M.L. Tomaszycki:** None. **E. Adkins-Regan:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.17/OO1

Topic: E.03. Behavioral Neuroendocrinology

Support: R01MH096983

1P50MH100023

P51-OD011132

Title: Genetic variation in the oxytocin receptor exerts robust regionally-selective control of expression to facilitate prairie vole pair bond formation

Authors: *L. B. KING[1,2,3], K. INOUE[1,2,3], L. J. YOUNG[1,2,3];
[1]Ctr. for Translational Social Neurosci, Yerkes Natl. Primate Ctr., [2]Silvio O. Conte Ctr. for Oxytocin and Social Cognition, [3]Dept. of Psychiatry, Emory Univ., Atlanta, GA

Abstract: Oxytocin modulates social information processing in the brain. Brain expression of the oxytocin receptor (OXTR), varies greatly between species and appears to contribute to diversity in social behavior. In the socially monogamous prairie vole, OXTR density is high in the nucleus accumbens (NAcc) compared to promiscuous vole species. OXTR signaling in the NAcc is essential for pair bond formation, and elevating OXTR in that region with a viral vector shortens the necessary cohabitation time for female prairie vole bonding. Despite a high species average, OXTR density in the prairie vole NAcc has a large variance relative to other brain regions. We have previously reported that this regionally selective expression variance is strongly associated with a genetic polymorphism, as evidenced by a three-fold allelic imbalance of OXTR messenger RNA from this region. Preliminary results suggested that the T-allele of our allelic imbalance marker SNP2, located in the 3' untranslated region of the OXTR, also predicts overall OXTR density in the NAcc. We therefore predicted that voles homozyogotic for the SNP2 T-allele would have a higher density of OXTR binding in the NAcc and would form pair bonds, as assessed by a partner preference, more readily than C-allele homozygote siblings. We established C/T heterozygous breeder pairs to generate offspring of all genotypes. Male offspring were tested for partner preference after a 6 hr cohabitation with an intact virgin female. Male prairie voles in our lab normally require a 24 hour cohabitation with mating to exhibit a partner preference. We then examined OXTR binding density in the brain. We found that male T/T voles (n=33) formed a partner preference, spending significantly more time huddling with their partner than the stranger (t-test, $p < 0.05$), while C/C siblings (n=37) did not. We also confirm the effect of SNP2 on OXTR density, T/T voles have a mean NAcc density 69% larger than that of C/C voles (t-test, $p < 0.05$). Thus we have identified a polymorphism with high predictive power over a social behavior phenotype through control of gene expression in a brain regions-specific

manner. Thus prairie voles serve as an excellent model to understand how genetic variants that are associated with behaviors relate to underlying physiology. This is important given the growing number of experiments relating non-coding SNPs in the human OXTR gene with endophenotypes and disorders of social behavior such as autism spectrum disorders.

Disclosures: **L.B. King:** None. **K. Inoue:** None. **L.J. Young:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

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Program#/Poster#: 832.18/OO2

Topic: E.03. Behavioral Neuroendocrinology

Support: MH096983 to LJY

P51-OD011132 to YNPRC

Title: Oxytocin-mediated FOS expression in social information processing brain areas in male prairie voles following cohabitation with a female

Authors: ***Z. V. JOHNSON**, Y. JAMAL, M. XIAO, L. J. YOUNG;
Yerkes NPRC, Atlanta, GA

Abstract: Pair bonding in prairie voles depends on numerous signaling molecules acting in different brain areas during a period of social interaction and mating between partners; however, it remains unclear how the transient signaling of these molecules ultimately gives rise to permanent alterations in behavior. One of these essential signaling molecules is oxytocin (OT), and has been shown to play a role in social behavior and information processing across species. In this study, we infused oxytocin receptor antagonist (OTA; N=20) or vehicle (N=19) into the cerebral ventricles of sexually naïve male prairie voles prior to a 30 minute exposure to an estrogen primed, sexually receptive female. Control animals received infusions but were not exposed to a female. 90 minutes after initial exposure, male subjects were euthanized and their brains were analyzed for expression of FOS protein using immunohistochemistry. FOS expression is typically associated with recent neuronal activation, robust changes in transcriptional activity, and long-term changes underlying synaptic plasticity in response to stimuli. FOS positive nuclei were quantified in brain areas hypothesized to comprise a social information processing network. Individual behavior was video recorded and scored for all 30

minute exposures. Initial analyses show significant elevation in FOS expression in the anterior olfactory nucleus, prefrontal cortex, and medial amygdala following 30-minute exposure to a female relative to unexposed controls, regardless of OTA or aCSF treatment ($p<0.05$). We found no significant differences in mean levels of FOS expression between treatment groups in these regions; there was a significant interaction effect of treatment (OTA vs. aCSF) and social investigation time on FOS expression in the medial amygdala ($p=0.0167$). We are conducting further analyses to address how OT signaling and behavior modulate neural activity throughout the social information processing network during acute exposure to a female. This work should provide insight into the neural mechanisms by which oxytocin contributes to pair bond formation in monogamous species.

Disclosures: Z.V. Johnson: None. **Y. Jamal:** None. **M. Xiao:** None. **L.J. Young:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

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Title: The effects of intensive meditation training on oxytocin, vasopressin, and attachment style

Authors: Q. A. CONKLIN[1], B. G. KING[1], A. P. ZANESCO[1], T. L. JACOBS[1], J. J. POKORNY[1], S. R. AICHELE[3], D. A. BRIDWELL[4], K. A. MACLEAN[5], K. L. BALES[2], P. R. SHAVER[2], E. L. ROSENBERG[1], B. A. WALLACE[6], E. FERRER[2], B. K. SAHDRA[7], *C. D. SARON[1];

[1]Ctr. for Mind and Brain, [2]Dept. of Psychology, Univ. of California At Davis, Davis, CA; [3]Univ. of Geneva, Geneva, Switzerland; [4]The Mind Res. Network, Albuquerque, NM; [5]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [6]Santa Barbara Inst. for Consciousness Studies, Santa Barbara, CA; [7]Australian Catholic Univ., Strathfield, Australia

Abstract: An emerging body of literature implicates the neuropeptides arginine vasopressin (AVP) and oxytocin (OT) in human social behavior, particularly with regard to human attachment. Other research highlights the potential for contemplative practices to enhance adaptive psychological traits and experienced well-being. Such findings may occur alongside concomitant changes in biological markers related to affiliation. Here we report the first study exploring the longitudinal effects of intensive meditation training on relations between attachment avoidance, a fundamental dimension underlying adult romantic attachment, and blood plasma levels of AVP and OT. All measures were obtained before and after a three-month Shamatha meditation retreat from participants randomly assigned to a training (n=30, 16 females) or matched wait-list control (n=30, 16 females) group. Partial correlations between pre-retreat attachment avoidance and levels of AVP or OT were calculated for the combined group of 60 participants to determine preexisting relations among these variables in this specific population. Given the sexually dimorphic roles of AVP and OT in human social behavior, we controlled for gender and found a significant relation between AVP and avoidant attachment, $r(48) = .317$, $p = .025$, but not between OT and attachment avoidance. Analyses were then conducted to explore the effects of training. A significant decrease in attachment avoidance was observed from pre- to post-retreat in the training group but not in the control group. However, there was no effect of training on the levels of AVP or OT. To determine if the observed change in attachment avoidance could in part be accounted for by change in AVP, four regression models were tested using change in AVP as a predictor of change in attachment avoidance for each gender in each group. Change in AVP significantly predicted change in attachment avoidance for the training group males, but not for control males, control females, or training group females. Parallel analyses were conducted using change in OT as a predictor, but these results were not significant. These findings support a pattern of complex dimorphic relations between these neuropeptides and dimensions of human attachment in a population of contemplative practitioners. They do not, however, suggest a clear mechanism for change relating to Shamatha meditation training. To further explore these patterns in a second cohort of practitioners, data collected during a one-month Vipassana retreat will be analyzed.

Disclosures: Q.A. Conklin: None. **B.G. King:** None. **A.P. Zanesco:** None. **T.L. Jacobs:** None. **J.J. Pokorny:** None. **S.R. Aichele:** None. **D.A. Bridwell:** None. **K.A. MacLean:** None. **K.L. Bales:** None. **B.K. Sahdra:** None. **P.R. Shaver:** None. **E.L. Rosenberg:** None. **E. Ferrer:** None. **B.A. Wallace:** None. **C.D. Saron:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

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Program#/Poster#: 832.20/OO4

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH-HD042882

UNO-GRACA

Title: Marmosets' responses to inequity following oxytocin manipulation

Authors: *A. MUSTOE[1], J. A. FRENCH[2];
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Abstract: One of the principal properties of primate cooperation is the egalitarian sharing of resources, which provides a foundation for exhibiting inequity aversion (IA). Marmosets (Callithrix sp.) serve as an exemplary model species because marmosets are cooperatively-breeding New-World primates who form socially monogamous pair-bonds and exhibit spontaneous prosocial behaviors. Thus, examining this cooperatively-breeding in marmosets, which is a rare social structure among primates, may elucidate important insight into the function and social context for IA. Across mammals, oxytocin regulates social bonds and interpretation of social signals, and oxytocin is an important neuroendocrine mechanism underlying cooperative behaviors. Further, marmosets possess a unique variant of the oxytocin sequence, where a proline substitution replaced leucine in position eight of the oxytocin nonapeptide. In this study, we examined how oxytocin influences food sharing and social behavior in multiple pair-bonded and non-pair-bonded opposite-sex marmoset dyads. Marmosets performed a prosocial choice task where donors provision food in both equitable and unequitable outcomes to themselves and their partners or strangers. We administered two oxytocin agonists (Pro8 and Leu8), an oxytocin antagonist, and saline controls to marmoset donors to evaluate the influence of oxytocin on IA. Marmosets do not differentially provision food to others in equitable ($F(2,6) = .82$, $p > .05$, $\eta 2 = .22$) or unequitable ($F(2,6) = .18$, $p > .05$,$\eta 2 = .06$) outcomes, and IA was not influenced by oxytocin ($F(6,18) = 1.38$, $p > .05$, $\eta 2 = .31$). Marmosets tested with strangers spent a marginally increased time in proximity with their homecage partner following testing compared to marmoset donors who were tested with their partner or tested alone ($F(2,6) = 4.59$, $p = .06$, $\eta 2 = .61$). Overall, marmosets do not show sensitivity to IA, but their social behavior following testing is influenced differently by the testing partner's social affiliation regardless of oxytocin treatment. Unlike many other primates, marmosets may tolerate unequal sharing in exchange for

maintaining functional cooperative-breeding structures. Future research will focus on the role of oxytocin on more socially-salient cooperative tasks and further disentangle marmosets' flexible behavior exhibited to pair-bonded and non-pair-bonded opposite-sex partners. This research was supported by NIH-HD042882 and UNO-GRACA.

Disclosures: **A. Mustoe:** None. **J.A. French:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.21/OO5

Topic: E.03. Behavioral Neuroendocrinology

Support: ONR Grant N000141210393

Title: Effect of oxytocin on rat neural activity and behavior in a reward-based task

Authors: L. A. DAVIES, A. FONTANINI, *G. LA CAMERA;
Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: The neuropeptide oxytocin (OT) is reportedly involved in the regulation of social behaviors by acting on structures of the fronto-limbic-striatal reward circuitry in humans - a phenomenon often associated with reduced activation of neural activity in amygdalar and striatal regions of the human brain. Recent data also suggest that socially reinforced learning depends on amygdala (AM) function and can be enhanced by OT. Consistently, recent studies in rodents have found that stimulation of OT fibers to the AM decreases amygdalar output to the nucleus accumbens. These brain regions are part of the neural circuitry engaged by reinforcement learning and other types of non-social reward, including cortical areas such as the orbitofrontal cortex (OFC), and suggest that OT may be involved in non-social reinforcement learning. To test this hypothesis, we have performed electrophysiological recordings in alert rats, and have previously reported a reduction of the spontaneous activity in a subset of neurons in the OFC and the basolateral nucleus of the amygdala (BLA) in the presence of OT. Here, we report on a preliminary investigation of the effect of OT on behavior and neural activity of rats engaged in a simple reward-based task. Long Evans rats were trained to press a lever for water reward in the presence of an auditory cue. After training, simultaneous electrophysiological recordings were performed from OFC and BLA neurons during behavior. In each session, the rats were infused intracerebroventricularly with aCSF (control) and then with OT (1ug/5ul icv), and the effect on

both behavior and neural activity was measured. In preliminary data from 1 rat so far, the generic reduction in spontaneous activity reported earlier seems to coexist with more sluggish behavior - as quantified by slower reaction times and more numerous error trials under OT compared to aCSF. This behavior was not present or was much reduced in control experiments where OT was replaced by a second infusion of aCSF, and thus it is not likely due to disengagement or satiation that could naturally emerge over time. We also observed the cessation of previously acquired responses to the cue predicting reward (or to the reward itself) in a larger number of neurons under OT compared to aCSF. These data suggest that OT may have a role in motivation and reward-based learning in a non-social context by altering the cortico-amygdalar-basal ganglia reward circuitry, in particular, by reducing OFC and AM activity associated to reward and reward-predictive cues.

Disclosures: L.A. Davies: None. **G. La Camera:** None. **A. Fontanini:** None.

Poster

832. Social Behavior: Oxytocin

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 832.22/OO6

Topic: E.03. Behavioral Neuroendocrinology

Title: Mechanisms underlying oxytocin's protective effects on phencyclidine-disrupted prepulse inhibition of the acoustic startle reflex

Authors: *M. E. RICH, H. K. CALDWELL;
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Abstract: One endophenotype of schizophrenia is impairment in sensorimotor gating, which reflect an inability to screen out unimportant stimuli, and lead to sensory inundation, disordered thinking, and cognitive fragmentation. Across species, sensorimotor gating can be measured using prepulse inhibition (PPI) of the acoustic startle reflex. We have previously found that Oxt is protective against phencyclidine (PCP)-induced disruptions of PPI. To confirm that the PCP-induced PPI effects in Oxt −/− mice are due to the absence of Oxt signaling, we found that central administration of Oxt to Oxt −/− mice was able to partially rescue the PCP-induced disruption of PPI. Based on work in Oxt receptor knockout (Oxtr −/−) mice, we have evidence that whatever protective effects are being provided by Oxt, that it may not be via the Oxtr; since Oxtr −/− mice do not do not appear to display increased vulnerability to the PPI-disrupting effects of PCP. Since Oxt has been found to also signal through the vasopressin 1a receptor

(Avpr1a), we hypothesized that Oxt was acting on the Avpr1a to mediate its protective effects on PCP-disrupted PPI. However, we were unable to further disrupt PCP-induced deficits in PPI in Oxtr −/− mice following the central administration of an Avpr1a antagonist. Finally, glutamate signaling has been shown to regulate PPI, and is important to the functioning of the CSPP circuitry. Because there is some evidence that Oxt may interact with this system, we also quantified NMDA receptor expression within the CSPP circuit in Oxt −/− mice, Oxtr −/− mice, and wildtype controls. We found that NMDAR1 expression was reduced in the amygdala of Oxt −/− mice, but not in Oxtr −/− mice. Preliminary data suggests that there are no differences in the expression of NMDR2A and NMDR2B subunits between Oxt −/− mice, Oxtr −/− mice, and wildtype controls. These data continue to support the hypothesis that disruptions of Oxt signaling, and possibly Oxt interactions with the glutamate system, may contribute to altered sensorimotor gating.

Disclosures: M.E. Rich: None. **H.K. Caldwell:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

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Program#/Poster#: 833.01/OO7

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK083452

Title: Neuropeptide Y inhibits visceral afferent activation of NTS catecholamine neurons through both Pre- and Post-synaptic mechanisms

Authors: *H. ZHAO, S. M. APPLEYARD;
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Abstract: Neuropeptide Y (NPY) is widely expressed in the central nervous system and has been demonstrated to participate in central mechanisms controlling food intake, blood pressure and energy homeostasis. The nucleus of solitary tract (NTS) is the recipient of sensory information from the gastrointestinal tract relayed centrally via the vagus nerve, and is thus a critical area in the control of food intake and energy balance. In particular, catecholamine (CA) neurons in the NTS are directly activated by visceral afferents and are modulated by various peripheral and central stimuli, making them ideally suited to respond to, and mediate, the effects of NPY at this level. In the present study we examined the effects of NPY on NTS-CA neurons,

using mice expressing a green florescent protein under the control of the tyrosine hydroxylase promoter to identify CA (TH-EGFP) neurons and patch-clamp techniques in horizontal slices that allow selective stimulation of the solitary tract (ST) containing vagal afferents. We found that NPY dose-dependently inhibited the ST-evoked excitatory post-synaptic current (eEPSC) in ~60% of the TH-EGFP neurons at all concentrations tested, with an EC50 around 30 nM. 100 nM NPY significantly reduced the amplitude of the first ST-EPSC in 6 of 10 neurons by an average of ~50%; this was accompanied by a significant increase in the pair pulse ratio from 0.42 to 0.63. NPY (100 nM) also caused a significant decrease of miniature ESPC (mEPSC) frequency in 5 of 9 neurons, on averaged to ~70% of the baseline frequency, suggesting a presynaptic mechanism of action. We saw similar effects in some non-CA neurons, but the effects were more variable and were not statistically significant across all neurons. In addition to these presynaptic effects, we also observed evidence for post-synaptic effects of 100 nM NPY in the majority of CA neurons. NPY caused a positive shift in the holding current, on average ~22 pA, which partially recovered following wash in 8 of 10 TH-EGFP neurons tested. This effect was greatly blunted when cesium was used in the internal solution instead of potassium (reduced to 4.5 pA); suggesting NPY increases a potassium conductance. In contrast, the cesium internal did not affect the presynaptic effects of NPY. Taken together, our findings demonstrate two cellular mechanisms by which NPY can inhibit NTS-CA neurons and provide insights into potential pathways underlying NPY's effects at the level of the hindbrain.

Disclosures: H. Zhao: None. **S.M. Appleyard:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 833.02/OO8

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK083452

Title: Nicotine effects on catecholamine neurons in the hindbrain are dependent on glucose concentration

Authors: *S. PAGE[1], M. ZHU[2], B. L. ROBERTS[2], S. APPLEYARD[2];
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Abstract: Visceral afferents carrying satiety information from the gastrointestinal system primarily terminate on neurons in the nucleus of the solitary tract (NTS). Catecholamine neurons in the NTS (NTS-CA) have been suggested to play a critical role in integrating and relaying satiety signals from the gut to many other nuclei in the brain. Nicotine has been shown to be a potent suppressor of appetite, and smoking cessation is associated with an increase in appetite and weight gain. Nicotine has been shown to strongly activate NTS neurons, including NTS-CA neurons, through both a presynaptic mechanism to increase glutamate release from visceral afferent terminals, and a direct post-synaptic action on NTS neurons. Serotonin activation of visceral afferent neurons through the 5-HT3 receptor was recently shown to be dependent on extracellular glucose concentration. In the current study, we tested whether or not nicotine responses are also altered by changes in glucose concentration. We used transgenic mice, which express enhanced green fluorescent protein under the control of the tyrosine hydroxylase promoter (TH-EGFP), to identify NTS-CA neurons in a horizontal NTS brain slice preparation. Whole cell patch-clamp recordings were made from NTS-CA neurons, and puffs of nicotine (200uM) were applied to the cell body using a picospritzer. Nicotine puffs induced both a direct inward current and increased the frequency of spontaneous excitatory post-synaptic currents (sEPSCs) in TH-EGFP neurons, as previously reported. Lowering bath glucose concentration from 5mM to 2mM reduced the amplitude of nicotine-induced currents in TH-EGFP neurons. In addition, the nicotine-induced increase in sEPSC frequency was reduced, suggesting a reduction in the size of the presynaptic effect of nicotine. Osmolarity was kept constant for all experiments. Restoring glucose concentration to 5mM returned both the nicotine-induced current amplitude and sEPSC response to control levels. The size of nicotine currents in isolated nodose ganglia neurons, which are the cell bodies of the visceral afferents located in the periphery, was also dependent on glucose concentration. Lowering bath glucose from 10mM to 5mM reduced the amplitude of the nicotine-induced current, and it was further reduced in 2mM glucose. The results of this study suggest that the ability of nicotine to activate NTS-CA neurons, both directly and indirectly by increasing glutamate inputs, is dependent on glucose concentration.

Disclosures: S. Page: None. **M. Zhu:** None. **B.L. Roberts:** None. **S. Appleyard:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

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Program#/Poster#: 833.03/OO9

Topic: E.07. Food Intake and Energy Balance

Support: NIH grant DK83449

NIH grant DK41301

Title: Meal structure of nocturnal feeding altered by a long-acting cholecystokinin agonist, (pGlu-Gln)-CCK-8 in rats

Authors: *L. WANG, 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). CCK agonist, (pGlu-Gln)-CCK-8 sulfated (peqCCK-8) is a long acting agonist. The prolonged actions may result from resistance to enzymatic degradation. This long acting agonist has been shown to reduce food intake, body weight and glucose levels in mice (Irwin N et al., Diabetology 2012, Biochem Pharmacol 2013, Biochim Biophys Acta 2013). We studied the effect of peqCCK8 on meal pattern of nocturnal feeding in rats using an automated feeding monitoring system and activation of brain sites by induction of Fos. SD rats on normal rodent chow were acclimated for 1 week to the automated episode feeding monitoring system (BioDAQ, Research Diets, Inc., New Brunswick, NJ). At 5-10 min before onset of dark phase, peqCCK-8 was injected intraperitoneally (ip) at 0.2, 0.6 and 1.8 nmol/kg (0.3, 1 and 2.5 μg/kg, n=8-9/group). Controls were received ip saline. CCK receptor 1 antagonist, devazepide (1 mg/kg) or vehicle (10% DMSO, 5% Tween-80 and 85% saline), were injected ip 15 min before ip injection of peqCCK-8. Fos immunoreactivity was processed in rat brains at 1 h after ip injection of saline or peqCCK-8 at 1.8 nmol/kg. Food intake was reduced by peqCCK-8 at 2 h after onset of dark phase in rats and 1.8 nmol was more potent than lower doses. Meal structure analysis of the first 2 h after ip injection of peqCCK-8 showed significantly reduced meal size and frequency, bouts and time spent on meals, and very prolonged latency to the 1st meal compared with saline (96.0 ± 13.3 vs. 5.4 ± 2.8 min), without significant change in meal duration. However, there are no significant changes in food intake and meal structure at 4 h after ip peqCCK-8. Of note, peqCCK-8 did not change the 1st meal size, intermeal interval and satiety ratio. Robust increase in Fos expression induced by ip peqCCK-8 was observed in the bed nucleus of stria terminalis, paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, external subnucleus of the lateral parabrachial nucleus, area postrema and nucleus of the solitary tract. The activated brain areas are similar to those induced by CCK-8 in rats and mice. Compared with vehicle, pretreatment with devazepide, a CCK1 receptor antagonist, completely blocked the prolonged latency to the 1st meal and altered 2-h meal structure in rats induced by ip injection of peqCCK-8 (1.8 nmol/kg). These data indicate that peqCCK-8 reduces food intake by prolonging the latency to the first meal in rats and activates brain circuits that inhibit initiation of feeding, this may result from its resistance to enzymatic degradation.

Disclosures: L. Wang: None. **J.R. Reeve:** None.

Poster

833. Integration of Peripheral Signals: Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: PRIN grant 2009ESX7T3

FIR grant RBFR12DELS_003

Title: High dietary fat intake influences the activation of specific hindbrain and hypothalamic nuclei by the satiety factor oleoylethanolamide

Authors: *B. TEMPESTA[1], A. ROMANO[1], E. KARIMIAN AZARI[2], A. MANSOURI[2], M. MICIONI DI BONAVENTURA[3], T. LUTZ[4], G. BEDSE[1], W. LANGHANS[2], S. GAETANI[1]; [1]Dept. of Physiol. and Pharmacol., Sapienza Univ. of Rome, Rome, Italy; [2]Physiol. and Behavior Lab., ETH Zurich, Schwerzenbach, Switzerland; [3]Sch. of Pharmacy, Pharmacol. Unit, Univ. of Camerino, Camerino, Italy; [4]Inst. of Vet. Physiol., Vetsuisse Faculty, and Ctr. of Integrative Human Physiol., Zurich, Switzerland

Abstract: Chronic exposure to a diet rich in fats changes the gastrointestinal milieu and alters responses to several signals involved in the control of food intake. Oleoylethanolamide (OEA) is a gut derived satiety signal released from enterocytes upon the ingestion of dietary fats. The anorexigenic effect of OEA, which requires intestinal PPAR-alpha receptors and is supposedly mediated by vagal afferents, is associated with the induction of c-fos in several brain areas involved in the control of food intake, such as the Nucleus of the Solitary Tract (NST) and the hypothalamic Paraventricular (PVN) and Supraoptic nuclei (SON). In the present study we investigated whether the exposure to a high fat diet (HFD) alters the hindbrain and hypothalamic responses to OEA. To this purpose we evaluated the effects of OEA at a dose that reliably inhibits eating (10 mg /Kg i.p.) on the induction of c-fos in the NST, Area Postrema (AP), PVN and SON in rats maintained either on standard chow or a HFD. We performed a detailed analysis of the different NST subnuclei activated by i.p. OEA and found that peripheral OEA strongly activates c-fos expression in the AP, NST and in the hypothalamus of both chow and HFD fed rats. The extent of c-fos expression was, however, markedly different between the two groups of rats, with a weaker activation of selected NST subnuclei and stronger activation of the PVN in HFD-fed than in chow-fed rats. HFD-fed rats were also more sensitive to the immediate hypophagic action of OEA than chow-fed rats. These effects may be due to a decreased

sensitivity of vagal afferent fibers that might mediate OEA's actions on the brain and/or an altered sensitivity of brain structures to OEA.

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Poster

833. Integration of Peripheral Signals: Regulators

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Program#/Poster#: 833.05/OO11

Topic: E.07. Food Intake and Energy Balance

Title: Subdiaphragmatic vagotomy ameliorates the obesity caused by genetic deletion of the melanocortin 4 receptor in the mouse

Authors: G. DEZFULI, R. GILLIS, B. XU, K. DRETCHEN, J. VERBALIS, *N. SAHIBZADA;
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Abstract: Background/Objectives: We tested the hypothesis that the dorsal motor nucleus of the vagus (DMV) is an essential component of the central melanocortin neural circuits that controls food intake (FI) and body weight (BW). Subjects/Methods: This hypothesis was tested in two separate studies, which examined the role of the DMV in the prevention and treatment of obesity using adult mice with a genetic deletion of the melanocortin 4 receptor (Mc4r -/-). In the first study, 3-5-month-old Mc4r -/- mice were used that had not yet reached full obesity, while in the second study 8-month-old Mc4r -/- mice were used that were severely obese. Results: In the first study, subdiaphragmatic vagotomy (SDV) prevented the obesity associated with this genotype compared to sham-operated mice. This was not correlated with a reduction in overall FI. SDV mice did have reduced cumulatively FI; however, this was only in the first week. In the second study, the severe obesity that is symptomatic of the adult Mc4r -/- genotype was ameliorated by SDV. As in the first study, this was not reflective of a decrease in FI as after the first week of treatment it normalized to that of the control groups. In addition to the accompanying loss in weight, SDV mice had increased energy expenditure (EE) and a decreased light cycle respiratory exchange ratio (RER). Analysis of the white fat-pad deposits in these mice also showed that they were significantly less than the control groups. Conclusions: Altogether, our data suggests that the weight-loss associated with SDV in Mc4r -/- mice is due to several mechanisms that include

an initial decrease in FI, increase in EE, and a reduction in RER favoring lipid utilization as an energy source. Furthermore, it underscores the role of the DMV as a key output of the central melanocortin system in chronic control of BW.

Disclosures: **G. Dezfuli:** None. **N. Sahibzada:** None. **R. Gillis:** None. **K. Dretchen:** None. **B. Xu:** None. **J. Verbalis:** None.

Poster

833. Integration of Peripheral Signals: Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: INRA package to M. Covasa

UEFISCDI PN-II-ID-PCE-2012-4-0608 no. 48/02.09.2013 to M. Covasa

Title: Impact of Roux-en-Y gastric bypass surgery on enteroendocrine cell differentiation, glucose sensing and gut microbiota composition

Authors: ***M. COVASA**[1,2,3], B. LANGELIER[2], V. DOUARD[2], F. DEVIME[2], P. LEPAGE[2], A. HAJNAL[4], J. BEIGLER[4];
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Abstract: Roux-en-Y gastric bypass (RYGB) is the most effective treatment for severe obesity and improvement of type 2 diabetes. Several mechanisms have been proposed for these effects that include changes in the gut hormones, nutrient signaling and microbiota. Therefore, we first examined the impact of RYGB on enteroendocrine cell differentiation by determining gene expression of the gut basic helix-loop-helix (bHLH) transcription factors in the ileum, colon, biliopancreatic and Roux limbs of sham and RYGB high fed rats at 3 and 6 months post-surgery. Second, we assessed glucose signaling by determining Tas1R3, 5-HT, SGLT-1, GLUT1 and GLUT2 gene expression in these intestinal segments. Finally, we examined microbiota composition via 16S rRNA454 pyrosequencing to determine changes in bacterial phylogeny and taxonomy in sham and RYGB operated rats. RYGB altered enteroendocrine cell differentiation through an increased expression of MATH1 and NGN3 in the ileum. However, HES1 expression

was slightly upregulated in the ileum while it decreased significantly in the Roux limb at 6 month after surgery. Similarly, PAX6 expression was upregulated in the biliary and Roux limbs at 6M. SGLT-1, 5HT and Tas1R3 were all slightly decreased in the ileum at 6M post-surgery. Analyses of gut microbiota showed significant changes in gut microbiota genera abundance at both 3 and 6 months after RYGB. There was an overall decrease of Firmicutes and a significant augmentation of Proteobacteria and Actinobacteria at 6M after surgery. Escherichia/Shigella were significantly increased in RYGB rats while Clostridium IV, Peptococcus, Blautia, Pseudoflavonifractor, Ruminococcus, Hydrogenoanaerobacterium and Insolitispirillum were all significantly decreased after the surgery. These results demonstrate that anatomical reorganization following gastric bypass surgery impacts regulation of transcription factors controlling enteroendocrine cell differentiation particularly in the ileum and common alimentary limb which in turn may be responsible for long term changes in nutrient signaling. Finally, RYGB resulted in significant long term shifts in gut microbiota composition that may influence gut peptide synthesis and release and contribute to weight loss maintenance. The relationship between gut microbiota composition and genes regulating enteroendocrine cell differentiation is under investigation.

Disclosures: **M. Covasa:** None. **B. Langelier:** None. **V. Douard:** None. **F. Devime:** None. **P. Lepage:** None. **A. Hajnal:** None. **J. Beigler:** None.

Poster

833. Integration of Peripheral Signals: Regulators

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Title: SerpinA3N expression in the hypothalamus is regulated by nutritional status, age and leptin

Authors: *L. M. WILLIAMS[1], C. GRANT[1], A. C. MORRIS[1], E.-M. BACHMAIR[1], C. KOCH[2], F. H. MCLEAN[1], A. MULLER[1], N. HOGGARD[1], B. DE ROOS[1], M. V. BOEKSCHOTEN[3], F. C. MCGILLICUDDY[4], C.-D. MAYER[5], H. M. ROCHE[4], M. MULLER[3], R. NOGUEIRAS[6], C. DIEGUEZ[6], A. TUPS[2];
[1]RINH, Univ. of Aberdeen, Aberdeen, United Kingdom; [2]Dept. of Animal Physiol., Philipps Univ., Marburg, Germany; [3]Nutrition, Metabolism and Genomics Group, Wageningen Univ., Wageningen, Netherlands; [4]Nutrigenomics Res. Group, Univ. Col. Dublin, Dublin, Ireland; [5]Biomath. and Statistics Scotland, Univ. of Aberdeen, Aberdeen, United Kingdom; [6]Dept. of Physiol., Univ. of Santiago de Compostela and CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Santiago de Compostela, Spain

Abstract: Energy balance is regulated by the hypothalamus but breaks down when animals are fed a high-fat diet (HFD) and obesity develops. HFD causes inflammation and damage to the hypothalamus apparent within days (Thaler JP, et al 2012 J Clin Invest 122:153-162. Williams LM 2012 Proc Nutr Soc 71:521-533). Using a microarray-based transcriptomics approach to identify novel genes regulated by HFD and leptin in the hypothalamus, mouse global array data identified SerpinA3N as a novel gene highly upregulated by both a HFD and leptin challenge as well as increasing age ($P < 0.001$ with no interaction between HFD, leptin challenge or age). *In situ* hybridisation confirmed these results demonstrating a wide distribution of SerpinA3N gene expression in the hypothalamus which was globally upregulated by HFD. Immunohistochemistry revealed that alpha-1-antichymotrypsin (the protein encoded by SerpinA3N) was localised to neurones, an unexpected finding, as induction of SerpinA3N has been shown to be a marker of astrogliosis (Zamanian JL, et al 2012 J Neurosci 32:6391-6410) and a HFD is reported to cause hypothalamic astrogliosis (Thaler JP, et al 2012 J Clin Invest 122:153-162. Buckman LB, et al 2013 J Comp Neurol 521:1322-1333.). The upregulation of SerpinA3N expression by HFD is blunted in IL-1 receptor 1 deficient (IL-1R1-/-) mice, implicating inflammation in its regulation. Nonetheless, hypothalamic gene expression of other markers of inflammation, IL-6, IL-1β and SOCS3 did not show any significant changes with HFD over 16 weeks. Intracerebroventricular (ICV) injection of alpha-1 antichymotrypsin acutely inhibited food intake in a concentration dependent manner but failed to alter long-term food intake. Gene expression of appetite regulatory peptides of mice treated with aACT showed little change apart from CART downregulation in the lateral hypothalamus indicating a response to decreased food intake rather than having a causative role. These data indicate a potential role for serpinA3N in energy balance regulation but its mechanism of action remains to be identified.

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Poster

833. Integration of Peripheral Signals: Regulators

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Program#/Poster#: 833.08/OO14

Topic: E.07. Food Intake and Energy Balance

Support: UGA Obesity Initiative

Georgia Research Alliance Eminent Scholar Endowment (CAB)

Title: High fat diet-induced alterations in hippocampal gene expression in adult female mice

Authors: *E. R. ENGLAND, S. KRISHNA, Z. LIN, J.-Y. YANG, M. DELLA-FERA, R. MEAGHER, D. HARN, C. DE LA SERRE, C. BAILE, N. FILIPOV;
Univ. of Georgia, Athens, GA

Abstract: Emily England[1], Saritha Krishna[2], Zhoumeng Lin[2], Jeong-Yeh Yang[3], Mary Anne Della-Fera[3], Richard B. Meagher[4], Donald A. Harn[5], Claire de La Serre[6], Clifton A. Baile[1,3,6], Nikolay M. Filipov[1,2] [1]Neuroscience Division, Biomedical and Health Sciences Institute [2]Department of Physiology and Pharmacology, College of Veterinary Medicine [3]Department of Animal and Dairy Science [4]Department of Genetics [5]Department of Infectious Diseases [6]Department of Foods and Nutrition University of Georgia, Athens, GA, USA **High fat diet-induced alterations in hippocampal gene expression in adult female mice** Consumption of a high fat diet (HFD) is considered to be a primary factor in obesity. Multiple rodent studies show a robust effect of HFD on the central nervous system but primarily use male animals to do so, leaving the female response to dietary change largely underreported. HFDs stimulate the mesolimbic dopamine-reward pathway and the hippocampus, specifically the ventral hippocampus, assists this process partly by encoding the context of the reward. Studies in male rats show that HFD negatively impacts hippocampal function and the integrity of the blood-brain barrier in proximity to the hippocampus. In this study young adult female C57BL/6 mice were exposed to HFD (60% kcal) or low fat diet (LFD, 10% kcal) for 6 weeks. Body weight was recorded weekly. Glucose/insulin tolerance tests were carried out at 5 weeks. After 6 weeks, the mice were sacrificed and the brains were removed, rapidly frozen, and sliced. Punches were taken from dorsal and ventral hippocampus and processed for gene expression and neurochemical analysis. After 6 weeks on their respective diets, the HFD-fed mice weighed 25% more than the LFD-fed mice. At 5 weeks, the HFD mice exhibited impaired glucose tolerance and peripheral insulin sensitivity. In both the dorsal and ventral hippocampus of HFD-fed mice, mRNA levels of brain-derived neurotrophic factor (BDNF) and the insulin receptor were

increased compared to their LFD counterparts ($p<0.01$). In the ventral, but not the dorsal hippocampus, mRNA levels of peroxisome proliferator-activated receptor alpha (PPARα) were decreased in the HFD mice compared to LFD ($p<0.05$). Neurochemically, the HFD-fed mice displayed changes in dopamine homeostasis only in the ventral part of the hippocampus. Our demonstration of dysregulated ventral hippocampal dopamine signaling and PPARα expression, which is novel, highlight the potential of HFD impact on the ventral hippocampus in contributing to the already demonstrated fatty acid-mediated dysregulation of the dopamine-reward system and its role in overconsumption and, eventually, obesity.

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Poster

833. Integration of Peripheral Signals: Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: DA024314

HD058638

Title: The role of nitric oxide synthase in the estrogenic attenuation of cannabinoid induced changes of energy homeostasis

Authors: *A. I. BORGQUIST[1], S. DO[2], C. MEZA[2], E. WAGNER[2];
[1]Grad. Col. of Biomed. Sci., [2]Col. of Osteo. Med. of the Pacific, Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Cannabinoids exert their effects on feeding behavior and metabolism in a sexually differentiated manner, with females being less sensitive than males. This is due, at least in part, to the activational effects of gonadal hormones, such as the estrogen-induced reduction in CB1 receptor density in the hypothalamus of ovariectomized female rats. While estrogen lowers CB1 receptor density, the reduced sensitivity to cannabinoids actions could also be attributed to estrogen receptor-mediated signaling pathways that culminate in the activation of nitric oxide synthase (NOS). We therefore tested the hypothesis that NOS plays an integral role in the

estrogenic attenuation of cannabinoid-induced changes in energy intake, energy expenditure and transmission at proopiomelanocortin (POMC) synapses. Whole animal experiments were carried out in ovariectomized female guinea pigs treated with either estradiol benzoate (EB; 10μg; S.C.) or its sesame oil vehicle (0.1mL; S.C.). EB per se decreased incremental food intake as well as oxygen consumption, carbon dioxide production and metabolic heat production as early as 2 hours post administration. Western blotting revealed that EB increased phosphorylation of neuronal nitric oxide synthase (nNOS) in the arcuate nucleus (ARC) micro-dissected from 1mm-thick hypothalamic slices measured 2 hours post treatment. Administration of the cannabinoid receptor agonist WIN 55,212-2 (3μg) into the third ventricle evoked hyperphagia as early as 1 hour post administration, which was blocked by EB and restored by the non-specific NOS inhibitor L-NAME (100μg; I.C.V) when the latter was combined with the steroid. Whole-cell patch clamp recordings showed that EB (100nM) rapidly diminished cannabinoid induced decreases in miniature excitatory post synaptic current frequency, which was mimicked by pre-treatment with the NOS substrate L-arginine (30μM) and abrogated by L-NAME (300μM). Collectively, these results indicate that the estrogen-induced decrease in energy intake is mediated, at least in part, by a decrease in cannabinoid sensitivity within the ARC feeding circuitry through a mechanism involving the activation of NOS.

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Poster

833. Integration of Peripheral Signals: Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: CONACyT Grant 243335

Title: Obesity and nociceptive pain in hypoestrogenic Wistar rats

Authors: O. A. JARAMILLO-MORALES[1], J. V. ESPINOSA-JUÁREZ[1], G. BRAVO[1], *F. J. LOPEZ MUNOZ[2];
[1]Farmacobiología, [2]CINVESTAV, Mexico, Mexico

Abstract: Background: In clinical, perception and pain threshold may increase or decrease depending on a few factors including hormones, sex, age, race, ethnicity or psychological state. Recent evidence had shown that the pain threshold during obesity may be altered, however, are

not well established mediators involved and their mechanism of action, so it is necessary to investigate whether the obesity is directly involved in pain disorders. The purpose of this study was to analyze the nociceptive pain using Plantar test method in obese Wistar rats with hypoestrogenism. Methods: Female Wistar rats were randomized in 2 groups (n= 10) and were subject to bilaterall ovariectomy. The animals were given hypercaloric diet (30% sucrose in drinking water) or water and standard laboratory chow ad libitum for 24 weeks. During this period, nociception was assessed weekly using the Plantar test method as well as the body weight. Results: Obese hypoestrogenic Wistar rats had significantly higher body weigth than the controls. During time course of thermal threshold for 24 weeks by induction of a hypercaloric diet (30% sucrose), the animals showed a biphasic response: 1) in the first 4 weeks was significantly decreased in thermal latency 2) of the week 12 to 18 was observed significantly increased in thermal latency compared to our control group. Conclusions: Our data shows that the increase in body weight and fat associated with a decrease hormone (estrogen) bring a biphasic response of nociception perception (hyperalgesia stage, and a stage of hypoalgesia) during induction of obesity in female Wistar rats hypoestrogenic (for a hypercaloric diet with 30% sucrose).

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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK080441

Title: Neuropeptide Y controls the sympathetic response to food deprivation by regulating synaptic plasticity at the level of the preganglionic input

Authors: *M. WANG, M. D. WHIM;
Cell Biol. and Anat., LSU Hlth. Sci. Ctr., New Orleans, LA

Abstract: Failure to avoid hypoglycemia can lead to ventricular arrhythmia, coma and death. When blood glucose levels fall, epinephrine is secreted by the neuroendocrine chromaffin cells in the adrenal medulla. Epinephrine contributes to the restoration of euglycemia by acting on

peripheral targets including the liver, pancreas and adipose tissue. How adrenal epinephrine secretion is regulated and whether food deprivation (a potential inducer of hypoglycemia) alters the sympathoadrenal response remains unclear. To address this issue we compared urine epinephrine levels from mice that were fed *ad lib* or fasted for 1 day. As previously reported, urine epinephrine levels were significantly higher after fasting. However the blood glucose levels did not change, consistent with a role for epinephrine in the maintenance of euglycemia. Because neuropeptide Y (NPY) is a transmitter that is co-released with epinephrine from chromaffin cells we repeated these experiments on NPY knockout (NPY k/o) mice. In contrast, under these circumstances food deprivation did not increase the urine levels of epinephrine and the mice were hypoglycemic. To determine how the loss of NPY prevented epinephrine release we quantified secretion from chromaffin cells *in vitro*. Catecholamine release was evoked by a train of voltage clamp depolarizations and monitored using carbon fiber amperometry. There was no difference in the amplitude of amperometric events between fed and food deprived wild type (wt) mice. However food deprivation significantly increased the amplitude of amperometric events in fasted NPY k/o mice, indicating that the catecholamine secretory capacity from isolated cells is negatively regulated by NPY. Because this could not explain the observed decrease in epinephrine release *in vivo* we next considered whether NPY alters the strength of the preganglionic → chromaffin cell synapse. Using acute adrenal slices we found that food deprivation was associated with an increase in the amplitude of the evoked EPSC monitored in chromaffin cells from wt mice. In contrast, the amplitude of the evoked EPSC was reduced in the fasted NPY k/o animals compared to fed littermates. Food deprivation led to a decrease in the paired-pulse ratio (PPR) in wt animals, but to an increase in the PPR in NPY k/o mice, consistent with the involvement of a presynaptic component. We conclude that food deprivation is associated with significant plasticity at the preganglionic → chromaffin cell synapse. The dominant affect appears to be an NPY-dependent increase in the strength of this synapse. This increases the adrenal discharge of epinephrine which is required to maintain euglycemia.

Disclosures: **M. Wang:** None. **M.D. Whim:** None.

Poster

833. Integration of Peripheral Signals: Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant HL091911

NIH Grant DK098841

Title: Minimal role of GPR30 in mediating the anti-dipsogenic and anti-natriorexigenic effect of estradiol in the female rat

Authors: *J. SANTOLLO, D. DANIELS;
Psychology, Univ. at Buffalo, SUNY, Buffalo, NY

Abstract: Estradiol (E2) decreases both water and saline intakes in the female rat and recent studies show that membrane-associated estrogen receptors (ER) are sufficient to decrease fluid intake. Because there are multiple ER subtypes that can localize to the cell membrane, it is unclear which receptor(s) are involved in decreasing fluid intake. Accordingly, we tested the hypothesis that activation of the recently identified membrane-associated ER, GPR30, is sufficient to decrease fluid intake in female rats. Female rats were ovariectomized and, with the exception of rats in Experiment 1, implanted with a chronic cannula aimed at the lateral ventricle. Behavioral testing started after a two-week recovery period, during which accurate cannula placement was verified. Experiment 1 tested whether GPR30 is sufficient to decrease overnight water intake by administering multiple doses (0, 6.25, 12.5, 25, 50 and 100 µg, subcutaneous) of the selective GPR30 agonist G1 during the 30 min period before dark onset. Neither 24 h water nor food intake was influenced by any dose of G1 (all p values > 0.05). Experiment 2 tested whether GPR30 is sufficient to decrease angiotensin-II (AngII) stimulated water and saline intakes. G1 (0, 12.5, 25, 50 µg) was administered (subcutaneous) 3.5 h before rats were injected (icv) with 100 ng AngII. Again, G1 had no effect on either water or saline intake during a 30-min drinking test after AngII treatment (all p values > 0.05). Because GPR30 also can produce rapid changes in neuronal activity, Experiment 3 tested for a rapid effect of GPR30 on AngII-induced water and saline intakes. G1 (0, 25 and 50 µg) was injected into the lateral ventricle and immediately after rats received a second icv injection of 100 ng AngII. Both doses of G1 produced a significant decrease in 30-min water intake ($p < 0.05$), but no change in saline intake was observed ($p > 0.05$). The final experiment tested for the ability for GPR30 to rapidly decrease food intake. Rats were food deprived overnight (16 h) and then injected icv with G1 (0, 25 and 50 µg). Subsequent food intake was measured 5, 10, 15, 30, 60 and 120 min and 24 h later. Preliminary data suggest that, again, activation of GPR30 does not decrease food intake. Together these data suggest that activation of GPR30 plays a minimal role in the inhibitory effect of E2 on ingestive behavior in the female rat.

Disclosures: J. Santollo: None. **D. Daniels:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 833.13/OO19

Topic: E.07. Food Intake and Energy Balance

Support: NSERC Discovery Grant

Title: Toxin-induced gustatory conditioning in rats: Examining the influence of a bitter taste on ingestion of a palatable sucrose solution containing a low level toxin (LiCl)

Authors: L. SZOTA[1], A. N. GOOD[2], M. KAVALIERS[1], *K.-P. OSSENKOPP[3];
[1]Psychology, [2]Grad. Neurosci. Program, [3]Univ. Western Ontario, London, ON, Canada

Abstract: Foraging animals must decide which foods, when ingested, will maximize nutrients and minimize toxins. In the laboratory lithium chloride (LiCl) has been shown to produce robust conditioned taste avoidances in rats in a dose dependent manner. We have previously shown that at very low LiCl concentration levels (0.005 to 0.02 M) rats exhibit a dose related reduction in consumption of a sucrose plus salt solution, findings which support the hypothesis that rats use a behavioral tolerance mechanism to regulate their intake of toxin. It was further observed that rats regulate to a threshold level (ID50) of 0.02 M. One important cue regarding the potential presence of a toxin in a food is bitter taste. The present study examined the influence of a bitter taste (quinine) on the consumption, licking behaviour, and palatability of a sucrose plus salt solution. Over 5 days (Phase 1) adult rats were presented with a 0.3 M sucrose solution containing either 0.02 M NaCl (control) or 0.02 M LiCl (toxin). In addition these solutions also contained either 0, 0.0001 or 0.0002 M quinine. These six groups (n = 6/group) were then presented with only their respective sucrose plus salt solution in Phase 2 (5 days, no bitter taste). Fluid intake, number of licks, as well as pattern of licks were quantified on each day. The results showed that the bitter taste influenced initial consumption by suppressing intake for both the control fluids (NaCl) as well as the toxin fluids (LiCl), relative to the quinine free fluids. The groups drinking sucrose plus NaCl and quinine then slowly increased intake over the 5 days of Phase 1. The groups drinking the sucrose plus toxin fluids exhibited strongly suppressed intake over the entire Phase 1. When the bitter taste was removed in Phase 2, the two LiCl groups with quinine in Phase 1 showed an initial large increase in intake which then returned to much lower levels on the 2nd and subsequent days. The two NaCl groups with quinine in Phase 1, exhibited the same level of consumption as the quinine free NaCl group in Phase 2. These findings show that bitter taste is treated as a danger cue when first encountered. If the postingestive effects of the food are positive (NaCl groups), then the bitter taste is increasingly recognized as safe. When the bitter taste is paired with aversive postingestive effects (LiCl) it becomes associated with the toxic effects and suppresses intake, which then temporarily increases when the bitter taste is removed. These findings suggest that although the bitter taste may serve as an initial cue for

danger, the subsequent significance of this signal is contingent upon post-ingestive feedback from the food being consumed.

Disclosures: **L. Szota:** None. **K. Ossenkopp:** None. **A.N. Good:** None. **M. Kavaliers:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 833.14/OO20

Topic: E.07. Food Intake and Energy Balance

Support: IBRC Graduate Research Award

Title: The effects of brain inflammatory cytokine on peripheral lipid metabolism

Authors: ***T. LEE**[1,2,3], K. KINZIG[1,3];
[1]Psychological Sci., [2]Nutr. Sci., [3]Ingestive Behavior Res. Ctr., Purdue Univ., West Lafayette, IN

Abstract: It has been demonstrated that the brain influences the hepatic very low-density lipoprotein (VLDL) secretion and plasma triglyceride level through the vagus nerve. Recent evidence suggests that low-grade inflammation occurs in the brain in high-fat diet feeding animals, even before weight gain or peripheral inflammation. In order to understand the role of inflammation in the brain on diet-induced obesity, we hypothesized that elevated proinflammatory cytokines in the brain can influence energy balance or peripheral lipid metabolism. Male Sprague Dawley rats received intracerebrovantricular injection of 0-20 pg TNFα through third ventricle cannula immediately prior to the onset of the dark cycle. We found that rats with 0.5 pg TNFα injection had slightly higher 24-hour chow intake and body weight. After daily injection for six days, these rats had significantly higher average daily food intake and higher plasma TNFα compared to the saline group. There were no differences in fat mass, lean mass, plasma glucose, insulin and triglyceride between TNFα and saline groups. Lipogenic and lipolytic genes expression in liver and white adipose tissue were also not different. In another long-term injection model, rats were implanted with an osmotic minipump subcutaneously, such that rats one group received TNFα continuously for a total of 0.5 pg/day TNFα for twenty six days, whereas the control group received continuous saline. Compared to the saline group, there were no differences in body weight change, food intake, fat mass, lean mass, plasma glucose. However, the hepatic fatty acid synthase (FAS) was lower and the epididymal adipose SREBP-1c was higher in the TNFα group compared to saline group. There

were no changes in lipolytic genes. These findings suggest that low-grade TNFα in the brain may have an acute effect on food intake stimulation, and that chronically elevated TNFα will increase fat storage. These findings contribute to the understanding of the role of brain inflammation on obesity and some possible mechanisms of energy balance.

Disclosures: T. Lee: None. **K. Kinzig:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 833.15/OO21

Topic: E.07. Food Intake and Energy Balance

Support: CNPq

FAPESP

IBRO

Title: Adrenalectomy reduces the sensitivity to central effects of insulin on food intake

Authors: *E. T. UCHOA, R. C. RORATO, S. G. RUGINSK, J. ANTUNES-RODRIGUES, L. L. K. ELIAS;
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Abstract: Adrenal insufficiency induced by adrenalectomy (ADX) induces marked hypophagia. Glucocorticoids are known to counter regulate the peripheral metabolic effects of insulin. However, the effects of glucocorticoids on central hypophagic action of insulin in the hypothalamus are not established. In the present study, we evaluated the effect of ADX on the effect of icv (lateral ventricle) injection of insulin on food intake and the expression of insulin receptor (InsR) mRNA in the hypothalamus of adrenalectomized (ADX) rats with and without corticosterone (B) replacement. In the first set of experiment, after icv surgery, male Wistar rats (200-250g, n=5-7 per group) were subjected to ADX or sham surgery. ADX animals received 0.9% NaCl as drinking fluid, and half of them also received corticosterone in the drinking fluid (B: 25mg/l, ADX+B). Seven days after surgery, animals were treated with icv injection of insulin (12 μM/5 μl) or vehicle (0.9% NaCl/ 5μl). Fifteen minutes after the injections, food consumption was determined during one hour. In a second set of experiment, seven days after surgery, sham, ADX and ADX+B animals (n=7-9 per group) were decapitated for trunk blood

and brain tissue collection, insulin plasma levels were determined by radioimmunoassay and InsR mRNA was determined by real time PCR. ADX/vehicle animals showed a decrease ($P<0.05$) of food intake, when compared to sham/vehicle and this response was partially reversed by B replacement. Central injection of insulin decreased ($P<0.05$) food intake in sham rats, with no effects in ADX rats. Though a nonsignificant trend was observed in the ADX+B group, corticosterone treatment did not rescue the hypophagic effect of insulin. ADX decreased ($P<0.05$) InsR mRNA expression in the arcuate (ARC), paraventricular (PVN), dorsomedial (DMH) and ventromedial (VMH) nuclei of the hypothalamus, compared to sham group. B replacement reversed this response in the VMH and partially reversed it ($P<0.05$) in the ARC, PVN and DMH. Plasma insulin plasma were lower ($P<0.05$) in the ADX group, when compared to sham and ADX+B animals. In conclusion, these data demonstrate that ADX reduces the sensitivity to the hypophagic effect of central insulin treatment, suggesting that circulating glucocorticoids are required for insulin-induced hypophagia. This effect is, at least in part, due to the reduction of InsR mRNA expression in the hypothalamus after ADX.

Disclosures: **E.T. Uchoa:** None. **R.C. Rorato:** None. **S.G. Ruginsk:** None. **J. Antunes-Rodrigues:** None. **L.L.K. Elias:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 833.16/OO22

Topic: E.07. Food Intake and Energy Balance

Title: Estrogen regulates neuronal glucose sensing phenotype transition in the ventromedial nucleus of the hypothalamus

Authors: *S. A. MOUSSATOV[1], M. STAVARACHE[1], M. KAPLITT[2];
[1]Weill Med. Col. of Cornell Univ., New York, NY; [2]mas2066@med.cornell.edu, New York, NY

Abstract: Glucose sensing neurons in the VMN play a crucial role in neural networks that govern energy homeostasis by directly using glucose and integrating inputs from other central and peripheral metabolic centers. While distinct neuronal populations in the VMN are not fully understood, they are functionally described as glucose-excited (GE), glucose-inhibited (GI), and non-responsive to glucose (NS). Here we report that estrogen signaling, via estrogen receptor alpha (ERα), as a key pathway regulating the function of these cells in both males and females. Within the cell ERα acts through multiple pathways by binding to genomic estrogen response

elements (ERE) or via ERE-independent cascades. To gain further insight into underlying mechanisms in glucose sensing, we have manipulated ERα in primary VMN neurons using AAV vectors designed to silence endogenous ERα through RNAi followed by restoration with an RNAi-resistant version encoding for WT ERα or ERE-dead mutant. Overexpression of WT ERα markedly increased the percentage of GI neurons and to lesser degree that of GE neurons as determined using Ca imaging. In contrast, ERα replacement with ERE-dead mutant had opposite effect, resulting in the majority of cells displaying the characteristics of GE neurons and a decline in the percentage of GI neurons. Importantly, the increase in the percentage of GE neurons could not be fully accounted for by their transitioning from the pool of NS cells, suggesting a possibility that at least some GE and GI neurons are capable of switching their phenotype. To directly address this hypothesis, we developed a modified Ca imaging procedure which allows repeated recordings of the same field days apart without loss of cell viability. Following 48-h exposure to estrogen but not vehicle, the majority of cells identified as GI neurons during the first recording displayed a response typical for GE cells. These observations thus suggest that at least some GE and GI neurons in the VMN are not distinct populations but rather have an inherent dual glucose sensing phenotype which is regulated by estrogen acting through ERα. This switch is delayed, as we observed no effect during acute estrogen treatment. Furthermore, the transition appears to engage different ERα pathways, as activation of the classical genomic ERE-dependent mechanism appears necessary for the GI phenotype, while ERE-independent mechanisms seem to regulate transition towards the GE phenotype. These data strongly implicate ERα as a critical factor governing the response of individual VMN neurons to glucose and suggest a direct link between estrogen and hypothalamic control of metabolism.

Disclosures: S.A. Moussatov: None. **M. Stavarache:** None. **M. Kaplitt:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

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Program#/Poster#: 833.17/OO23

Topic: E.07. Food Intake and Energy Balance

Support: KHT R&D Grant A111436

Title: Myeloid-specific SIRT1 deletion aggravates a high-fat diet-induced hepatic steatosis and induces systemic inflammation

Authors: *G. ROH[1], K. KIM[1], H. KIM[1], R. HEO[2], H. SHIN[2];
[1]Dept. of Anat., [2]Dept. of Anat. and Convergence Med. Sci., Gyeongsang Natl. Univ. Sch. of Med., Jinju, Korea, Republic of

Abstract: Macrophage-mediated local inflammation is involved in the development of insulin resistance and metabolic syndrome. Recent studies have shown that organ-specific deletion of SIRT1 aggravates local inflammation and insulin resistance in dietary and genetic obesity. However, regulatory mechanisms of SIRT1 in macrophages on systemic inflammation remain unclear. Here, we investigated macrophage SIRT1 function in obesity by using myeloid-specific SIRT knockout (KO) mice that fed with a high-fat diet (HFD). We examined HFD-induced phenotype changes and found that SIRT deletion has significantly increased body weight and the fat and liver mass. KO mice were disturbed in glucose and lipid homeostasis and exhibited severe insulin resistance and hepatic steatosis. SIRT1 deletion significantly increased the levels of acetylated NF-kB and nuclear NF-kB induced by HFD. TNFα was decreased in KO mice and significantly increased by HFD; the HFD-induced changes in KO mice were considerably high compared to changes in WT mice. We showed that hepatic expression levels of 4-HNE, a lipid peroxidation marker and glucose transporter 4 have also significantly increased in HFD-fed KO mice. Further, the hepatic fibrosis and collagen levels were elevated in HFD-fed KO mice. HFD-fed KO mice displayed severe inflammation in macrophage-derived cells in adipose tissue, spleen and pancreas, but not in the hypothalamus. We conclude that SIRT1 plays an important role in stimulating obesity-induced systemic inflammation and increasing risks of developing insulin resistance, hepatic steatosis, and other metabolic syndromes.

Disclosures: G. Roh: None. **K. Kim:** None. **H. Kim:** None. **R. Heo:** None. **H. Shin:** None.

Poster

833. Integration of Peripheral Signals: Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 833.18/OO24

Topic: E.07. Food Intake and Energy Balance

Support: FP7 Project 278603

Title: Identification of FKBP51 as a novel susceptibility gene in metabolic dysfunction

Authors: *G. BALSEVICH[1], N. C. GASSEN[1], C. W. MEYER[2], T. REIN[1], F. HAUSCH[3], A. CHEN[1], M. H. TSCHÖP[2], M. V. SCHMIDT[1];

[1]Max-Planck-Institut für Psychiatrie, Munich, Germany; [2]Inst. for Diabetes and Obesity, Helmholtz Zentrum Munich, Munich, Germany; [3]Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: FKBP51 is well recognized for its regulatory role on stress reactivity via modulation of glucocorticoid receptor sensitivity. More recently, FKBP51 has been shown to regulate additional signaling cascades though direct protein-protein interactions. Through its ability to regulate such diverse biological processes, FKBP51 has been identified as a target gene involved in the etiology of several disease states, including psychiatric disorders, Alzheimer's disease, and various cancers. Interestingly, many of these diseases are stress-related, and are often accompanied by an altered metabolic phenotype. In addition, mice deficient in FKBP51 were shown to display a lean phenotype. We therefore aimed to characterize the possible role of FKBP51 in metabolic regulation using FKBP51 knockout (51KO) mice under control (chow) and high fat diet (HFD) conditions. 51KO mice presented reduced body weight, decreased fat mass, and increased lean mass. Furthermore, 51KO mice were resistant to diet-induced obesity and diet-induced glucose-intolerance. Indirect calorimetry revealed that the weight-adjusted daily energy expenditure was significantly higher in 51KO mice compared to WT mice. By contrast, a pair-feeding experiment revealed that food intake was not contributing to the lean phenotype induced by the absence of FKBP51. Together, our data indicate that the increase in energy expenditure resulting from FKBP51 deficiency may underlie the long-term maintenance of energy balance under HFD conditions. To explore potential underlying mechanisms, we investigated interaction partners of FKBP51. Using co-immunoprecipitation, we demonstrated that FKBP51 acts as a scaffolding protein to regulate select intracellular signaling pathways mediating whole body energy balance. Our findings identify FKBP51 as a novel regulator of body weight control, which may represent a promising target for anti-obesity drug development.

Disclosures: G. Balsevich: None. **N.C. Gassen:** None. **C.W. Meyer:** None. **T. Rein:** None. **M.H. Tschöp:** None. **M.V. Schmidt:** None. **A. Chen:** None. **F. Hausch:** None.

Poster

834. Neuropeptide Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 834.01/OO25

Topic: E.07. Food Intake and Energy Balance

Support: RDC grant from East Tennessee State University, RDC # E82248

Title: Recombinant leptin up-regulates the secretion of luteinizing hormone and the expression E-cadherin and β-catenin in the ovary of dietary-induced obese infertile rats: mechanism of action

Authors: *E. OTUKONYONG[1], *S. CAMPBELL[2], *T. NATHANIEL[3];
[1]Dept. of Hlth. Sci., [2]Biomed. Sci., East Tennessee State Univ., Johnson City, TN; [3]Biomed. Sci., Univ. of South Carolina, Greenville, SC

Abstract: Infertility is one of the complications of obesity. It affects men and women alike as well as the lower animals. Studies have shown that administration of leptin reversed infertility, but uptil this moment, the exact mechanism by which leptin does this is not fully understood. It has been reported that E-cadherin and β-catenin proteins are expressed in the rat ovary during ovulation and folliculogenesis. Since leptin is a ploteotropic hormone, we hypothesized that leptin given peripherally can upregulates the secretion of luteinizing hormone and the expression of E-cadherin and β-catenin in dietary-induced obese infertile rats to restore fertility. Female Spraque-Dawley rats were fed either high-fat diet (HFD) or regular rat chow diet (RCD). Food intake and body weight were measured twice weekly and oestrus cycles monitored daily for 15 weeks until their estrous cycles became irregular. 100 µg of leptin was administered intraperitoneally (i.p.) as 1 ml to HFD-fed rats (n=8) with irregular cycle while the control rats HFD (n=8) and RCD (n=8) with irregular cycles were treated with normal saline. Blood was collected by decapitation and serum obtained for hormonal analysis of luteinizing hormone by enzyme-linked immunosorbent assay (ELISA). Ovary was harvested and checked for ovulation and then processed by western blotting for the E-cadherin and β-catenin expression. Leptin treatment decreased food intake and body weight and restored regular estrous cycle and ovulation and upregulated E-cadherin and β-catenin expression and the blood levels of luteinizing hormone (LH) in all the 8 rats compared with the control which had irregular oestrus cycle, no ovulation, increased body weight and food intake and a down-regulated E-cadherin and β-catenin expression and LH levels. The results suggest a novel mechanism for leptin action in reversing infertility in obese-infertile rats.

Disclosures: E. Otukonyong: None. **S. Campbell:** None. **T. Nathaniel:** None.

Poster

834. Neuropeptide Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 834.02/OO26

Topic: E.07. Food Intake and Energy Balance

Support: USDA/NIFA AFRI 11303067

Title: Anorexic chicks have stress-dependent orexigenic responses to exogenous neuropeptide Y injection

Authors: *J. YI, P. B. SIEGEL, E. R. GILBERT, M. A. CLINE;
Virginia Tech., Blacksburg, VA

Abstract: Neuropeptide Y (NPY) has been extensively studied for its role in regulation of food intake in both mammals and chickens. Previously we reported that chicks selected for low body weight (LWS chicks, containing anorexic individuals) were insensitive to exogenous NPY's orexigenic effect while those selected for high body weight (HWS chicks, all of which become obese) were highly sensitive. To further explore these effects we hypothesized that the loss of NPY function in LWS chicks was due to increased anorexic tone caused by stressor exposure. In experiment 1, LWS chicks that had been exposed to a stressor immediately following hatch did not increase food intake after ICV NPY injection at 5 days post-hatch, however LWS chicks without stressor exposure increased food intake. Both stressor-exposed and non-exposed HWS chicks responded to ICV NPY injection with increased food intake. To explore this stress-associated sensitivity to NPY, mRNA expression of appetite-related neuropeptides and their receptors in the whole hypothalamus was quantified. The statistical model included the main effects of line (LWS vs. HWS), treatment (vehicle vs. NPY), stress (stressor vs. non-stressor exposed) and all interactions on mRNA abundance. There was an interaction of treatment × line × stress on agouti-related peptide (AgRP) mRNA abundance, where LWS chicks, regardless of stressor-exposure, expressed greater quantities of mRNA in response to injection of NPY as compared to vehicle-injected chicks. There were greater quantities of corticotrophin-releasing factor (CRF) and oxytocin mRNA in stressor-exposed as compared to non-exposed chicks. Expression levels of melanocortin receptor 4 and pro-opiomelanocortin mRNA were greater in HWS as compared to LWS chicks. Abundance of CRF receptor 2 mRNA increased in NPY-injected chicks relative to vehicle-injected chicks. In summary, these results show that LWS chicks have a stress-dependent food intake response to NPY. Exposure to stress at hatch is associated with changes in expression of CRF and oxytocin mRNA later in life and in LWS but not HWS chicks, injection of NPY is associated with up-regulation of AgRP mRNA. Further studies will explore the potential epigenetic mechanisms underlying these effects.

Disclosures: J. Yi: None. **P.B. Siegel:** None. **E.R. Gilbert:** None. **M.A. Cline:** None.

Poster

834. Neuropeptide Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 834.03/OO27

Topic: E.07. Food Intake and Energy Balance

Title: Elucidating the orexigenic mechanism of prolactin-releasing peptide in chicks

Authors: *G. WANG, E. R. GILBERT, M. A. CLINE;
Virginia Tech., Blacksburg, VA

Abstract: Prolactin-releasing peptide (PrRP) is an endogenous hypothalamic peptide that when exogenously administered causes decreased food intake in rats; however, when administered to chicks it causes a potent increase in food intake. The central mechanisms mediating the appetite-associated effects of PrRP are poorly understood in any species, especially so in chicks. Thus, our purpose was to further elucidate the orexigenic mechanism of PrRP in chicks. Chicks that received intracerebroventricular (ICV) injection of PrRP at a dose as low as 3 pmol increased food intake up to 60 min following injection. Those treated with higher doses, up to 188 pmol, increased food intake throughout the entire 180 min observation period while water intake was not affected by any dose. Whole hypothalamus was collected from 188 pmol PrRP-injected chicks, total RNA isolated, reverse transcribed, and real-time PCR performed. Chicks that received ICV PrRP injection had decreased hypothalamic oxytocin and orexin mRNA abundance compared to the vehicle-injected chicks. To ascertain macronutrient influence on PrRP-induced food intake, chicks were raised on 3 isocaloric (3,000 kcal ME/kg) diets: a standard, high fat (60% kcal ME derived from lard) or high protein (30 % crude protein), and PrRP was ICV-injected. Chicks fed the high fat diet had a higher magnitude of PrRP-induced food intake increase (24% vs. 80%, standard vs. high fat) while those that consumed the high protein diet had a reduced threshold response (188 pmol vs. 3 pmol lowest efficacious dose for standard vs. high protein). When chicks had access to all the three diets simultaneously, ICV PrRP injection did not shift the preference. In conclusion, ICV PrRP injection increases food intake in chicks and is associated with decreased expression of hypothalamic oxytocin and orexin mRNA, and dietary macronutrient composition influences sensitivity and magnitude of response.

Disclosures: G. Wang: None. **E.R. Gilbert:** None. **M.A. Cline:** None.

Poster

834. Neuropeptide Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 834.04/OO28

Topic: E.07. Food Intake and Energy Balance

Title: *In vivo* ghrelin release from the arcuate nucleus measured by push-pull perfusion

Authors: *E. ALI[1,2], C. CAYER[3,2], M. WELLMAN[1], A. ABIZAID[1], J. S. JAMES[2], Z. MERALI[3,2];
[1]Carleton Univ., Ottawa, ON, Canada; [2]Inst. of Mental Hlth. Res., Ottawa, ON, Canada; [3]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Ghrelin is a stomach-derived neuropeptide involved in appetite, food intake and energy balance through the interaction of its receptor, growth hormone secretagogue receptor, which are widely distributed in the brain. Total ghrelin is represented as, the active form, n-octanoyl-modified ghrelin, and its inactive counterpart, des-acyl ghrelin. Ghrelin is mainly synthesized in the stomach however both types have been discovered in the arcuate nucleus (ARC) of the hypothalamus in rats and mice at low levels. Previous research has indicated that ghrelin neurons in the hypothalamus project to orexigenic neurons and anorexigenic neurons, each with competing effects on feeding. The ARC plays a crucial role in the regulation of food intake and energy homeostasis and its relationship with ghrelin makes it a key target for metabolic conditions such as obesity. However, the local release of ghrelin at the ARC and whether it is the main source of ghrelin in the central nervous system has never been elucidated. The objective of the present study is to assess the local release of ghrelin at the ARC using *in vivo* push-pull perfusion. Sprague Dawley rats (n=8) were anesthetized and implanted with push-pull perfusion probes aiming at the ARC. Artificial cerebrospinal fluid (aCSF) was perfused at a flow rate of 6 µl/min. After the collection of three baseline samples (collected every 20 minutes), potassium (145mM) was added to the perfusion medium, and another six samples were then collected. Radioimmunoassay analysis revealed that the addition of potassium to the aCSF elicited an immediate and robust (80%) increase in total local ghrelin release at the ARC. These results constitute the first measurement of *in vivo* central ghrelin release which will hopefully provide future insight into the role of brain endogenous ghrelin in appetite and energy balance.

Disclosures: E. Ali: None. **C. Cayer:** None. **M. Wellman:** None. **A. Abizaid:** None. **J.S. James:** None. **Z. Merali:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.05/OO29

Topic: E.07. Food Intake and Energy Balance

Support: NIH/NIDDK NORC Grant Number P30 DK050456

Title: Spontaneous physical activity (SPA) following optogenetic manipulation of lateral hypothalamic orexin neurons is dependent on the circadian cycle

Authors: *K. PARKER[1], C. E. PEREZ-LEIGHTON[2], C. J. BILLINGTON[1], C. KOTZ[1]; [1]Univ. Minnesota, Minneapolis, MN; [2]Ctr. for Integrative Med. and Innovative Sci., Univ. Andres Bello, Santiago, Chile

Abstract: The neuropeptide, orexin A (OXA, or hypocretin 1) has been shown to mediate multiple behaviors including energy homeostasis, arousal, reward, feeding, and spontaneous physical activity (SPA) (Harris and Aston-Jones, 2006; Kotz, 2006; Tsujino and Sakurai, 2009). Modulation of SPA by the orexin neurons is a complex process that involves multiple brain regions. Previous studies clearly demonstrate a role of orexin in SPA and orexin neuron projections to the rostral lateral hypothalamus (rLH) as a key mediator of the effects on SPA. This research has established orexin and orexin receptors as prime targets for mediating SPA and demonstrates their possible role in the development of obesity. Importantly, consideration should be made for the variation in responsiveness to endogenous OXA release as it may have a critical role in the propensity for SPA. The following studies examined the effects of optogenetic manipulation of the OXA neurons and the role of the circadian cycle on the activity of orexin neurons involved in SPA. In the present studies, we used optogenetic stimulation to control the excitation or inhibition of OXA neurons within the caudal lateral hypothalamus (cLH) and examined SPA throughout the circadian cycle. Male C57BL/6J mice were anesthetized and then unilaterally injected with a lentivirus expressing light sensitive channel, Channelrhodopsin-2 (ChR2) into the cLH. Additionally, a Cre-driven transgenic mouse line was used to express CRE in orexin cells and CRE/LOX system to generate transgenic mice expressing either Ch2R or NpHR (halorhodopsin) in orexin neurons. All animals were implanted with a fiber optic probe aimed at the cLH. Following recovery, animals were placed in activity monitors and allowed to acclimate to the test environment for 24 hours. Following acclimation, activity was recorded for 24 hours to establish baseline activity levels prior to stimulation. During testing, animals received optical stimulation over the 24-hr period; four cycles of consecutive measurements, each 6 hours in duration (1 cycle = 2 hr pre-stimulation / 2 hr stimulation/ 2 hr post-stimulation). The cycle of stimulation began 6 hours into the light cycle. Results indicate that optogenetic activation of the OXA neurons significantly increased SPA in mice expressing ChR2 only within the third interval of stimulation during the late dark phase of the circadian cycle. The current data suggest that selective activation of lateral hypothalamic OXA neurons can be sufficient to increase SPA, but this general activation is dependent on the circadian cycle.

Disclosures: **K. Parker:** None. **C.E. Perez-Leighton:** None. **C.J. Billington:** None. **C. Kotz:** None.

Poster

834. Neuropeptide Regulators

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 834.06/OO30

Topic: E.07. Food Intake and Energy Balance

Support: CIHR MOP 84409

CIHR RNL-132870

RDC #5404.1171.102

CIHR/RDC Doctoral Fellowship

Title: Time-dependent plasticity of synaptic transmission in orexin neurons following palatable diet exposure

Authors: V. LINEHAN, *M. HIRASAWA;
Div. of Biomed. Sci., Mem. Univ., St. John's, NL, Canada

Abstract: Orexin (hypocretin) neurons of the lateral hypothalamus increase both energy intake and expenditure to promote overeating while preventing weight gain. Interestingly, orexin neurons are activated only by brief exposures to a palatable diet but not with chronic overeating. We hypothesized that this change in orexin activation after brief and chronic palatable diet feeding would be reflected in changes in synaptic transmission to orexin neurons. We used whole cell patch-clamp recordings in hypothalamic slices from male Sprague Dawley rats fed high fat Western Diet (WD) or a control low fat diet to study pharmacologically isolated evoked excitatory postsynaptic currents (eEPSCs) and miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs, respectively). After a one-week feeding, when rats overate WD without causing greater weight gain compared to controls, the amplitude of mEPSCs was increased, suggesting a postsynaptic potentiation of glutamatergic input. On the other hand, we found no change in the amplitude or frequency of mIPSCs, suggesting that this diet specifically modulated excitatory transmission to activate orexin neurons. However, we found that in the WD group, post-tetanic potentiation of eEPSCs was attenuated and LTD could be expressed, which was not observed in controls. These changes in activity-dependent plasticity of excitatory inputs

onto orexin neurons may represent a homeostatic response to intense synaptic activity. In contrast, with a four-week feeding, when WD rats began to gain excess weight compared to controls, the amplitude of mEPSCs was no longer different between groups and synapses could no longer express LTD, suggesting that changes observed at one week were transient. However, we found that the frequency of both mEPSCs and mIPSCs was significantly higher in the WD group. Interestingly, although we found an increase in mEPSC frequency, we noticed a significant increase in the paired pulse ratio of eEPSC. This would suggest that the number of excitatory inputs showed a delayed increase while their average vesicular release probability was decreased compared to controls. In conclusion, palatable diet induces time-dependent plasticity in orexin neurons. The postsynaptic potentiation of glutamatergic input after brief palatable diet exposure may activate orexin neurons. In contrast, with chronic feeding, synaptic remodeling may lead to the diminished activation of orexin neurons. Therefore, while orexin neurons may contribute to the initial overeating of a palatable diet, relatively lessened orexin signaling during chronic feeding would reduce energy expenditure and lead to weight gain.

Disclosures: V. Linehan: None. **M. Hirasawa:** None.

Poster

834. Neuropeptide Regulators

Location: Halls A-C

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Program#/Poster#: 834.07/OO31

Topic: E.07. Food Intake and Energy Balance

Support: KAKENHI 23300142

KAKENHI 23115103

KAKENHI 24790192

Title: The physiological role of orexin neurons in feeding and metabolism revealed by pharmacogenetic activation and chronic ablation

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Abstract: Orexin neurons in the hypothalamus regulate energy homeostasis by coordinating various physiological responses. Although past studies have shown the role of the orexin peptide, orexin neurons contain not only orexin but also other neurotransmitters such as glutamate and dynorphin. In this study, we examined the physiological role of orexin neurons in feeding behavior and metabolism by pharmacogenetic activation and chronic ablation. We generated novel orexin-Cre mice and utilized Cre-dependent adeno-associated virus vectors to express Gq-coupled modified GPCR, hM3Dq or diphtheria toxin fragment A in orexin neurons. We observed selective expression of hM3Dq in orexin neurons and confirmed that synthetic ligand of hM3Dq, clozapin-N-oxcide, activates orexin neurons expressing hM3Dq *in vitro* and *in vivo*. Pharmacogenetic stimulation of orexin neurons simultaneously increased locomotive activity, food intake, water intake and the respiration exchange ratio. Accordantly, strong ablation of orexin neurons resulted in decreased food and water intake, while mild ablation had almost no effect on these parameters. Our results indicate that orexin neurons play an integral role in regulation of both feeding behavior and metabolism and that this regulation is so robust because more than 80% of orexin neurons were ablated before significant changes in feeding and metabolism emerged.

Disclosures: A. Inutsuka: None. **A. Inui:** None. **S. Tabuchi:** None. **M. Lazarus:** None. **A. Yamanaka:** None. **T. Tsunematsu:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.08/OO32

Topic: E.07. Food Intake and Energy Balance

Support: PHS DK 040498

PHS DK 081546

Title: Contribution of hindbrain catecholamine neurons to orexin-induced feeding

Authors: *A.-J. LI, Q. WANG, H. DAVIS, S. RITTER;
Washington State Univ., Pullman, WA

Abstract: Both lateral hypothalamic orexinergic neurons and hindbrain catecholaminergic neurons contribute to feeding behavior. In addition, both phenotypes are widely distributed in the

brain and their terminal sites are in many cases overlapping. In the hindbrain, both orexin receptor subtypes (OX1R and OX2R) have been found in close proximity to dopamine-β-hydroxylase (DBH)-expressing cell bodies, raising the question of whether orexin stimulates feeding by activating catecholamine neurons. We tested this hypothesis in the present study. First, we implanted rats with fourth ventricular (4V) cannulas and tested feeding in response to 4V injection of orexin (0.5 nmol). Orexin stimulated feeding in rats, and this stimulation was abolished in rats given paraventricular hypothalamic injections of the retrogradely-transported immunotoxin, anti-DBH-saporin, which targets and destroys DBH-expressing neurons. We then examined hindbrain c-Fos expression in normal rats in response to 4V injection of the same orexin dose that stimulated food intake. Using multiple immunofluorescent labels and confocal microscopy we found that most of the orexin-induced c-Fos-immunoreactive (-ir) neurons in the dorsomedial and ventrolateral medulla were DBH-ir and, moreover, that orexin-ir varicosities were situated in close proximity to the Fos-expressing DBH-ir soma. Together these results suggest that orexin stimulates feeding, at least in part, by activating hindbrain catecholamine neurons.

Disclosures: **A. Li:** None. **Q. Wang:** None. **H. Davis:** None. **S. Ritter:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.09/PP1

Topic: E.07. Food Intake and Energy Balance

Support: CONICYT - CONCURSO NACIONAL APOYO AL RETORNO DE INVESTIGADORES DESDE EL EXTRANJERO 82130017

Title: Interaction between orexin and opioid/non-opioid dynorphin in PVN in the regulation of food intake and physical activity

Authors: *C. E. PEREZ-LEIGHTON, M. SARMIENTO, L. VELAZQUEZ;
CIMIS, Facultad de Medicina, Univ. Andres Bello, Santiago, Chile

Abstract: The orexin/dynorphin (ox/dyn) neurons are central in regulating energy balance. These neurons promote energy expenditure and obesity resistance. Although most research has focused on the role of orexin peptides, these neurons also release dynorphin peptides. How orexin and dynorphin peptides act together to modulate energy balance is not clear. Classically,

the biological effects of dynorphin peptides, such as DYN-A_{1-13}, are mediated through opioid receptors. However, there are biologically active dynorphin peptides, such as DYN-A_{2-17}, that do not act through opioid receptors. Recent electrophysiological data suggest the net effect of orexin and opioid dynorphin peptides is excitatory. Therefore, we hypothesize that orexin and dynorphin peptides will act in concert to promote food intake and physical activity. 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 the orexin peptide orexin-A and opioid/non-opioid dynorphin peptides in the PVN. We tested the effects of non-opioid DYN-A_{2-17} and its interaction with orexin-A on food intake and physical activity. To this end, Balb/c mice (n = 4) were cannulated unilaterally aiming at PVN and, using a repeated measures design, injected with DYN-A_{2-17} (0, 2.5, 5 nmol). DYN-A_{2-17} increased physical activity (one-way repeated measured ANOVA, $F_{[2,6]} = 4.59$, $P = 0.06$) and significantly increased food intake (one-way repeated measured ANOVA, $F_{[2,6]} = 7.35$, $P = 0.02$). Co-injection of DYN-A_{2-17} (1.25 nmol) with orexin-A (150 pmol) into the PVN suggested a potentiation of the response compared with an injection of either peptide alone, however this effect did not reach statistical significance ($P = 0.15$). To the best of our knowledge, this is the first demonstration of a role of non-opioid dynorphin actions in behaviors associated with energy balance. We are confirming examining the interaction between orexin-A and opioid dynorphin peptide DYN-A_{1-13} in food intake and physical activity. These experiments will improve our understanding of the mechanisms by which the orexin/dynorphin neurons control energy balance. .

Disclosures: C.E. Perez-Leighton: None. **M. Sarmiento:** None. **L. Velazquez:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.10/PP2

Topic: E.07. Food Intake and Energy Balance

Support: PAPIIT IN306711

PAPIIT IN224214

Title: The increase of α-MSH inmunoreactivity in the arcuate nucleus of rats after an acute exposition to a high fat diet

Authors: *D. D. DIAZ-URBINA[1], F. CORTES-SALAZAR[1], J. O. SUAREZ-ORTIZ[1], R. E. ESCARTÍN-PÉREZ[1], C. ESCOBAR-BRIONES[2], V. E. LÓPEZ-ALONSO[1], J. M. MANCILLA-DÍAZ[1];
[1]Facultad De Estudios Superiores Iztacala- UNAM, Tlalnepantla, Mexico; [2]Facultad de Medicina-UNAM, México, D.F, Mexico

Abstract: Melanocortins play an inhibitory role in food intake and energy expenditure regulation, and this process is affected by the dietary composition. The chronic consumption of high-fat (HF) diets decreased the pro-opiomelanocortin (POMC) mRNA expression as well as the α-melanocortin releasing hormone (α-MSH) immunoreactivity in the arcuate nucleus of rats; accordingly, decrease of melanocortin signaling has been associated to obesity. In contrast, acute exposition to HF diets has been related to increase of POMC mRNA expression; however the corresponding changes in α-MSH immunoreactivity remain unknown. Consequently, in the present study we assessed the effects of sub-chronic exposition (10 days) to a HF diet on food intake, body weight, fat accumulation (adipose tissue), as well as cFos and α-MSH inmunoreactivity in the arcuate nucleus of rats. Two independent groups of male Wistar rats were feed with a HF diet (45 % kcal of fat, n=10) or the control diet (standard lab chow, n=10) during 10 days, and food intake as well as body weight were recorded during this period of time. The last day of the sub-chronic exposure to the experimental (or control) diet (day 10), behavioral observations were conducted during a 60-min period at the beginning of the dark cycle using the behavioral satiety sequence (BSS) analysis. After the behavioral observations subjects were decapitated, and brain slices (40 μm) containing the arcuate nucleus were prepared for double immunohistochemistry (cFos and α-MSH). Also adipose tissue (abdominal, gonadal and retroabdominal) was removed and weighted. Finally, we count cFos and/or α-MSH positive neurons in the arcuate nucleus. We found that animals exposed to the HF diet decreased progressively the food intake (g) during the 10 days (the initial 4 days the caloric intake was higher in the high-fat diet rats). Despite body weight did not change significantly between groups, adipose tissue (g) increased in the animals exposed to the HF diet. Immunoreactivity to α-MSH as well as α-MSH and cFos increased in all evaluated regions of arcuate nucleus of rats feed with the high-fat diet. Finally, the behavioral analysis revealed reduction of time of resting and an increase in the time spent grooming in the high-fat diet group. In conclusion, the animals exposed to t diet decrease the food consumption to compensate the higher energy density contained in the food; however, fat accumulation is not prevented even if the behavioral activity is increased too. Furthermore, increase of α-MSH and cFos positive neurons suggests that the acute exposition to the high-fat diet increase the melanocortin signaling in order to inhibit food intake and prevent body weight gain.

Disclosures: D.D. Diaz-Urbina: None. **F. Cortes-Salazar:** None. **J.O. Suarez-Ortiz:** None. **R.E. Escartín-Pérez:** None. **C. Escobar-Briones:** None. **V.E. López-Alonso:** None. **J.M. Mancilla-díaz:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.11/PP3

Topic: E.07. Food Intake and Energy Balance

Support: NIH Intramural Program: NIDDK/DEOB

Title: Melanocortin-4 receptor in satiety and hunger: Influence from limbic regions

Authors: *E. S. WEBBER, M. KRASHES;
Natl. Inst. of Health/NIDDK/DEOB, Bethesda, MD

Abstract: Research has shown that the melanocortin system is intimately involved in energy homeostasis. Specifically, melanocortin-4 receptors (MC4Rs) have been strongly implicated in satiety. These receptors are sensitive to the anorexigenic agonist alpha-MSH and orexigenic antagonist agouti-related peptide hormone (AgRP). AgRP neurons within the arcuate nucleus of the hypothalamus ($AGRP^{ARC}$) project to a variety of brain regions, a number of which evoke increases in food intake under acute photostimulation of terminal fields. Two of these hunger-promoting projections innervate limbic areas: the lateral hypothalamus (LH) and the bed nucleus of the stria terminalis (BNST). Research has shown dense populations of MC4R neurons within the LH and BNST in close proximity of these $AGRP^{ARC}$ efferents. The current study set out to examine whether $AGRP^{ARC} \rightarrow$ LH and $AGRP^{ARC} \rightarrow$ BNST "hunger" projection neurons act to inhibit downstream MC4R neurons within the LH and BNST, respectively and whether these MC4R neurons signal satiety. Employing chemogenetic strategies to stereotaxically target either stimulatory or inhibitory DREADD receptors in distinct limbic regions, we tested the role of $MC4R^{LH}$ and $MC4R^{BNST}$ in regulating food intake. Furthermore, optogenetic techniques were used to examine whether these $AGRP^{ARC} \rightarrow$ MC4R cell types were anatomically and functionally connected. Specifically, effects of simultaneous photostimulation of AgRP terminal fields and downstream MC4R neuron bodies were compared against photostimulation of AgRP terminal fields alone. These results will lead to a better understanding of the physiological neural circuits underlying appetite.

Disclosures: E.S. Webber: None. **M. Krashes:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.12/PP4

Topic: E.07. Food Intake and Energy Balance

Support: PSI2011-24943

BFU2011-27492

CIBEROBN

Title: Neonatal estradiol treatment modulates hypothalamic expression of anorexigenic proopiomelanocortin (POMC) in undernourished female rats

Authors: P. COLLADO[1], B. CARRILLO[1], B. DÍEZ[1], F. HERNANDEZ-NUÑO[2], P. ARGENTE-ARIZÓN[2], F. DÍAZ[2], *J. A. CHOWEN[2], H. PINOS[1];
[1]PSICOBIOLOGY, UNIVERSIDAD NACIONAL DE EDUCACIÓN A DISTANCIA (UNED), MADRID, Spain; [2]Hosp. Infantil Universitario Niño Jesús, Dept. of Endocrinol., Madrid, Spain

Abstract: Recent studies have shown that pre- and postnatal food restriction have severe effects when the animals become adults on the morphology and peptide expression of structures controlling the system involved in appetite regulation. These effects are suggested to be due to an alteration in the development of the neural circuits that control feeding behavior or to metabolic and/or physiological imbalances. Several findings indicate a possible inhibitory role of estradiol in the modulation of feeding behavior. Taking into account that undernutrition during development alters the expression of peptides that control food intake, and that there is a possible interaction between estrogen and peptides to control energy expenditure and feeding behavior, in the present study we analyzed if early postnatal administration of estradiol modulates the effects that undernutrition produces on body weight and hypothalamic proopiomelanocortin (POMC) mRNA expression in adult female rats. Three groups of female rats were analyzed: subjects in the control group (C) (n= 11) were fed prenatally and postnatally with a normal chow diet (20% casein), the undernourished group (LP; n=9) were submitted prenatally and postnatally to a low protein and caloric restricted diet (8% casein and 30% caloric restriction with respect to chow diet) and; the undernourished-treated with estradiol group (LP+EB; n=10) was submitted prenatally and postnatally to the same diet as the LP group, and treated from postnatal day 6 (P6) until P13 with a s.c. dose of 0.4mg/kg estradiol benzoate (EB). A statistically significant effect was found on body weight. The control group had a greater body weight (mean=164.64 ± 3.70) than the undernourished groups LP (mean=101.44 ± 5.32) and LP+EB (mean=119.90 ± 3.62)

($p<0.05$ in all cases). Moreover, treatment with EB early in development lead to an increase in body weight with respect to the undernourished group ($p<0.05$). Analysis of hypothalamic POMC mRNA levels showed that undernourishment decreased hypothalamic mRNA levels of POMC (control group;mean=100 ± 10.41; LP group, mean=52.03 ± 5.42), but administration of EB recovered the levels of this peptide in the hypothalamus (LP + EB group, mean=92.37 ± 4.19) ($p<0.05$). Results of the present work show a modulatory effect of estradiol on body weight and hypothalamic POMC mRNA expression in undernourished female rats. These findings suggest a regulatory role for estradiol in the development of neurophysiological control of metabolism in female rats.

Disclosures: **P. Collado:** None. **B. Carrillo:** None. **B. Díez:** None. **F. Hernandez-Nuño:** None. **P. Argente-Arizón:** None. **F. Díaz:** None. **J.A. Chowen:** None. **H. Pinos:** None.

Poster

834. Neuropeptide Regulators

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK080441

Title: Fasting regulates the expression of AgRP in the mouse sympathoadrenal system

Authors: *R. GUPTA, M. D. WHIM;
Cell Biol. and Anat., LSUHSC, New Orleans, LA

Abstract: Agouti-related protein (AgRP) is a neuropeptide that has a very limited distribution. In the CNS it is synthesized by a small population of neurons in the arcuate nucleus of the hypothalamus. These neurons co-express neuropeptide Y and are involved in the control of feeding. In the periphery the main site of expression appears to be the adrenal but there is disagreement about whether AgRP is present in the steroidal cells in the adrenal cortex or the neuroendocrine chromaffin cells in the medulla. The presence of AgRP in this gland is intriguing given the involvement of the adrenal in the response to food deprivation. In the present study we examined the distribution of AgRP in the mouse adrenal gland and determined whether the expression of AgRP was altered in response to metabolic stress. RT-PCR confirmed the presence of AgRP in the adrenal medulla. When adrenal cryosections were stained with two different AgRP antibodies, cells within the adrenal medulla were immunoreactive. Co-staining with a

variety of chromaffin cell markers including tyrosine hydroxylase, PNMT and neuropeptide Y confirmed that the AgRP-ir cells were mainly epinephrine-synthesizing chromaffin cells. Similar results were found using *in vitro* cultures of the adrenal medulla. We next examined the levels of AgRP-ir in chromaffin cells from matched littermates that were fed *ad lib* or food deprived for 1 day. The latter led to a significant increase in AgRP-ir when quantified either from adrenal sections or from cells isolated in culture. As an alternative approach we then repeated these experiments using AgRP-Cre::tdTomato mice. There was no adrenal expression of tdTomato in fed mice but in a subset of fasted animals (4 of 10 experiments) there was an induction of expression in chromaffin cells. The reason for this variability is not clear, but it was not correlated with the fasting plasma levels of glucose or ketone bodies. Carbon fiber amperometry was used to confirm that the tdTomato-expressing cells could secrete the catecholamines and were thus functional chromaffin cells. These results are consistent with the idea that AgRP is a co-transmitter with epinephrine and neuropeptide Y in adrenal chromaffin cells and its release may therefore play a role in the sympathetic response to fasting.

Disclosures: R. Gupta: None. **M.D. Whim:** None.

Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: FAPESP # 2012/23488-6

AFIP

CAPES

Title: Prolonged sleep restriction associated with hypercaloric diets alters expression of leptin and NPY hypothalamic receptors

Authors: *D. SUCHECKI[1], D. P. VENANCIO[2];
[1]Psychobiology, Univ. Federal De Sao Paulo, Sao Paulo, Brazil; [2]Psychobiology, Univ. Federal de Sao Paulo, Sao Paulo, Brazil

Abstract: Reduction of sleep time in humans has been associated to increased incidence of metabolic syndrome and obesity. In the rat, sleep restriction results in augmented food intake,

but loss of body weight. We reasoned that the later may be due to intake of regular chow, since humans report craving for hypercaloric foods. The present study sought to investigate whether association of prolonged sleep restriction and hypercaloric diet results in increased body weight and changes in peripheral and central mediators of hunger and satiety. Wistar male rats were distributed in two main groups: 1) Control, not sleep-restricted (CTL) and 2) Sleep restricted (SR, 21 days, platform method, for 18 h/day, from 4 pm to 10 am). During the sleep restriction period, CTL and SR groups were fed regular chow (CTL+Reg and RS+Reg), chow enriched with sacarose (73.4%; CTL+Sac and RS+Sac) or enriched with pork's fat (45%; CTL+Fat and RS+Fat), as recommended by Diet Research®. Body weight gain was assessed every day at 10 am, whereas food intake was measured at 10 am and 4 pm. After the end of sleep restriction period, rats were decapitated and blood and brain, collected for determination of leptin plasma levels and hypothalamic expression of leptin and NPY (Y1r, Y2r and Y5r) receptors. CTL groups gain weight with hypercaloric diets, whereas RS groups lost weight, regardless of diet. Intake of fat-rich diets was greater in both groups, and more so for RS+Fat than CTL+Fat rats; nonetheless, leptin levels were lower in RS+Fat group than in its respective CTL group, which had the highest levels. Sacarose-rich diet impaired regulation of hypothalamic leptin receptors expression for no difference in leptin plasma levels was observed between CTL and RS groups, but expression of leptin receptors was lower in RS+Sac rats than in CTL+Sac and RS+Reg rats. Hypercaloric diets increased Y1r expression in CTL, but reduced it in RS rats. Expression of Y2r and Y5r was reduced in CTL+Fat compared to the other CTL groups, whereas both enriched diets lowered expression in RS groups. These results indicate that prolonged sleep restriction represents such a catabolic state that even when rats are offered hypercaloric foods they lose weight. Interestingly, RS associated to both hypercaloric diets induced dysregulation of peripheral and central regulators of satiety. Moreover, induction of obesity in RS animals remains a challenge and requires further investigation to be accomplished.

Disclosures: D. Suchecki: None. **D.P. Venancio:** None.

Poster

834. Neuropeptide Regulators

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Program#/Poster#: 834.15/PP7

Topic: E.07. Food Intake and Energy Balance

Title: Elucidating the anorexigenic central mechanism of adrenocorticotropic hormone in chicks (Gallus gallus)

Authors: *S. SHIPP, J. YI, E. R. GILBERT, M. A. CLINE;
Virginia Tech., Blacksburg, VA

Abstract: Adrenocorticotropic hormone (ACTH), consisting of 39 amino acid residues, is most well known for its involvement in an organism's stress response. It also participates in appetite regulation, as exogenous ACTH causes decreased food intake in rats. However, its anorexigenic mechanism is not well understood and its effect on appetite is not reported in the avian class. Thus the present study was designed to evaluate ACTH's effect on chick food intake and to elucidate the mechanism mediating this response. Chicks that received intracerebroventricular (ICV) injection of 1, 2, and 4 nmol of ACTH reduced food intake, under both ad libitum and 180 min fasted conditions. Water intake was also reduced in ACTH-injected chicks. Following 2 nmol ACTH injection, c-Fos immunoreactivity was quantified in key appetite-associated hypothalamic nuclei including the ventromedial hypothalamus, dorsomedial hypothalamus, periventricular nucleus, lateral hypothalamic area (LHA), arcuate nucleus (ARC) and the parvo- and magno-cellular portions of the paraventricular nucleus. ACTH-injected chicks had increased c-Fos immunoreactivity in the LHA and ARC. Hypothalamus was collected, total RNA isolated, and reverse transcription and real-Time PCR performed to measure mRNA abundance of some appetite-associated factors. Quantities of neuropeptide Y, pro-opiomelanocortin, glutamate decarboxylase 1, melanocortin receptor 4, and urocortin 3 mRNA were not affected by ACTH treatment. However, expression of corticotrophin releasing factor (CRF), urotensin 2 (UT2), agouti-related peptide (AgRP), and orexin (ORX) mRNA decreased in the hypothalamus of ACTH-injected chicks relative to vehicle-injected chicks. In conclusion, ICV ACTH causes decreased food intake in chicks, and is associated with LHA and ARC activation, and a decrease in hypothalamic mRNA abundance of CRF, UT2, AgRP and ORX.

Disclosures: S. Shipp: None. **J. Yi:** None. **E.R. Gilbert:** None. **M.A. Cline:** None.

Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: MRC grant MR/J013293/1

Title: Anatomical distribution of GLP-1 receptor expressing cells throughout the mouse central nervous system

Authors: S. C. CORK[1], F. REIMANN[2], F. M. GRIBBLE[2], *S. TRAPP[1];
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Abstract: Glucagon-like peptide 1 (GLP-1) is emerging as a key regulator of energy metabolism and food intake. As a central neuropeptide, GLP-1 is released from a discrete population of neurons in the brainstem which target key nuclei involved in metabolic control and reward throughout the brain[1,2]. Once released, GLP-1 binds to GLP-1 receptors (GLP-1R), however the precise expression pattern of these receptors in the mouse brain is currently unknown. Here we use a novel transgenic mouse model expressing Cre-recombinase under the control of the *glp1r* promoter with a ROSA26-EYFP reporter to map GLP-1R expression throughout the murine brain. GLP-1R-Cre mice were transcardially perfused with 0.1M PB followed by 4% paraformaldehyde. Brains were removed and 30µm thick coronal sections were cut from the rostral end of the 3rd ventricle to the spinomedullary junction before immunofluorescent detection of EYFP. YFP-positive cells were found throughout the rostrocaudal extent of the brain in areas equivalent to those previously reported in rat[3]. Specifically, high numbers of YFP-positive cells were found in the amygdala, subfornical organ (SFO), paraventricular nucleus of the hypothalamus (PVN), dorsomedial hypothalamus (DMH), arcuate nucleus (ARC), substantia nigra (SN), rostral ventrolateral medulla (RVLM) and area postrema (AP). Lower levels of expression were observed in the nucleus of the solitary tract (NTS), thalamic paraventricular nucleus (PVT) and ventral tegmental area (VTA). These regions correlate with areas shown to receive high levels of input from brainstem GLP-1 neurons[2]. Furthermore a proportion of YFP-positive neurons in the NTS, AP, RVLM, PVN, DMH but not VTA or SN were found to co-localise with tyrosine hydroxylase immunoreactivity. No co-localisation was found in any nuclei when co-stained for glial fibrillary acidic protein. Interestingly, YFP positive neurons were also found in areas devoid of PPG-neuron projections, such as the hippocampus and cortex, raising the question whether these areas may respond to GLP-1 of non-neuronal origin. This study comprises the first comprehensive description of GLP-1R expression in the mouse CNS and provides information about the phenotype of GLP-1R expressing cells. The use of Cre-recombinase in cells expressing GLP-1R provides a novel molecular handle on these neuron populations, which will aid future investigations into their physiological role in the brain. 1. Llewellyn-Smith *et al,* 2011. *Neuroscience*; 180: 111-121 2. Llewellyn-Smith *et al*, 2013. *Neuroscience;* 229: 130-143 3. Merchenthaler *et al*, 1999. *J Comp Neurol;* 403: 261-280

Disclosures: S.C. Cork: None. **S. Trapp:** None. **F. Reimann:** None. **F.M. Gribble:** None.

Poster

834. Neuropeptide Regulators

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 834.17/PP9

Topic: E.07. Food Intake and Energy Balance

Support: NIH grant R00 DA024719

Title: Selective activation of hindbrain GLP-1 neurons reduces food intake but has little effect on glucose or metabolic homeostasis

Authors: *M. M. SCOTT[1], P. S. LAMBETH[1], D. M. WARTHEN[1], K. W. WILLIAMS[3], R. P. GAYKEMA[2];
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Abstract: Glucagon-like peptide-1 (GLP-1) plays a prominent role in the control of feeding, glucose and energy homeostasis. As GLP-1 is produced and acts both in the periphery and in the CNS, it has been difficult to dissect the peripheral from the central actions of this peptide. Brain derived GLP-1 is believed to be implicated in conveying satiety signals leading to reduction in food intake or meal termination, and may contribute to stress- and illness-induced anorexia and conditioned taste aversion. Neurons producing GLP-1 are almost exclusively situated in the caudal portion of the nucleus of the solitary tract (NTS) and innervate multiple brain regions implicated in feeding and energy homeostasis. In this study we selectively expressed the Cre-dependent Gq-coupled modified hM3Dq receptor, a designer receptor exclusively activated by designer drugs (DREADD) in GLP-1 neurons through adeno-associated viral delivery of this gene construct into the NTS of GLP-1::Cre transgenic mice. These GLP-1 DREADD transgenic mice, as well as un- operated and Cre-negative controls, received intraperitoneal injection of clozapine-N-oxide (CNO), the specific ligand used to activate the DREADD receptor, and were tested for overfeeding on palatable food, re-feeding after fasting, glucose and insulin tolerance, metabolic changes, corticosterone production, conditioned taste aversion, and behaviors related to stress and anxiety. CNO treatment diminished (but not abolished) food intake in the overfeeding and fast-refeeding experiments but had little or no effect on many of the other measurements. The expression and functionality of the DREADD receptor was confirmed using immunohistochemical verification of the presence of the co-expressed mCherry and Fos induction in the NTS GLP-1 neurons following CNO treatment prior to sacrifice. In conclusion, GLP-1 neurons in the NTS reduce feeding in both in sated and food-deprived conditions thus confirming the anorectic role of central GLP-1 action.

Disclosures: M.M. Scott: None. **P.S. Lambeth:** None. **D.M. Warthen:** None. **K.W. Williams:** None. **R.P. Gaykema:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant UL1TR000071

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Title: Diet drugs of the future? Small-molecule neuromedin U receptor 2 agonists suppress food intake in animal models

Authors: C. R. BENZON[1], N. YE[1], S. B. JOHNSON[3], J. M. KASPER[1], D. L. MCCUE[2], J. ZHOU[1], *J. D. HOMMEL[1];
[1]Pharmacol. and Toxicology, [2]Neurosci., Univ. of Texas Med. Br., Galveston, TX; [3]MD Anderson Cancer Ctr., Houston, TX

Abstract: Safe and effective approaches for the treatment of obesity are needed. An innovative target in this regard is neuromedin U (NMU), a peptide shown to suppress food intake and cause weight loss. These effects of NMU may be related to actions at the NMU receptor 2 (NMUR2) in the hypothalamus, particularly in the paraventricular nucleus (PVN) which is enriched for NMUR2. Centrally administered NMU has been shown to reduce food intake in rats, but a lack of small-molecule NMU receptor agonists limits our options for pharmacological investigation of NMUR2. Recently, we identified two small-molecule NMUR2 agonists that were functional *in vivo*. We found that acute subcutaneous administration of the compounds significantly decreased rats' 24-hour intake of obesogenic food compared to controls. One compound also significantly decreased intake of non-obesogenic food. Furthermore, 24-hour activity measurements in the home cage show that both compounds significantly decrease locomotor activity. These data indicate that NMUR2 may be a valid target for small-molecule based therapeutics to treat obesity. Progress in drug discovery will provide new tools for the study of NMUR2 signaling in the central nervous system and provide new insights into complex behaviors like feeding.

Disclosures: C.R. Benzon: None. **N. Ye:** None. **D.L. McCue:** None. **J.M. Kasper:** None. **J. Zhou:** None. **J.D. Hommel:** None. **S.B. Johnson:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH R15 DK 96541

Title: GnIH stimulates food intake when injected directly into the DMN and LH, but not the Arc, PVN, or DBB, in a dose-dependent manner

Authors: *A. M. MEELKER[1], A. L. PORTER[2], A. SCHENK[2], C. CAMPBELL[2], G. S. FRALEY[2];
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Abstract: Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic neurohormone with two major functions in mammals; GnIH both inhibits gonadotropin synthesis and release and stimulates food intake. Although much attention has been given to GnIH's role on gonadotropin release, little is known about the neuromechanisms by which GnIH stimulates feeding behavior. This study mapped functional sites of GnIH actions that lead to increased food intake. Adult male Sprague Dawley rats were stereotaxically cannulated into various areas of the hypothalamus: LH, PVN, DMN, ARC, or DBB (n = 20 per hypothalamic site). GnIH (50 ng or 100 ng in 10 nl volume of vehicle; n = 10 per injection type) was injected into the specific brain area. Food pellets were weighed and then placed into the cage. Food intake was measured after an hour. Injection was repeated in a crossover design to give a final n =20 for each GnIH and vehicle. One week later, a final injection of GnIH or vehicle was given (n=10) and the rats were euthanized thirty minutes later using 1mL FatalPlus (400 mg/kg pentobarbital). The rats were then decapitated and blood plasma was collected for LH analyses. The brains were extracted and static fixed in 4% paraformaldehyde then cryoprotected in 25% sucrose PB. The brains were sections at 40 micrometers and Nissl stained. The cannula placement was mapped and correlated to the food intake data. All data were analyzed by ANOVA and a $p < 0.05$ considered significant. At no dose did GnIH stimulate food intake when injected into the Arc, PVN or DBB. We found that the low dose (50 ng) of GnIH significantly ($p = 0.05$) stimulated food intake in the DMN compared to vehicle injections. Paradoxically, the higher dose of GnIH inhibited food intake when injected into the DMN or LH compared to vehicle-treated rats. GnIH injections into the DBB and Arc significantly ($p < 0.05$) reduced plasma levels of LH compared to vehicle controls. These data suggest that although some inhibitory effects of GnIH on feeding behavior occurred in LH injections, the DMN may be a primary site for GnIH action on food intake.

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Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: the Intramural Research Program of the NIH, NIDDK

Title: Drug-induced activation of a Gs-coupled designer GPCR in hypothalamic AgRP neurons stimulates chronic food intake and weight gain in mice

Authors: K. NAKAJIMA[1], *Z. CUI[2], M. KRASHES[1], B. LOWELL[3], J. WESS[1];
[2]NIMH, [1]NIH-NIDDK, Bethesda, MD; [3]Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Obesity has emerged as a worldwide health problem. Understanding the molecular mechanisms of appetite regulation is important to develop new approaches for the treatment of obesity. Agouti-related peptide (AgRP)-expressing neurons located in the arcuate nucleus of the hypothalamus play a key role in triggering appetite. AgRP neurons release three chemically different orexigenic molecules: GABA, Neuropeptide Y (NPY), and AgRP. The activity of AgRP neurons is modulated not only by synaptic ion channels but also by G-protein coupled receptors (GPCRs). GPCRs are linked to several major functional classes of heterotrimeric G proteins, primarily Gq, Gs, and Gi. Recent evidence indicates that activation of Gq-coupled receptors expressed by AgRP neurons, such as the ghrelin receptor, leads to acute feeding in mice. The present study was designed to examine whether activation of a Gs-coupled GPCR expressed by AgRP neurons modulates food intake. To address this issue, we selectively expressed a Gs-coupled designer GPCR (Rs = Gs DREADD) in AgRP neurons. Importantly, the Rs designer GPCR cannot be activated by endogenous ligands but only by an exogenously administered drug, clozapine-N-oxide (CNO), an otherwise pharmacologically inert compound. Selective expression of Rs in AgRP neurons was achieved by injecting a Cre-dependent adeno-associated virus containing the Rs coding sequence into the arcuate nucleus of AgRP-ires-Cre knockin mice. After a single intracerebroventricular injection of CNO, we observed a robust increase in food intake that continued for several days, associated with a significant weight gain. Interestingly, this phenotype was not observed when CNO was co-injected with an anti-AgRP antibody. By contrast, inhibition of NPY receptor signaling did not suppress the Rs-mediated

stimulation in food intake. In addition, activation of Rs had not orexigenic effect in mice carrying the Ay agouti mutation which renders mice insensitive to AgRP. These results suggest that existence of an orexigenic Gs→AgRP pathway in AgRP neurons which leads to chronic food intake. Drugs able to inhibit signaling via Gs-coupled GPCRs endogenously expressed by AgRP neurons may prove useful for the treatment of obesity.

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Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: CIHR

Title: Acute intracerebroventricular administration of relaxin-3 differentially affects food intake and HPA axis activity in male and female rats

Authors: ***C. LENGLOS**, J. CALVEZ, G. GUÈVREMONT, A. MITRA, E. TIMOFEEVA; CRIUCPQ - Univ. Laval, Quebec, QC, Canada

Abstract: Relaxin-3 is a newly discovered neuropeptide which is notably known to produce an orexigenic effect in rats. Previous studies have shown that relaxin-3 intracerebroventricular (ICV) injection increases feeding in male rats but its effect in females remains unknown. Moreover, our previous study shows that chronic stress and food restriction sex-specifically regulates relaxin-3 expression in rats and this neuropeptide could thus have a different effect in female versus male rats. The aim of the present study is to investigate relaxin-3 effects on food intake after its ICV injection at different doses in male and female rats. Twenty male and female Sprague-Dawley rats received once a week, over three weeks, 50, 200 and 800 pmol of relaxin-3 or vehicle in within-subject counterbalanced design. Food intake (standard chow) was measured every 30 minutes during two hours after injection. One hour after 200 pmol relaxin-3 injection, plasma and brains were collected to assess corticosterone level and CRF mRNA expression, respectively. Results show that post injection food intake significantly increased by low (200 pmol) and high (800 pmol) doses of relaxin-3 in female and male rats, respectively. Moreover, this feeding response appeared earlier in female (30 minutes post injection) than in male rats (60

minutes post injection) and gradually increased during two hours of post-injection in female rats while it reached a plateau in male rats after 90 minutes post injection. One hour after 200 pmol relaxin-3 injection, corticosterone level relative to baseline tend to be higher only in male, compared to control. Inversely, corticotropin-releasing factor (CRF) mRNA expression in the parvocellular part of the paraventricular nucleus of the hypothalamus was significantly higher in female relaxin-3 injected than control. In conclusion, because females demonstrated earlier feeding response to a lower dose that persisted longer than in males, this study shows that acute ICV administration of relaxin-3 induces sex-specific effects on food intake in rats. Moreover, measurement of corticosterone and CRF mRNA expression reveal a sex specific effect of relaxin-3 on the activation of the hypothalamo-pituitary adrenal axis.

Disclosures: **C. Lenglos:** None. **J. Calvez:** None. **G. Guèvremont:** None. **A. Mitra:** None. **E. Timofeeva:** None.

Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: CIHR

Title: Sex-specific effects of chronic intracerebroventricular administration of relaxin-3 on food intake, body weight and HPA axis activity

Authors: ***J. CALVEZ**, C. LENGLOS, G. GUEVREMONT, A. MITRA, E. TIMOFEEVA; Laval Univ., Québec, QC, Canada

Abstract: Relaxin-3 (RLN3) is a neuropeptide that is thought to play a role in modulating physiological functions such as food intake and stress. Our recent results suggest a sex-specific regulation of the central RLN3 system. While acute and chronic central administration of RLN3 has been shown to increase feeding and body weight and to stimulate the neuroendocrine stress axis in male rats, it has never been tested in female rats. Our goal was thus to examine the role of RLN3 on food intake regulation, body weight and hypothalamo-pituitary adrenal (HPA) axis activity in both male and female rats by using chronic intracerebroventricular (icv) administration of RLN3. Two groups of male and female rats received vehicle or human RLN3 (400 pmol/d) during 14 days. During all the experiment, the RLN3 rats displayed persistently

higher body weight than control rats and this increase was significantly greater in female than male rats. Accordingly, the RLN3 rats demonstrated higher intake of chow compared to the vehicle-treated rats and this hyperphagia persisted in female rats during all the infusion period whereas male rats showed an increase of food intake only during the first week of treatment. Additionally, decrease of mRNA expression of the RLN3 receptor RXFP3 was observed in the paraventricular nucleus (PVN) of the hypothalamus of RLN3 female rats suggesting a modulation of the HPA axis by RLN3. Although no effect of RLN3 on the expression of corticotrophin-releasing factor (CRF) in the PVN of the hypothalamus was observed, RLN3 female rats displayed greater plasma corticosterone levels than control. In conclusion, female rats exhibited greater hyperphagia, overweight and activation of the HPA axis than male rats when chronically icv infused with RLN3. Further experiments are needed to determine the involvement of RLN3 in the higher prevalence of emotional and stress-induced eating in women compared to men.

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Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK054032

NIH Grant DK043225

Title: R-spondin 1 is up-regulated by leptin and increases β-catenin signaling in the hypothalamus

Authors: ***J.-Y. LI**, B. CHAI, Z. LI, W. ZHANG, M. W. MULHOLLAND;
Dept Gen Surgery, Univ. Michigan, Ann Arbor, MI

Abstract: Objective: Leptin, a peptide hormone produced and secreted by the white adipose tissue, acts in the hypothalamus to inhibit food intake. Our previous study revealed that the secreted protein R-spondin 1 and its cognate receptor leucine-rich repeat-containing G-protein-coupled receptor-4 (LGR4) are expressed in the hypothalamic area, and injection of R-spondin 1

into the third brain ventricle inhibited food intake. In the present study we examined the effect of leptin on R-spondin 1 gene expression. Recently it was found that activation of LGR4 by R-spondin 1 potentiated Wnt/ β-catenin signaling. Glycogen synthase kinase 3β (GSK3β) plays a key role in Wnt/ β-catenin signaling pathways. Phosphorylation of β-catenin by GSK3β leads to its degradation, while inhibition of GSK3β by Wnt results in the accumulation of β-catenin. GSK3β is consecutively active whereas phosphorylation at serine 9 inhibits its activity. GSK3β activity is elevated in Lep-ob and high fat diet induced obese mice. In the present work we also examined the effect of R-spondin 1 on the GSK3β activity and β-catenin level in the hypothalamus of rat. Methods: *In situ* hybridization and immunofluorescence were used to examine the mRNAs and proteins in brain sections. Double *in situ* hybridization was use to determine colocalization. Western blot was used to determine protein levels in hypothalamic extracts and cell line. Results: R-spondin 1 mRNA colocalized with the functioning long form leptin receptor (ObRb) in the ventromedial nucleus of hypothalamus (VMH). VMH R-spondin 1 mRNA was decreased in obese Zucker rats, which have a defected leptin receptor; Intravenous injection of leptin increased VMH R-spondin 1 mRNA in Sprague Dawley rats, indicating that R-spondin 1 gene is up-regulated by leptin. Wnt receptor frizzled 3 mRNA is expressed in VMH and colocalizes with LGR4. Fasting reduced the phosphorylation of GSK3β at serine 9, implying that GSK3β activity was elevated, and in the mean time decreased β-catenin levels in rat hypothalamus. Third brain ventricle injection of R-spondin 1 enhanced phosphorylation of GSK3β at serine 9, and increased the nuclear accumulation of β-catenin in rat hypothalamus. In the hypothalamic neuronal neuropeptide Y cell line, R-spondin 1 potentiated Wnt3a stimulated β-catenin levels. Conclusions: These data indicate that R-spondin 1 acts down-stream of leptin and potentiated Wnt/β-catenin signaling via the inhibition of GSK3β in the hypothalamus.

Disclosures: J. Li: None. **B. Chai:** None. **Z. Li:** None. **W. Zhang:** None. **M.W. Mulholland:** None.

Poster

834. Neuropeptide Regulators

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Title: Dnmt3a in Sim1 neurons is necessary for normal energy homeostasis

Authors: *D. KOHNO[1], S. LEE[2], M. J. HARPER[2], K. KIM[3], H. SONE[4], G. FAN[5], J. K. ELMQUIST[2];
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Abstract: Obesity rates continue to rise throughout the world. Recent evidence has suggested that environmental factors contribute to altered energy balance regulation. However the role of epigenetic modifications to the central control of energy homeostasis remains unknown. To investigate the role of DNA methylation in the regulation of energy balance, we investigated the role of the de novo DNA methyltransferase, DNMT3a, in the paraventricular nucleus of the hypothalamus (PVH). DNMT3a expression levels were decreased in the PVH of high-fat-fed mice. Mice lacking DNMT3a specifically in the Sim1 neurons, which are expressed in the forebrain, including PVH, became obese with increased amounts of abdominal and subcutaneous fat. The mice were also found to have hyperphagia, lowered locomotor activity, and glucose-intolerance with increased serum insulin and leptin. Furthermore, these mice developed hyper-LDL-cholesterolemia when fed a high-fat diet. Gene expression profiling and DNA methylation analysis revealed that the expression of tyrosine hydroxylase and galanin were highly upregulated in the PVH of Sim1-specific Dnmt3a deletion mice. DNA methylation levels of the tyrosine hydroxylase promoter were decreased in the PVH of the deletion mice. These results suggest that DNMT3a in the PVH is necessary for the normal control of body weight and energy homeostasis and that tyrosine hydroxylase is a putative target of DNMT3a in the PVH. These results provide evidence for a role for DNMT3a in the PVH to link environmental conditions to altered energy homeostasis.

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Poster

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Title: Galanin-like peptide (GALP) facilitates thermogenesis via synthesis of prostaglandin e2 by astrocytes

Authors: F. TAKENOYA[1,2], K. HARUAKI[4], S. HIRAKO[3], N. WADA[3], J. WATANABE[3], T. RYUSHI[5], *S. SHIODA[3];
[1]Hoshi Univ. Sch. Pharm Pharmaceut Sci., Tokyo, Japan; [2]Anat, [3]Anat., Showa Univ. Sch. Med., Tokyo, Japan; [4]Kiryu Univ., Gunma, Japan; [5]Daito Bunka Univ., Saitama, Japan

Abstract: Galanin-like peptide (GALP) is a 60 amino acid neuropeptide that was isolated from porcine hypothalamus. Intracerebroventricular (i.c.v.) administration of GALP leads to a decrease in food intake and body weight, moreover GALP induces an increase in core body temperature. To determine whether GALP-induced thermogenesis occurs in the brain, indices of thermogenesis, including the whole body O2 consumption rate, heart rate, skin temperature, and core body temperature, were measured when GALP was i.c.v. injected, either alone or following i.c.v. or intravenous (i.v.) pretreatment with the COX inhibitor, diclofenac. Performed using c-Fos, a marker of neuronal activation, and anti-glial fibrillary acidic protein (GFAP), an astrocytic marker. Moreover, gene expression of the rate-limiting enzymes for PGE2 biosynthesis was sequentially measured in primary cultured astrocytes from the rat's brain using real-time PCR with TaqMan probes, following treatment with GALP. The rats were i.c.v. injected with GALP or saline, after which oxygen consumption, heart rate, and body temperature. The animals were also pretreated with the cyclooxygenase (COX) inhibitor, diclofenac, via i.c.v. or i.v. injection. The c-Fos expression in brain was also examined after injection of GALP, and the levels of COX and prostaglandin E2 synthase (PGES) mRNA in primary cultured astrocytes, treated with GALP, were analyzed by using RT-PCR. The i.c.v. injection of GALP caused biphasic thermogenesis and the effect was blocked by pretreatment with centrally (i.c.v.), but not peripherally (i.v.) administered diclofenac. The strong c-Fos immunoreactivity was observed in astrocytes in the periventricular zone of the third ventricle. GALP treatment also increased COX-2 and cytosolic PGES, but not COX-1, microsomal PGES-1, or microsomal PGES-2 mRNA levels in cultured astrocytes. These results suggest that GALP elicits thermogenesis via a prostaglandin E2-mediated pathway through astrocytes.

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Poster

834. Neuropeptide Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: BLR&D BX001686

R01DK080782

T32DK083250

Title: Impact of environmental enrichment on individual food intake and expression of hypothalamic genes related to energy balance

Authors: ***E. NOBLE**[1,2], A. DIEULEVEULT[1], C. DUFFY[1], R. LEE[1], J. NIXON[1,3], T. BUTTERICK[1,3], C. BILLINGTON[1,3,2], C. KOTZ[1,3,2], C. WANG[1,3];
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Abstract: In rodents, an enriched environment (EE) incorporates both social (group housing) and inanimate (toys) components, and sometimes includes voluntary physical exercise (running wheel). EE including physical exercise elevates hypothalamic brain derived neurotrophic factor (BDNF) expression and promotes a leaner phenotype, but this effect may be dependent on the physical exercise component. Hypothalamic orexin stimulates physical activity, and environments promoting physical activity may induce changes in basal levels of orexin. Hypothalamic BDNF can reduce food intake and elevate energy expenditure. We hypothesized that an enriched environment, which incorporates social enrichment and toys, reduces energy intake and promote resistance to obesity while elevating hypothalamic BDNF and orexin. Sprague Dawley rats (n=32) were randomized into 2 groups of equal body composition, and placed in EE or standard housing (S). EE cages (17x17 inch) included toys and crinkle bedding and housed 4 animals per cage. S rats were individually housed with a single nylabone. Toys were removed during scheduled meal times, and order was rotated upon replacement. Once a week cages were cleaned and toys were switched between cages, such that each group of animals

interacted with each set of toys during two separate weeks. For both S and EE, feeding took place during scheduled meal times for one hour at the onset of the light cycle and for 4 hours during the initial phase of the dark cycle. EE cages were cleared of toys and bedding during meal times and transparent partitions were placed in the cages so that individual food intake could be monitored. Body weights were recorded every 48 hours and body composition was measured every 2 weeks. After 8 weeks animals were sacrificed, and hypothalamic nuclei were dissected and stored at -80°C. We are currently in the process of analyzing rostral, caudal lateral, ventromedial, paraventricular and the arcuate nucleus hypothalamic nuclei for orexin and BDNF expression using qRT-PCR. In obesity research, animals are often housed individually to accurately obtain measurements of individual food intake. Our method of segregating animals during scheduled meal times facilitated the observation that EE including social enrichment reduces feeding ($p<0.0001$) and body weight ($p<0.01$). Previous studies using EE without a social component (toys only) have reported that EE confers no changes in food intake or body weight in individually housed rats. Together these data suggest that social enrichment may be required for EE-induced weight and feeding reductions.

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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant F32NS077643

Title: Satiety mechanisms during the fall transition to hibernation in the thirteen-lined ground squirrel

Authors: *C. P. SCHWARTZ[1], M. HAMPTON[2], M. T. ANDREWS[1];
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Abstract: Hibernation is a survival adaptation utilized by many mammalian species to endure periods of low food availability and unfavorable weather conditions. Obligate hibernators, like the thirteen-lined ground squirrel (Ictidomys tridecemlineatus), enter hibernation annually regardless of environmental conditions, including access to food. During the hibernation season,

thirteen-lined ground squirrels survive for many months without consuming any food, despite regular, spontaneous arousals to normothermia. They abstain from eating during hibernation even when provided food in an artificial laboratory environment, which strongly supports the existence of strong satiety signals and/or suppression of a food-seeking response. In lieu of eating, hibernating ground squirrels rely on white adipose tissue stores and a depressed metabolism to survive. Because of this, the squirrels are hyperphagic during the spring and summer, doubling and even tripling their body weight. However, in early fall, the squirrels' food consumption drops by 53% on average over the course of only 2-3 weeks, dropping over 90% in some cases. This fall transition period to hypophagia occurs reliably in the lab, even when the squirrels are held in constant conditions (LD 12:12) with ad libitum access to food and water. The molecular mechanisms underlying this robust seasonal hyperphagic-hypophagic switch could be very valuable in understanding how satiety is controlled, particularly in the presence of abundant food. The hypothalamus is known to control feeding behavior and seasonal rhythms, and so we used the Illumina HiSeq 2000 system to compare hypothalamic gene expression between hyperphagic and hypophagic ground squirrels. Overall, we identified 9923 protein-coding transcripts. Of these, 143 were significantly differentially expressed, meaning they had 1) a false discovery rate < 0.05, 2) at least a 25% change and 3) a minimum of 100 counts. 58 genes were upregulated in the hypophagic animals, including the anorexigenic TRH (thyrotropin releasing hormone) and LEPR, encoding the receptor for leptin, a well-known satiety signal produced by the white adipose tissue. Interestingly, there was no significant change in the well-known orexigenic genes agouti-related peptide (AGRP) or neuropeptide Y (NPY), despite a substantial reduction in food intake. This experiment provides a unique look at the genes underlying the rapid phenotypic transition that hibernators undergo in the fall. Not only does this work give us insight about satiety mechanisms, but it also provides some clues about how the brain changes in preparation for the upcoming hibernation season.

Disclosures: C.P. Schwartz: None. **M. Hampton:** None. **M.T. Andrews:** None.

Poster

835. Functional Mechanisms of Attention II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 835.01/PP20

Topic: F.01. Human Cognition and Behavior

Support: Coordination for the Improvement of Higher Education Personnel (CAPES)

Title: Attentional bias for food images among obese adults with and without binge eating disorder

Authors: *K. R. VIACAVA[1], M. DELUCHI[1], R. GONÇALVES[1], F. COSTA[2], R. FRIEDMAN[3], L. BIZARRO[1];
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Abstract: Obesity is one of the most serious health problems today, and implicit cognitive processes, such as attentional bias, have a potential role on its development and maintenance. Attentional bias is a tendency to focus attention on a particular class of stimuli over others due to its motivational value, and can elicit consumption. Attentional bias for unhealthy foods indicates altered functioning of the reward system and it is related to increased BMI over time. Although Binge Eating Disorder (BED) can affect up to 30 % of obese patients, few studies have investigated attentional bias in this subgroup. Moreover, distinctive neuronal systems are responsible for initial orientation and maintained attention; thus, the salience of food-related cues might be affected along with this process. Therefore, the aim of this study was to investigate the presence of attentional bias along the attentional process for unhealthy food in a clinical sample of obese adults, comparing the performance of obese patients with and without BED. Participants were in a waiting list for bariatric surgery (BMI > 30 kg / m²), with (n = 22) and without (n = 29) BED. They completed a visual probe task in which pairs of images of food and non-food concealed a probe for either 100, 500 or 2000 ms. A faster reaction time to the probe when it is preceded by an image of food indicates attentional bias. All participants showed attentional bias (M=32,85 ms) during the initial orientation (100 ms), and absence of bias (M=0,62 ms) in the maintenance of attention (2000 ms). Only the group with BED showed bias for food images (M=26,54 with BED vs. M=1,79 without BED) when they were presented for 500 ms. These results indicate that these outpatients presented a motivational ambivalence to stimuli of food. Although not directly addressed, bias for foods can be reduced by treatment. However, obese subjects with BED required more time to employ cognitive strategies that decrease the attentional bias for food. Obese individuals with BED seem to be more vulnerable to salience of food-related stimuli, which in turn might contribute to reported worse outcomes after bariatric surgery.

Disclosures: K.R. Viacava: None. **M. Deluchi:** None. **R. Gonçalves:** None. **F. Costa:** None. **R. Friedman:** None. **L. Bizarro:** None.

Poster

835. Functional Mechanisms of Attention II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 835.02/PP21

Topic: F.01. Human Cognition and Behavior

Title: Paying (for) attention: Effects of distraction, time-on-task, and monetary incentive in younger and older adults

Authors: *Z. LIN, C. LUSTIG;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Adult aging is heuristically associated with reductions in attentional control, but performance may be influenced by a number of factors including the type of attention assessed (e.g. sustaining attention vs resisting distraction) and participants' motivation. We used the Continuous Temporal Expectancy Task (O'Connell et al., 2009), a duration-discrimination task that shows rapid time-on-task performance declines, to assess young (n = 32, M age = 18.63 yrs) and older (n =32; M age = 70.37 yrs) adults' ability to sustain attention, and manipulated whether a nearby laptop was silent or playing videos to assess distractor vulnerability. Older adults performed better than young adults overall and had less severe time-on-task declines. Debriefing questionnaires suggested that boredom and lack of engagement by young adults drove this paradoxical age difference. To test this directly, we added an incentive condition in which participants could earn up to $10, with $.05 deducted for every error. (n = 32 young adults, M age = 18.71 yrs; n = 32 older adults, M age = 72.03 yrs.) Our results suggest this incentive may improve young adults' performance so that they now perform as well as older adults in the non-incentivized condition, but has no or even detrimental effects on the performance of older adults. Distraction did not increase as a function of time-on-task and was not influenced by incentive condition for either age group. Results suggest that different aspects of attentional control differ in their sensitivity to age differences in ability and motivation.

Disclosures: Z. Lin: None. **C. Lustig:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH079388

Title: Effects of cueing on anticipatory distractor suppression

Authors: *A. RAJAN[1], X. WEN[2], Y. LIU[3], M. DING[1];
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Abstract: Selective attention enhances the processing of behaviorally relevant targets and suppresses the processing of behaviorally irrelevant distractors. Employing cueing paradigms, past research has reported preparatory neural activity in sensory areas to facilitate target processing. To what extent cueing elicits preparatory brain activity in anticipation of distractor suppression remains less well understood. In this study we investigated this problem by recording high density scalp EEG from normal healthy subjects performing a cued spatial attention task. On each trial, the cue, in the form of a centrally presented color arrow, indicated the spatial location of the target (left or right hemifield) as well as the probability of a distractor appearing in the opposite hemifield (high or low). The high cue had 80% validity of predicting a distractor (valid cueing) whereas for the low cue this probability was 20% (invalid cueing). Both targets and distractors were Gabor patches and subjects were asked to report the orientation of the target. The following results were found. First, the reaction time was significantly longer for distractor-present trials relative to distractor-absent trials for both types of cueing, demonstrating the behavioral cost of distraction. Second, contrary to past reports, the reaction time for the invalidly cued distractor-present trials was not significantly longer than the validly cued distractor-present trials. Third, defining cueing-derived benefit in distractor suppression as the difference in distractor-caused reaction time cost between valid cueing and invalid cueing, we found that the subjects who benefited from valid cueing exhibited longer reaction time in validly cued distractor-absent trials than invalidly cued distractor-absent trials. The opposite pattern was observed for the remaining subjects. Fourth, a model of limited resources, for which both anticipatory target facilitation and distractor suppression compete, was proposed to explain these behavioral results. Fifth, distinct patterns of alpha lateralization were observed for the two groups of subjects, providing a neural explanation of the observed behavioral effects. Key words: Attention, EEG, alpha, distractor suppression, target facilitation

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Poster

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Support: Grant-in-Aid for Scientific Research (B) (25285206) from the Japan Society for the Promotion of Science (JSPS)

Title: Increased difficulty in visual discrimination enhances attentional capture by visual but not auditory deviant stimuli when they appear in different sequence from task relevant stimuli

Authors: *F. SUGIMOTO[1], J. KATAYAMA[2];
[1]Kyushu Univ., Fukuoka, Japan; [2]Kwansei Gakuin Univ., Nishinomiya, Japan

Abstract: An abrupt onset of a stimulus captures attention automatically even when the stimulus is task irrelevant. This capture of attention is enhanced when a large amount of attentional resource is allocated to a task. For example, distractor stimuli in a three-stimulus oddball task elicit P3 component of event-related brain potentials (ERPs), and its amplitude increases when the discrimination between the standard and target stimuli is difficult. In our previous studies, we found that increased difficulty in visual discrimination enhanced the amplitude of the P3 for both visual and auditory distractor stimuli. These studies indicate that allocating the attentional resource to the visual modality enhances the processing of not only visual but also the auditory task-irrelevant deviant information. However, there is a possibility that the enhancement of the capture of attention occurred because the distractor stimuli were presented in the same oddball sequence as the standard and target stimuli. Considering this possibility, we examined if the difficulty of the visual discrimination affected the amplitude of the P3 for visual and auditory distractors when they appeared independently of the sequence of task relevant stimuli. In the experiment, ERP was recorded while 12 participants were performing a two-stimulus oddball task by discriminating visual target stimuli (20%) from visual standard stimuli (80%). The intervals of the stimulus onsets were 1200 ms. Visual distractors were presented in the visual distractor condition, and pure tones were presented as distractors in the auditory distractor condition. The distractors appeared after 500, 600, or 700 ms from an onset of the standard and target stimulus with the probability of 12%. In the both distractor conditions, easy and difficult conditions were set depending on the difficulty of the visual discrimination. Both the visual and auditory distractors elicited the distractor P3. However, although the amplitude of the visual distractor P3 increased in the difficult condition compared to the easy condition, the amplitude of the auditory distractor P3 was not different between the conditions. This study indicates that although the resource allocation to the visual modality enhances the processing of deviant visual stimuli regardless to their timing of appearance, the processing of task-irrelevant auditory stimuli are not affected when they are not in the same stimulus sequence as the task-relevant visual stimuli.

Disclosures: **F. Sugimoto:** None. **J. Katayama:** None.

Poster

835. Functional Mechanisms of Attention II

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Topic: F.01. Human Cognition and Behavior

Title: The temporal transition of attentional allocation for deviant events reflected by distractor P3 distribution

Authors: ***F. MORIMOTO**[1], J. KATAYAMA[2];
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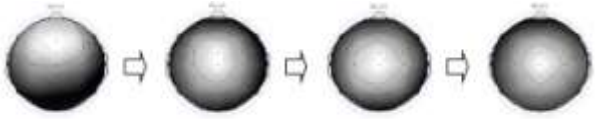
Abstract: In daily life, as well as in experimental situations, human attention momentarily shifts to infrequent events rather than constantly. Temporal transitions in attention to infrequent, deviant events were investigated by using ERP P3s elicited by visual stimuli. Participants pressed a button during a three stimuli oddball task to discriminate targets from deviant and standard stimuli. A standard (80%; blue circle), an infrequent deviant (15%; combination of the standard circle and two lateral red squares) and an infrequent target (5%; small blue circle) were presented in a random series, once every 1.2 seconds for a duration of 120 ms. Participants performed the task for approximately 32 min: in four 8-minute blocks, and their ERPs were recorded. Results indicated that infrequent stimuli elicited distractor P3s in all the blocks. Over a designated time period, the scalp distribution of the distractor P3 extracted by ICA changed from frontal and central to parietal and the amplitude of the distractor P3 attenuated, whereas this tendency was not seen for the target P3. Detailed analysis of this change of amplitude and distribution indicated that in each block, the accumulated number of deviant stimuli, rather than the position of deviant stimuli influenced this change. Previous studies have reported that the distractor P3 with central distribution reflects passive aspects of attention and the target P3 with parietal distribution does active aspects. These results indicate that task-irrelevant deviation captured attention at the beginning, and were subsequently treated as task-relevant deviations.



Disclosures: **F. Morimoto:** None. **J. Katayama:** None.

Poster

835. Functional Mechanisms of Attention II

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Topic: F.01. Human Cognition and Behavior

Support: SFB779-TPA1

Title: A comparison between the neural sources of target selection and distractor suppression

Authors: ***S. E. DONOHUE**[1,2], H. STRUMPF[2], M. A. SCHOENFELD[1,2], J.-M. HOPF[1,2]; [1]Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany; [2]Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: To find a target in a visual scene, attention must be deployed to the relevant target features. In addition to finding the relevant features, any distracting/irrelevant features that are present must be suppressed. These cognitive processes have been shown to entail two underlying electrophysiological components that together comprise the N2pc: The target negativity (N_T) and the distractor positivity (P_D). The N_T is a contralateral negativity that occurs around 200 ms and reflects the shift of visual attention to a target. The P_D has been observed contralateral to a distractor as a relative positivity, and occurs within approximately the same time-range. Although the source of the N2pc has been localized to have an initial parietal source followed by a ventral-occipito-temporal source, it is not clear if both the target processing and distractor processing originate from this source, or if they include differing sources as the underlying neural mechanisms of enhancement vs. suppression differ. To examine the sources of these two components, we conducted a study using both electroencephalography (EEG) and magnetoencephalography (MEG) with 24 human participants. Subjects searched for a pop-out-color target among homogenous distractors, with one of the distractors being a different color from the rest of the distractors and from the target (an attentional pop-out). Scalp-recorded electrophysiological data confirmed that the task elicited both an N_T contralateral to the target and a P_D contralateral to the distractor, both occurring around 200 ms post-stimulus onset. Source analysis showed that both of these processes elicited similar parietal and ventral-occipital-temporal activity (with slightly different timing); however, the primary observable difference was that the polarity (i.e., current influx/efflux) was inverted. These data suggest that although similar neural sources are recruited for target enhancement and distractor suppression,

the activity at these sources is substantially different, given the differences observed in polarity. The N2pc, therefore, likely comprises a complex cascade of attentional control mechanisms that direct attention to the relevant locations in a search display and enhance the processing there while suppressing other locations to which attention may be drawn, thereby generating successful target identification in visual search.

Disclosures: S.E. Donohue: None. **H. Strumpf:** None. **M.A. Schoenfeld:** None. **J. Hopf:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.07/QQ2

Topic: F.01. Human Cognition and Behavior

Title: Preference for high sensation images is correlated with self-reported risk taking but not with interference with pre-attentional processing

Authors: *J. L. EVENDEN[1], B. CONNOR[2];
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Abstract: The activities usually associated with high risk taking or excitement seeking are typically unsuitable for laboratory studies. The present study was designed to investigate the potential for using presentation of pictures containing images of high or low sensation activities as a surrogate marker for the activities themselves. To do this, we presented pictures of action sports (AS+) or social occasions (SO+) paired with similar images with low levels of action (AS-, SO-) in a dot probe task (DPT) to 245 college students as part of multi-component test battery. Participants were also asked to report which of the two pictures they preferred. At the end of the battery, study participants also completed several self-report questionnaires including the sensation seeking personality test (SSPT) and the sensitivity to punishment and reward questionnaire (SPSRQ). As this was a new task, data were randomly assigned to two samples, one for exploratory analyses and one for confirmatory analyses. Only correlations which reached statistical significance in both samples were considered scientifically significant. We found a positive correlation between SSPT Excitement Seeking and preference for AS+ and SO+ pictures, and between SSPT Risk Seeking and preference for AS+ pictures. There was also a positive correlation between Sensitivity to Reward and preference for AS+ pictures and a negative correlation between Sensitivity to Punishment and preference for the same pictures.

There were no significant correlations in either sample between these personality scales and accuracy of dot detection, or between picture preference and DPT accuracy. Separate analyses of the DPT demonstrated that AS+ and SO+ pictures interfered more with dot detection than AS- and SO- pictures. These results suggest that sensation-related images are processed at at least two different levels, which are independent of one another - a preconscious level where they compete for attentional resources, and a conscious level where they are incorporated into a self-generated personality profile. These results have important implications for treatment of mental health problems associated with high sensation seeking, such as substance abuse, since sensation-associated stimuli may capture attention to a degree which is not accessible to consciousness, and unrelated to individuals own view of themselves.

Disclosures: J.L. Evenden: A. Employment/Salary (full or part-time):; WiltonLogic LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); WiltonLogic LLC. **B. Connor:** None.

Poster

835. Functional Mechanisms of Attention II

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust postdoctoral fellowship

Title: Computational models of thalamocortical unsupervised attentional selection and competitive learning

Authors: *R. N. CARDINAL;
Psychiatry, Univ. Cambridge, Cambridge, United Kingdom

Abstract: Mammalian brains represent a large amount of sensory information, yet only a small proportion is selected for attention or represented in active consciousness. Nonetheless, small changes in arbitrary aspects of sensory input can gain attention. Like the active ‘workspace’ of consciousness, some aspects of the mechanisms for learning are of limited capacity. Although some large-scale structures are known to be involved in attentional control, little is known about the way information is represented and selected at the level of a thalamocortical module. However, psychological and biological constraints allow some details of this processing to be inferred. A mechanism is proposed in which each cortical module receives a set of weighted

inputs corresponding to an 'observed' (bottom-up or sensory) input value, plus inputs corresponding to an 'expected' (top-down) value. The module's principal output to other modules is, in the simplest situation, suggested to reflect a combination of these two inputs. In addition, a global competitive process is suggested, hypothetically mediated through thalamic nuclei, through which activity enhancement is provided to a subset of cortical modules. The competition for this enhancement is suggested to be driven by the observation/expectation discrepancy computed by each cortical module, plus influences from systems that govern higher-level attentional processes such as spatial attention. Thus a subset of information, having a high probability of behavioural relevance by virtue of its unexpectedness, receives enhanced information processing, additional support for long-range polysynaptic transmission, and a speed advantage for learning. However, this enhancement is energetically costly. As the consequences of an unexpected stimulus become learned, this additional support is needed less, improving the energetic efficiency with which predictable information is processed and allowing the enhancement to be deployed elsewhere. The proposed architecture is based principally on the propagation of error-enhanced 'state' signals and is compared to theories based on the propagation of signed prediction error.

Disclosures: R.N. Cardinal: None.

Poster

835. Functional Mechanisms of Attention II

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Topic: F.01. Human Cognition and Behavior

Support: Leon Levy Foundation

American Psychiatric Foundation

MH086466-04

MH018870-25

Title: Myelination of the Dorsal Attention Network

Authors: *G. H. PATEL[1,2], E. C. JAMERSON[1], D. C. JAVITT[1,2], V. P. FERRERA[1,2]; [1]Columbia Univ., New York, NY; [2]New York State Psychiatric Inst., New York, NY

Abstract: The dorsal attention network in humans comprises frontal and parietal areas involved in the control of spatial attention. These areas are present in both hemispheres, and include an area at the anterior end of the intraparietal sulcus (aIPS) and an area at the junction of the precentral and superior frontal sulci often referred to as the frontal eye-fields (FEF). Additional areas often are activated in attention tasks, including an area in the inferior frontal sulcus labeled here as inferior FEF (iFEF) and an area in the anterior inferior sulcus (aIFS), but whether they are members of the dorsal attention network or not is unclear. In macaques, dense myelination can be used to distinguish the areas thought to be homologous to the human dorsal attention network, the lateral intraparietal area (LIP) and the frontal eye-fields (FEF). By combining a recently described myelin-mapping neuroimaging technique with fMRI, we examined whether areas activated during a spatial attention task also shared the property of dense myelination. We collected T1w and T2w anatomical images from seven subjects, which were used to both create a cortical surface representation and to map myelin density in cortex using the T1w/T2w ratio. We also collected BOLD-fMRI data from these subjects while they performed a visual search task requiring the covert monitoring and processing of visual stimuli presented in the visual periphery. The myelin map and BOLD activation maps were both projected to the individual's own cortical surface, and the average myelin density was calculated within the areas activated in this task and compared to the average myelin density of the whole cortical surface. We found that areas within the dorsal attention network demonstrated increased myelin density. For instance, aIPS in all seven subjects had a significantly higher T1w/T2w ratio than the brain as a whole (aIPS group mean: 1.439 (CI: +/-.046); whole brain group mean: 1.326 (CI: +/-.003), $p<.001$). The dense myelination extended to areas that were outside of the dorsal attention network but still activated in this task, such as iFEF (group mean 1.441 (CI: +/-.043), $p<.001$). The shared property of dense myelination may indicate that these frontal and parietal areas perform similar functions and are all part of the dorsal attention network, similar to the homologous network in macaques.

Disclosures: G.H. Patel: None. **E.C. Jamerson:** None. **D.C. Javitt:** None. **V.P. Ferrera:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Science Foundation Ireland 09-RFP-NES2382)

Title: Pre and post-target spatial attention influences the rate of evidence accumulation during perceptual decision making

Authors: *G. LOUGHNANE[1], D. P. NEWMAN[2], M. A. BELLGROVE[2], E. C. LALOR[1], S. P. KELLY[3], R. G. O'CONNELL[1];
[1]Trinity Col., Dublin, Ireland; [2]Monash Univ., Melbourne, Australia; [3]City Col. of New York, New York, NY

Abstract: Complementary discoveries in mathematical psychology and neurophysiology have gone a long way towards exposing the neural mechanisms underpinning perceptual decision making but we still know relatively little about how these mechanisms interface with related brain systems such as those governing attention. Recent work on human EEG has isolated a centro-parietal positivity (CPP) that provides access to the distinct parameters of the neural decision process (e.g. onset time, evidence accumulation rate and decision threshold). The purpose of this study was to examine the impact of spontaneous attentional fluctuations on the properties of the CPP within a spatial attention paradigm. Participants monitored two patches of randomly moving dots, one in either visual hemifield, for coherent motion targets. Hemispheric lateralization of parieto-occipital α-band activity in the pre-target period was used as an index of the relative distribution of spatial attention resources to each hemi-field. We found that α-lateralization was predictive of reaction times (RTs) to targets, such that a relative decrease in contralateral α-power was associated with faster RTs. Analysis of the CPP revealed that relatively decreased contralateral α-power was specifically associated with a faster rate of perceptual evidence accumulation. Furthermore, in the post-target onset ERP a posterior lateralized component was observed (approx. 250 to 300ms) before CPP onset (approx. 350ms) with similar characteristics to the well-known N2pc. An increase in the amplitude of this component was associated with a faster build-up of the CPP in the initial stages of evidence accumulation, indicative of an early orienting mechanism. These novel findings indicate that both pre and post-target onset attention affect a specific aspect of decision-making, i.e. speed of evidence accumulation.

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Poster

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Center for the Developing Brain at the Child Mind Institute

NIMH R01 MH081218

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NIMH R21 MH084126

Title: Intrinsic brain indices of threat bias

Authors: E. J. HO[1], E. T. MARCELLE[1], D. O'CONNOR[1], D. J. LURIE[1], R. H. TOBE[2], B. L. LEVENTHAL[2], *F. CASTELLANOS[3,2], N. A. FOX[4], M. P. MILHAM[1,2];
[1]Ctr. for the Developing Brain, Child Mind Inst., New York, NY; [2]Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY; [3]NYU Child Study Ctr., NYU Ctr. Neurodevelopmental Disorders, NEW YORK, NY; [4]Dept. of Human Develop., Univ. of Maryland, College Park, MD

Abstract: Pathophysiologic and treatment models of anxiety consistently highlight the presence of an exaggerated attentional bias toward threat. Among the various paradigms and behavioral observations that have provided an empirical basis for this hypothesis, the dot probe task has generated the greatest attention in recent years--in large part due to its ability to demonstrate increases in attention to threatening stimuli, as well as decreases in the ability to disengage from them. While dot probe indices of facilitation and bias are rapidly becoming the targets for treatment interventions, their neural correlates remain underspecified. Resting-state fMRI (R-fMRI) has proven to be a powerful method for examining brain-based traits. Thus, the present work attempts to use R-fMRI to identify brain-based indices of variations in attentional bias among individuals. In order to accomplish this, we administered the dot probe task to 35 individuals from a larger community-ascertained cohort (ages 6-85), for which resting state fMRI and deep psychiatric phenotyping were also obtained. Consistent with established protocols for the dot probe, pairs of happy/neutral, threat/neutral, and neutral/neutral faces were presented, after which the probe appeared either behind the neutral face or behind the emotion-eliciting face.Threat bias and facilitation were calculated for each individual, yielding notable variation among participants. R-fMRI indices examined included: fractional ALFF (fALFF), Regional Homogeneity (ReHo), and Voxel-mirrored Homotopic Connectivity (VMHC). We found that threat bias was positively associated with subgenual VMHC and negatively related to

right postcentral gyrus ReHo. For threat facilitation, we found positive associations with cerebellum VMHC and fALFF, and negative associations with dorsomedial prefrontal fALFF.

Disclosures: **E.J. Ho:** None. **E.T. Marcelle:** None. **D. O'Connor:** None. **D.J. Lurie:** None. **R.H. Tobe:** None. **B.L. Leventhal:** None. **F. Castellanos:** None. **N.A. Fox:** None. **M.P. Milham:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.12/QQ7

Topic: F.01. Human Cognition and Behavior

Title: Impact of extreme stress on processing of cognitive and mental events

Authors: *J. E. WILLIAMS;
Dept. of Psychology, Eastern Illinois Univ., CHARLESTON, IL

Abstract: Research has shown that high stress situations cause alterations in physiological & behavioral responding (Williams & Ettinger, 2008). Psycho-neuroimmunology has shown that stress can alter immune processes in the body (O'Leary, 1990) and are often responsible for deterioration of not just physical, but cognitive performance as well, including tunnel vision, auditory exclusion, time distortions, loss of fine and complex motor skills. Little research has studied the relationship between existing psychological characteristics and ongoing physiological responses present during un-tethered, high-stress context situations. The ability of the human body to withstand stress depends on many factors, and in some cases the body responds to stress in the same ways it responds to illness (Kalat, 2001). Many studies also use static test environments that limit true responding *in situ*ations. The purpose of this research was therefore to collect and analyze physiological and psychological relational data in a field simulation combat exercise. One-hundred-ninety-five subjects from a southwest law enforcement district participated in a project performed by the Behavioral Physionics laboratory of Eastern Illinois University. All subjects provided informed consent and the study was conducted in accordance with the ethical care and treatment guidelines set forth by the American Psychological Association. Subjects were tested in a vacant school complex, with multiple assailant/hostage interactions, using a standard 2x2 entry formation. Simunition weapons were utilized and full combat gear/shielding was worn by all participants. All subjects wore Polar 380i downloadable heart rate watch monitors for the duration of the exercise, and heart rate and blood pressure

indices were collecting before and after the exercise. Personological, psychopathological and post-survival stress survey data was collected. Results indicate that the high stress context of the simulation caused a significant disruption in subjects ability to perceive cognitively and to respond in an appropriate manner. Subjective emotional and physical responses were altered and both auditory and visual perceptions were distorted during the simulation. Heart rate and blood pressure were significantly altered by the combat experience. This research demonstrates that collecting physiological response data along with psychological measurement enhances our understanding of human performance factors (physiological and cognitive).

Disclosures: J.E. Williams: A. Employment/Salary (full or part-time):; Eastern Illinois University.

Poster

835. Functional Mechanisms of Attention II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 835.13/QQ8

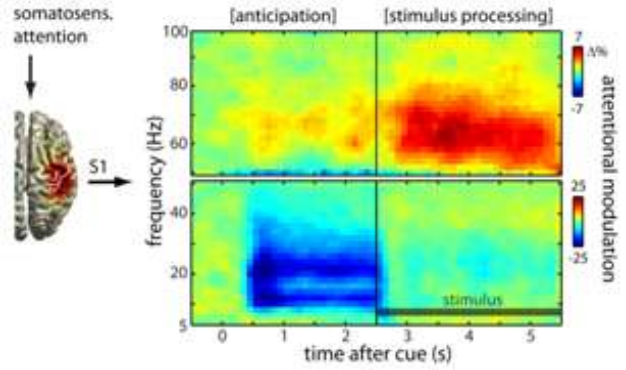
Topic: F.01. Human Cognition and Behavior

Title: Attentional modulations of somatosensory alpha, beta and gamma oscillations dissociate between anticipation and stimulus processing

Authors: *E. MARIS[1], S. SZEBÉNYI[2], F. VAN EDE[2];
[1]Donders Inst. For Brain, Cognition, and Behavior, Nijmegen, Netherlands; [2]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands

Abstract: What are the spectral signatures of somatosensory attention? Here we show that the answer to this question depends critically on the sensory context in which attention is deployed. We recorded magnetoencephalography (MEG) in humans and investigated tactile spatial attention in two different sensory contexts: in anticipation and during the processing of sustained tactile stimuli. We observe a double dissociation between these contexts and two key electrophysiological correlates of attention: in anticipation we primarily observe an attentional suppression of contralateral alpha and beta oscillations (8-12 and 15-30 Hz, respectively), whereas during stimulus processing we primarily observe an attentional amplification of contralateral gamma oscillations (55-75 Hz). This dissociation is well explained by the different neural states that occur prior and during the stimulus, and on which attention can exert its influence. In line with analogous observations in the visual modality, this suggests that the neural implementation of attention must be understood in relation to context and existing brain states.

Consequently, different signatures of attention may contribute to perception in different contexts and, as our data reveals for the attentional modulation of alpha oscillations, these are not always required for attention to improve perception. At the same time, these data demonstrate that the attentional modulations of alpha and gamma oscillations (during, respectively, attentional orienting and attentional selection), are generalizable phenomena across the different sensory modalities.



Disclosures: **E. Maris:** None. **F. van Ede:** None. **S. Szebényi:** None.

Poster

835. Functional Mechanisms of Attention II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 835.14/QQ9

Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research in Japan Society for the Promotion of Science (#25350707)

Title: Eliciting a perceptual-motor execution without cognitive processing by modulating attentional focus

Authors: *H. KATSUMATA;
Daito-Bunka Univ., Higashi-Matsuyama, Japan

Abstract: Visuo-motor performances of: (1) reaching toward an object and grasping it by an index finger-thumb pinch grip (Grasping), and (2) mimicking the visually perceived object size by the pinching motion (Matching) are fooled by visual perception about the object, when the

object structure induces visual illusion about its size (the Ebbinghaus illusion). In Grasping and Matching, the task requirement is the same such that the pinching aperture needs to be produced with respect to the visually perceived size of a target object. However, previous studies reported that Matching is always susceptible to the size-illusion, as opposed to Grasping, in which the illusion effect depends on a task situation. This suggests that Grasping is produced not only via cognitive processing as in Matching, but also without relying on it. According to the functional anatomy of two visual streams at cortical level and visuo-motor performances by patients with the injured temporal cortex, Grasping can be executed without cognitive processing and immune to the size-illusion. Therefore, the present study investigates a prediction that if Grasping needs to be executed under cognitively demanding situation, in which attention needs to be allocated to another cognitive task in parallel with the primary task (Grasping), it can be done without relying on cognitive processing and may not be affected by the size-illusion. 16 participants performed Grasping and Matching with and without the secondary task of visually discriminating a number in relation to the number shown previously. The positions of index finger and thumb during the task were recorded by a motion capture system for calculating the index finger-thumb distance. The aperture size in Matching was biased as the size-illusion effect predicts, regardless of the secondary task. This confirms the nature of Matching such that it requires cognitive processing about the object size to produce the aperture movement. As opposed to it, the aperture in Grasping with the secondary task was not affected by the illusion, even though it was scaled with respect to the different sizes of target objects. These results suggest different perceptual-motor processes for the qualitatively same task goal, depending on task situations.

Disclosures: H. Katsumata: None.

Poster

835. Functional Mechanisms of Attention II

Location: Halls A-C

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Program#/Poster#: 835.15/QQ10

Topic: F.01. Human Cognition and Behavior

Support: NSF Graduate Research Fellowship

NIH Grant R01-NS051048

Title: Rapid bilateral detection of a visual target precedes the lateralized focusing of attention

Authors: *M. L. GAMBLE, B. A. ZULKIEWICZ, M. G. WOLDORFF;
Duke Univ., Durham, NC

Abstract: The ability to successfully navigate and interact within our auditory and visual environments requires a mechanism through which we are able to selectively extract relevant information and discard irrelevant input. While selective attention to a particular relevant stimulus in a scene has been widely studied, the time course of neural mechanisms underlying the identification of and focusing of attention towards a particular stimulus is less well understood. To investigate, with high temporal resolution, the neural time course involved in target detection and processing in vision, we recorded EEG from participants while they were engaged in a novel temporally and spatially distributed visual-array search paradigm. The temporal distribution of the stimulus presentation and the high temporal resolution of EEG enabled the selective extraction of neural responses to the individual items in the array. The participants' task was to identify a pre-defined target popout from a temporally distributed search array comprised of ten rapidly presented ellipses (50 ms duration each, 16 ms ISI, total trial time 660 ms) that were randomly presented to the left and right of fixation. Of the 10 ellipses, 8 were grey, one blue, and one red. Participants had to detect the color-popout ellipse (either blue or red) designated as a Target for that run, focus their spatial attention towards it, and discriminate whether it was oriented vertically or horizontally. Comparison of the neural responses to the popout ellipse when it was designated as the Target (vs. Nontarget) showed a bilateral pattern of activation from 120-160 ms post-stimulus consisting of an occipital negativity followed by a central positivity. This activity was immediately followed by the lateralized N2pc, the hallmark parietal-occipital negativity that is contralateral to a visual target and reflects the focusing of visuo-spatial attention. This neural-response pattern, a bilateral activation reflecting the identification of a relevant target followed by a lateralized activation reflecting the shifting of attention towards that target, parallels the pattern recently reported in an auditory-search study. More specifically, Gamble & Woldorff (2014) reported very rapid auditory Target detection, reflected by a bilateral negativity over frontocentral scalp sites, which was followed by a shift of auditory spatial attention towards the relevant target, reflected by the contralateral N2ac. The present visual-search results implicate a mechanism of a bilaterally-represented template that facilitates early detection of a relevant visual target stimulus, prior to the deployment of visuo-spatial attention.

Disclosures: M.L. Gamble: None. **B.A. Zulkiewicz:** None. **M.G. Woldorff:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.16/QQ11

Topic: F.01. Human Cognition and Behavior

Support: NIH/NINDS R01 NS048527-08

NIH/NCATS UL1 TR 000424-06

NIH/NCATS P41 EB015909-13

Autism Speaks Foundation

Title: ADHD symptom severity correlates with different brain regions in children with autism spectrum disorder (ASD), ADHD, and comorbid ASD/ADHD

Authors: *D. SHOOK[1], A. BROWN[1], D. CROCETTI[2], S. MOSTOFSKY[2], C. STOODLEY[1]; [1]American Univ., Washington, DC; [2]Kennedy Krieger Inst., Baltimore, MD

Abstract: Children with autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) share several overlapping symptoms and neuropsychological features. The DSM-5 now allows for a comorbid diagnosis, increasing the importance of understanding how the neural correlates of these disorders converge and diverge. The aim of the current study was to analyze grey matter (GM) differences and the relationship between severity of ADHD symptoms and GM in children with ASD, ADHD, and comorbid ASD and ADHD. The analyses included 13 children with ASD only (age M=10.5 years), 15 children with ADHD only (age M=10.6 years), 15 children with both ASD/ADHD (age M=10.6 years), and 15 age- and gender-matched typically developing children (age M=10.5 years). Parents completed the DuPaul and Connors rating forms of ADHD symptoms. Voxel based morphometry (VBM) was conducted using T1-weighted MPRAGE structural MRI scans. VBM comparisons with the TD group and GM correlations with the composite parent rating scale scores were performed for each group. Analyses were thresholded at $p<0.001$ (uncorrected) and $k>100$. Relative to the TD group, the ASD and ADHD groups both showed reduced GM in the left superior frontal gyrus (SFG); the ADHD and ASD/ADHD groups both showed increased GM in the left middle frontal gyrus (MFG). Aside from these regions of overlap, each group showed distinct patterns of GM differences: the ASD group showed greater GM in bilateral precuneus and left inferior frontal gyrus (IFG), and reduced GM in the right MFG; the ADHD group showed greater GM in left MFG, but reduced GM in left inferior parietal, cingulate, and left precentral regions; the ASD/ADHD group showed greater GM in right MFG and IFG, but reduced GM in the right precuneus and cuneus. The regions in which GM correlated with ADHD symptom scores also differed between the groups. In the ADHD group, the more severe the ADHD symptoms, the more reduced the GM in the right insula extending into the parahippocampus. In the ASD group, greater ADHD symptoms were related to decreased GM in cerebellar (VIIIB and IX) cortices.

As with the structural findings, the ASD/ADHD group did not show an "additive" (ADHD+ASD) pattern, but rather increased ADHD symptoms were associated with increased GM in left lingual and left orbitofrontal gyri but decreased GM in left precuneus and superior frontal regions. Thus, the ASD/ADHD group showed a unique profile of GM variation in relation to symptoms of ADHD compared with the non-comorbid ASD and ADHD groups. These preliminary findings suggest that similar behavioral symptoms of inattention and hyperactivity may have different neural correlates in these disorders.

Disclosures: D. Shook: None. **A. Brown:** None. **D. Crocetti:** None. **S. Mostofsky:** None. **C. Stoodley:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.17/QQ12

Topic: F.01. Human Cognition and Behavior

Support: NIEHS ES021624

Title: Iron deficiency modulates behavioral, physiological, and cortical responses to variations in perceptual and mnemonic workload

Authors: *L. R. TATE[1], M. J. WENGER[2,3], L. E. MURRAY-KOLB[4], J. D. HAAS[3]; [2]Psychology/Cellular & Behavioral Neurobio., [1]Univ. of Oklahoma, Norman, OK; [3]Div. of Nutritional Sci., Cornell Univ., Ithaca, NY; [4]Nutritional Sci., The Pennsylvania State Univ., University Park, PA

Abstract: Iron deficiency (ID) is a highly prevalent micronutrient deficiency, affecting individuals in both developing and developed countries. ID with and without anemia has been shown to have deleterious effects on physical performance of both strenuous and non-strenuous tasks and on general worker productivity. Studies using animal models have demonstrated that physical endurance and the concentrations of oxidative enzymes and respiratory proteins all decrease in ID without anemia. In studies with humans, reductions in endurance have been documented in ID with and without anemia, with effects being seen in measures of energy expenditure and work efficiency. In contrast, little has been done to examine analogous questions in perceptual and cognitive work. The present study involved 37 college-aged females (20 ID as indicated by serum ferritin < 20 ng/ml, and 17 controls matched for age, education and physical

activity level) who performed a visual Sternberg task with a concurrent mental math task. Workload in the Sternberg task was varied by way of [a] the complexity of the visual stimuli and [b] the number of stimuli to-be-encoded on each trial. While performing the visual Sternberg task, three classes of dependent measures were collected: [a] behavior (accuracy and latency of responses); [b] eletroencephelography (EEG); and [c] physiology. Behavioral performance was quantified using the capacity coefficient (Townsend & Wenger, 2004), a summary measure on the distribution of latencies that is intensity of effort and cumulative work performed. EEG activity was quantified in terms of spectral power, specifically power in gamma-band activity, which has been related to effortful attention. The physiological data considered in detail here were the heart rate data, which were summarized in terms of changes with the onset of and increases in cognitive workload (i.e., manipulated difficulty of Sternberg), and changes across periods of fixed levels of workload. We obtained statistically-reliable effects of workload in all three classes of variables, along with reliable relationships among these three classes of variables, also as a function of variations in task difficulty. Critically, ID reliably moderated those relationships. The results suggest that ID has deleterious effects on the ability to respond to variations in perceptual and cognitive workload that parallel those documented for variations in physical workload.

Disclosures: L.R. Tate: None. **M.J. Wenger:** None. **L.E. Murray-Kolb:** None. **J.D. Haas:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.18/QQ13

Topic: F.01. Human Cognition and Behavior

Support: Department of Veterans Affairs Grant P01 NS015655

NIH Grant RO1 NS070856

Title: Regionally-specific correlations between the integrity of the cortical cholinergic input system and vulnerability to attentional distraction in Parkinson's disease

Authors: *K. KIM[1], M. MÜLLER[2], N. BOHNEN[3], M. SARTER[1], C. LUSTIG[1];
[1]Dept. of Psychology, [2]Radiology, [3]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Although the primary symptoms of Parkinson's disease (PD) are due to loss of midbrain dopaminergic neurons, a substantial portion of patients also exhibit loss of forebrain and brain stem cholinergic neurons. This reduction in cholinergic function, measured using [11C] methyl-4-piperidinyl propionate (PMP) PET, has been associated with reduced executive and attention function and a propensity for falls (Bohnen et al. 2012). We investigated PD patients' attentional performance in the presence of different types of distractors and the relationships between performance and PET measures of cortical cholinergic integrity. Our previous report demonstrated that performance in a visual signal-detection task in the presence of a global distractor that reduced the perceptual salience of the signal (distractor condition sustained attention task (dSAT); Demeter et al., 2008) is correlated with the integrity of the cortical and thalamic cholinergic afferents. In contrast, resistance to a content-rich distractor in a different sustained attention task that lacked a bottom-up visual signal (Continuous Temporal Expectancy Task (CTET) with video distractor; O'Connell et al., 2009; Berry et al., 2013) was correlated specifically with the integrity of the cortical cholinergic input system. Here we present a detailed regional analysis of the relationships between cortical levels of cholinergic function and performance in these tasks and distractor conditions. Greater distractor effects in dSAT were associated with lower cholinergic activity in the bilateral frontal and temporal regions, with a trend towards right-hemisphere lateralization. In contrast, greater distractor effects in CTET were associated with lower bilateral cholinergic activity in parietal and temporal regions. This evidence supports the hypothesis that different components of the cortical cholinergic projection system mediate the attentional performance in the presence of different types of distraction. Such more refined analyses will also assist in determining the regionally-specific loss of the cortical cholinergic input system and the nature of the attentional vulnerabilities that are closely related to the propensity for falls observed in PD patients with cholinergic cell loss.

Disclosures: K. Kim: None. **M. Müller:** None. **N. Bohnen:** None. **M. Sarter:** None. **C. Lustig:** None.

Poster

835. Functional Mechanisms of Attention II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 835.19/QQ14

Topic: F.01. Human Cognition and Behavior

Support: Student Blugold Commitment Differential Tuition Funds

Title: EEG Beta activity during exposure to alcohol stimuli predicts drinking-related problems in women

Authors: *D. S. LELAND, H. M. BADZINSKI, R. M. FISCHER, C. M. GUTSMIEDL, P. M. JOHNSON, L. WEG FERNANDEZ, C. A. FILTZ, A. R. BRANDT, J. C. DOYLE; Psychology, Univ. of Wisconsin-Eau Claire, Eau Claire, WI

Abstract: EEG activity in the Beta range (12-35 Hz) is associated with increased arousal and attention and is higher in binge drinking than non-binge drinking students when at rest (Courtney & Polich, 2010). We recorded EEG and analyzed Beta power in student drinkers (18 female, 11 male) under 3 conditions: 1) at rest, 2) looking at full non-alcohol beverage containers, 3) looking at full alcohol beverage containers. We also administered the Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001) as a measure of drinking-related problems and operationalized drinking intensity as the average of the first and second largest number of drinks consumed during the past 6 months (within a 2-hour period). There were no sex differences in drinking problems (AUDIT scores) or drinking intensity. Females but not males showed greater Beta power in the alcohol condition than the non-alcohol and at-rest conditions. Further, in females there was a positive correlation between AUDIT scores and the differences in Beta power between alcohol and non-alcohol stimulus conditions. Findings suggest that Beta reactivity to alcohol stimuli may predict alcohol problems, not merely drinking intensity. Unclear, however, is why these patterns were found in females only and whether Beta differences reflect underlying vulnerability to alcohol problems or are a consequence of drinking problems.

Disclosures: D.S. Leland: None. **H.M. Badzinski:** None. **R.M. Fischer:** None. **C.M. Gutsmiedl:** None. **P.M. Johnson:** None. **L. Weg Fernandez:** None. **C.A. Filtz:** None. **A.R. Brandt:** None. **J.C. Doyle:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.20/QQ15

Topic: F.01. Human Cognition and Behavior

Support: NIH grant R01-MH060415 to M.G.W.

NIH grant R01-NS051048 to M.G.W.

Title: The context-dependent interplay between proactive and reactive mechanisms for distraction filtering in the human brain

Authors: *F. MARINI[1,2], E. DEMETER[2], K. C. ROBERTS[2], L. CHELAZZI[1], M. G. WOLDORFF[2];
[1]Dept. of Neurolog. and Movement Sci., Univ. of Verona, Verona, Italy; [2]Ctr. for Cognitive Neurosci., Duke Univ., Durham, NC

Abstract: Distracting events can negatively impact task performance. Cognitive-brain systems are both proactively recruited to limit potential interference and reactively invoked when distraction is encountered. Recent behavioral evidence indicates that engaging a strategic, supramodal filtering mechanism can mitigate distraction, although this comes with a "filtering cost" of overall slower response times (Marini, Chelazzi & Maravita, 2013). Here, we investigated distraction-related proactive and reactive brain activity in a hybrid block/event-related fMRI study. Participants performed a flanker task discriminating the direction of a central target arrow in the presence and absence of congruent or incongruent flanking distracter arrows. The study included "Pure" blocks where the flanking distracter stimuli were always absent and "Mixed" blocks where the flanking distracters were present on 80% of trials. Mixed blocks were either mostly congruent (flankers congruent to the central target on 60% of trials) or mostly incongruent (flankers incongruent on 60% of trials). In Mixed vs. Pure blocks, an extended set of activations in the dorsal and ventral frontoparietal attention networks revealed the proactive (blockwise) recruitment of a distraction-filtering mechanism invoked in the face of potential distraction. Moreover, block-related activation in the right middle frontal gyrus (rMFG) correlated positively with the behavioral distraction-filtering cost and negatively with the conflict-related behavioral cost (incongruent vs. congruent distracters). Analysis of event-related, reactive brain activity showed that: 1) incongruent (vs. congruent) trials elicited greater responses in regions likely involved in trying to dynamically counteract distraction and conflict, including rMFG and anterior cingulate cortex; and 2) Mixed-block distracter-absent trials vs. Pure-block trials in which distracters were always absent evoked greater bilateral occipito-parietal activations, suggesting differential attentional orienting processes. Finally, analyses in lower-level visual cortex (bilateral V1/V2, bilateral V3, left lateral occipital complex) revealed that distracters evoked less activation in the mostly incongruent vs. in the mostly congruent mixed blocks, presumably reflecting tonic response suppression due to sustained proactive filtering mechanisms. Thus, our results help delineate the brain mechanisms underlying both proactive and reactive filtering of potential and actual distraction, respectively, as well as the context-dependent relationship of these mechanisms with each other and with behavioral performance.

Disclosures: F. Marini: None. **E. Demeter:** None. **K.C. Roberts:** None. **L. Chelazzi:** None. **M.G. Woldorff:** None.

Poster

835. Functional Mechanisms of Attention II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 835.21/QQ16

Topic: F.01. Human Cognition and Behavior

Title: Analysis of color temperature to improve intelligent productivity-The effect for psychological states and brain activity-

Authors: *N. ONISHI, U. YAMAMOTO, M. MIKI, T. HIROYASU;
Doshisha Univ., Kyotanabe, Kyoto, Japan

Abstract: [Purpose] The aim of this study is to derive appropriate light environment to get a better work for office worker. Prior research mentioned that color temperature of office light effected on psychological states like fatigue. Therefore we investigate the psychological states before the start of experiment and discuss the effect of color temperature on brain function and working efficiency. [Methods] Thirty-four healthy adult male (age, 21.5 ± 0.5 years) participated in this study. Before the start of experiment, they completed POMS (Profile of Mood States) that was used to evaluate the psychological states. After that, subjects were exposed to two types of lights (high color temperature at 7966 ± 65 K and low color temperature at 3226 ± 28 K) during the GO/NOGO task. In order to investigate brain activity during sustained attention, the subjects performed the GO/NOGO task. We investigated the GO/NOGO task performance and cerebral blood flow (CBF) change during sustained attention. The performance was evaluated on the basis of an error rate and reaction time (RT) of the GO/NOGO task. Reaction of the subject was counted as error when the reaction time of go trials exceeds 500 ms. In addition, fast 10% was calculated the average of reaction times among the 10% of the fastest reaction times in each subject. We measured CBF changes in the inferior frontal cortex (IFC) under sustained attention using fNIRS (functional near-infrared spectroscopy). [Results and Discussion] We classified subjects into two groups ; “Vigor” and “non-Vigor”. Any significant difference between Vigor and non-Vigor was not seen in RT ($p>.05$). However, fast 10% was faster in Vigor group than non-Vigor group under both of lights. In non-Vigor group, CBF decreased or unchanged under both of lights. On the other hand, in Vigor group, CBF significantly increased under Blue light ($p<.05$). These results suggest that Vigor group demonstrated a greater IFC activation and high score when exposed to high color temperature than non-Vigorous group. [Conclusion] This study demonstrated that the effect of color temperature differs from psychological states of the start of experiment. The results suggest that, when exposed to high color temperature, vigorous people performed fast responses and more activated in IFC.

Disclosures: **N. Onishi:** None. **U. Yamamoto:** A. Employment/Salary (full or part-time):; Doshisha University. **M. Miki:** A. Employment/Salary (full or part-time):; Doshisha University. **T. Hiroyasu:** A. Employment/Salary (full or part-time):; Doshisha University.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.22/QQ17

Topic: F.01. Human Cognition and Behavior

Support: PSC-CUNY Award # 66061-00 44

Title: Cognitive difficulties in urban population of cigarretes smokers

Authors: ***A. SHEVORYKIN**, D. ROBLES, J. MOSES, R. D. MELARA;
City Col. of New York, New York, NY

Abstract: Understanding the social, psychological, and neurological mechanisms that drive addicts to crave and seek drugs of abuse is an essential first step in developing effective treatments for addictions. Nicotine dependence from tobacco smoking remains among the most common form of addiction in the United States and worldwide, with 250 million packs of cigarettes consumed each day and 5.4 million individuals die each year from smoking-related causes (CDC, 2012). Despite numerous behavioral and pharmacological techniques currently available to quit cigarette smoking, the relapse rate for nicotine addiction is among the highest of any addictive substance, with 75-80% of quitters relapsing within six months (Zhou, Nonnemaker, Sherrill, et al., 2009). The goal of this study was to examine the neural and behavioral correlates of tobacco craving in cigarette smokers during the performance of a cognitive task. A group of smokers and a group of nonsmokers (matched in age, education and gender) performed a version of the flanker conflict task (discriminate lines preceded and followed by matching or mismatching lines) in the presence of one of four visual cues (smoking, positive, negative, and neutral faces) while EEG was recorded. Smokers refrained from cigarettes for one hour prior to testing. The flanker effect - an index of the magnitude of distraction from conflict - was measured as the difference in reaction time on congruent (matching) versus incongruent (mismatching) trials. The flanker effect to neutral cues was comparable in smokers and nonsmokers. However, the flanker effect to smoking cues was significantly greater in smokers. The results suggest that tobacco-related visual cues are disruptive to normal processes of selective attention in cigarette smokers. Therefore, as potential

mechanisms influencing these processing-related bias in smokers can be identified and analized, the substance abuse intervention approach could become more effective at targeting and reducing relapse rates among the smoking population. References Centers for Disease Control and Prevention. (2012). Smoking & Tobacco Use. Retrieved from http://www.cdc.gov/tobacco/
Zhou, X., Nonnemaker, J., Sherrill, B., Gilsenan, A.W., Coste,F., West, R. (2009). Attempts to quit smoking and relapse: Factors associated with success or failure from the ATTEMPT cohort study. Addictive Behavior, 34, 365-373

Disclosures: **A. Shevorykin:** None. **D. Robles:** None. **J. Moses:** None. **R.D. Melara:** None.

Poster

835. Functional Mechanisms of Attention II

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Program#/Poster#: 835.23/QQ18

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH060415 to M.G.W.

Title: Reward- and conflict-induced interference: Guiding and biasing of visual attention

Authors: *C. GIATTINO[1], B. VAN DEN BERG[1,2], F. B. LEE[1], M. M. LORIST[2], M. G. WOLDORFF[1];
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Abstract: Features in the world around us are constantly biasing our attention. When these features are associated with reward (and are thus more salient), they can bias our attention, thereby promoting - or sometimes impairing - cognitive task performance, depending on the relevance of those features for our goals. Here we examined how visual attention and conflict processing interact with reward associations. Participants performed a version of the Erikson flanker paradigm in which the central target letter could be surrounded by symmetric flankers (e.g., XXYXX [incongruent] or YYYYY [congruent]) or asymmetric ones (e.g., XXYYY, left congruent, right incongruent), while neurophysiological (EEG) data was recorded, from which we extracted time-locked event-related potential (ERP) and event-related spectral (ERSP) responses. Additionally, two of a set of four letters were associated with high reward and the other two with low reward, all of which could act either as targets or flankers in different trials. Participants could gain money depending on their response time (RT) performance. Results showed that RTs increased as the number of incongruent flankers increased (bilateral congruent

RTs < asymmetric incongruent RTs < bilateral incongruent RTs), as well as when the target letter was associated with high reward compared to low reward. In addition, if flanking incongruent letters were associated with high (vs. low) reward, participants responded more slowly, suggesting a reward-induced increase in attentional distraction. Asymmetrical conflict effects were observed in the ERPs with a lateralized negative deflection (at ~250-400 ms), followed by an increase in oscillatory power in the alpha band (8-12 Hz) substantially later (at ~600-900 ms), suggesting an initial distraction-induced attentional orienting toward the conflicting letters, followed by an active suppression of that input. In addition, preliminary analyses indicated that bilateral high-reward flankers elicited enhanced attentional biasing, as reflected by a decrease in occipital alpha. Finally, the analyses suggested that high-reward targets (irrespective of flanker reward) increased attentional biasing activity frontocentrally, manifested by a decrease in oscillatory beta-band activity (12-18 Hz). Together, these results reveal the different mechanisms by which reward can guide attention to suppress the processing of irrelevant distracting features (e.g., incongruent flankers) and to enhance the processing of relevant features (e.g., reward-associated targets) via top-down biasing.

Disclosures: C. Giattino: None. **B. van den Berg:** None. **F.B. Lee:** None. **M.M. Lorist:** None. **M.G. Woldorff:** None.

Poster

836. Decision-Making and Stress

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 836.01/QQ19

Topic: F.01. Human Cognition and Behavior

Support: MRC Career Development Award to OJR

Title: The impact of stress on financial decision-making

Authors: *O. J. ROBINSON, R. BOND, J. P. ROISER;
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Abstract: Stress has wide-ranging impacts upon cognitive function. It can be a contributing factor to mood and anxiety disorders, but it can also be an adaptive response to threatening environments. Prior work has demonstrated clear impacts of stress upon lower-level cognitive function: biasing attention towards unexpected and potentially threatening information and instantiating a negative affective bias. However, the impact that these changes have on higher-

order decision-making processes is as yet unclear. In this study we examined the impact of a translational stress induction - threat of unpredictable shock - on two financial decision making tasks (temporal discounting (N=36) and a framed gambling task (N=83)). Manipulation efficacy was also tested via replication of a simple face emotion perception task (N=34). Subjects reported feeling significantly more anxious under threat of shock ($t=16.1, p<0.001$) and we replicated a stress*valence interaction in face emotion perception ($F=7.4, p=0.01$), providing evidence that our manipulation was successful. Moreover, our gambling task revealed a significant predicted framing effect ($F=82, p<0.001$) and we showed a predicted temporal-discounting effect ($F=47, p<0.001$) but neither effect was impacted by stress (framing $p=0.6$; discounting: $p=0.31$). However, both tasks revealed clear reaction time effects; on the temporal discounting task, subjects showed stress-induced speeding in the loss but not gain domain ($F=4.1, p=0.050$) and on the gambling task, subjects were faster to gamble with uncertain (but not certain) choices under stress ($F=7.1, p=0.009$). Thus, stress did not impact choice per se, but did significantly increase the speed of choices in aversive or unpredictable contexts. Stress may therefore have significant lower-level perception and response effects, whilst leaving higher-level executive function unperturbed.This is consistent with the notion that stress increases harm-avoidant behaviour but suggests that this does not necessarily come at a cost of impaired or altered financial choices.

Disclosures: O.J. Robinson: None. **R. Bond:** None. **J.P. Roiser:** None.

Poster

836. Decision-Making and Stress

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01MH091864

Title: Maternal buffering of human amygdala-prefrontal circuitry specifically during childhood

Authors: *D. G. GEE[1], L. GABARD-DURNAM[1], E. H. TELZER[2], K. L. HUMPHREYS[1], B. GOFF[1], M. SHAPIRO[1], J. FLANNERY[1], D. S. LUMIAN[1], D. S. FARERI[1], C. J. CALDERA[1], N. TOTTENHAM[1];
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Abstract: Mature amygdala-medial prefrontal cortex (mPFC) circuitry provides regulation of affect in adulthood; however, this circuitry is late to develop. In (semi-) altricial species, caregivers provide potent regulation of affect in the absence of mature regulatory circuitry, buffering against stress reactivity and emotional over-arousal. The present investigation examined the effects of maternal stimuli on human amygdala-mPFC circuitry and related emotion regulation behaviors. Children (n=23; ages: 4-10) showed greater suppression of right amygdala reactivity in the presence of maternal stimuli, which had no effect on adolescents' (n=30; ages: 11-17) amygdala reactivity (independent samples t-test for mother versus stranger: p=.049). In the absence of maternal stimuli, children exhibited an immature connectivity pattern. However, in the presence of maternal stimuli, connectivity exhibited a mature pattern (i.e., negative connectivity) resembling the adolescent pattern (mother/stranger condition x age group interaction: p=.034). This pattern of responding suggests that children are able to recruit more mature patterns of connectivity when in the presence of maternal stimuli. Maternal effects on amygdala-mPFC circuitry were associated with maternal buffering effects on behavior, such that affect-related regulation skills during an emotional face go/nogo were improved (i.e., fewer commission errors) when children were in the presence of their mother (p=.015). Individual differences emerged as well such that greater maternal influence on amygdala-mPFC circuitry was associated with lower separation anxiety, more secure attachment, and more modulation of behavioral regulation by the mother in daily life. Taken together, the present findings suggest a neural mechanism through which caregivers modulate children's regulatory behavior by inducing a mature pattern of amygdala-prefrontal connectivity and buffering against heightened amygdala reactivity.

Disclosures: D.G. Gee: None. **L. Gabard-Durnam:** None. **E.H. Telzer:** None. **K.L. Humphreys:** None. **B. Goff:** None. **M. Shapiro:** None. **J. Flannery:** None. **D.S. Lumian:** None. **D.S. Fareri:** None. **C.J. Caldera:** None. **N. Tottenham:** None.

Poster

836. Decision-Making and Stress

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Program#/Poster#: 836.03/QQ21

Topic: F.01. Human Cognition and Behavior

Support: Emotions & Choice: R01AG039283

Title: Decision Making under Stress: acute stress effects how we learn to trust others

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Abstract: Many of our everyday social exchanges and decisions are made under stressful conditions. Although previous work suggests that non-social risky choices can be compromised under stress, little is known about how we navigate social exchanges under acute stress. The goal of the work presented here was to examine the interaction between acute stress, measured by increases in cortisol (a neuroendocrine marker of stress response) and social decision-making. Subjects played both a non-social gambling game and a trust game with multiple partners_where trust was operationalized as the willingness to invest in partners who can reciprocate monetary exchanges or not_after either normal or stressful manipulations (cold-pressor task). Subjects not under stress showed no differences between money spent to gamble and money entrusted to partners. In contrast, stressed subjects spent more money gambling but less money in the trust game, exhibiting increased risk taking but less trustworthy behavior. To decompose how stress differentially affects these cognitive processes, we modeled the effect of feedback_winning or losing in the gambling task and partners who reciprocated or chose not to in the trust task. Results reveal that non-stressed subjects only attend to feedback in non-social contexts (gambling task), essentially believing that every incremental loss will lead to a greater chance of winning on the next trial (i.e gambler's fallacy). However, subjects under acute stress were sensitive to feedback in both social and non-social contexts. That is, similar to subjects in the control condition, stressed subjects were strongly biased by the belief that multiple losses would result in a subsequent win. But unlike subjects in the control condition, stressed subjects were highly sensitive to social feedback, such that subjects engaged in more trusting behavior after engaging with partners who did not reciprocate. Together, these results suggest that increased stress disrupts efficient and successful processing of social feedback.

Disclosures: O. Feldmanhall: None.

Poster

836. Decision-Making and Stress

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Program#/Poster#: 836.04/QQ22

Topic: F.01. Human Cognition and Behavior

Support: R01MH068376

Title: Stressful life events blunts medial prefrontal activity to rewards in mdd

Authors: *P. KUMAR[1,2], G. M. SLAVICH[3], L. H. BERGHORST[4], M. T. TREADWAY[1,2], N. H. BROOKS[1], S. J. DUTRA[5], D. N. GREVE[6], D. A. PIZZAGALLI[1,2];
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Abstract: Background: Major depressive disorder (MDD) is often preceded by stressful life events and lab-induced stress has been shown to reduce reward processing, which is a core index of anhedonia. Therefore, understanding the neurobiological effects of life stress could provide important insights into the neural correlates of MDD. To this end, we investigated whether the neural correlates of reward processing in MDD were moderated by the number and severity of stressful life events. Methods: Twelve MDD and 10 healthy individuals performed a monetary incentive delay task under baseline (no-stress) and stress conditions during functional MRI. Stress manipulation involved a social-evaluative component (negative feedback about task performance) and sudden $5 penalty deductions. Life stress was assessed using the Life Events and Difficulties Schedule (LEDS), which was used to derive a total perceived stress score (TPSS). A whole brain correlation across all subjects was run between TPSS and neural correlates in response to reward feedback. Parameter estimates were extracted from reward vs. neutral feedback contrast (from run1: no stress and run 2: stress) and correlation analyses were performed using SPSS to investigate further. Results: A significant negative correlation was observed between the neural activation change in the frontal cluster (including the medial prefrontal cortex, mPFC) in response to reward feedback under stress compared to baseline, and TPSS (x=6, y=56, z=2, Z = 3.2, cluster size = 772 voxels). Post-hoc analyses revealed this negative correlation was mainly driven by MDD (r=-0.72, p=0.013). Further analyses revealed that this difference was prominent at the baseline (Run 1) condition, with MDD having a significant negative correlation in this region, whereas controls showed a non-significant positive relationship. With acute stress, there was significant positive correlation with subjective stress score across subjects (p = 0.008), mainly driven by HC (p = 0.013) and a similar direction in the MDD sample (p = 0.2). The above correlations became stronger after accounting for gray matter reductions in MDD. Conclusion: The current relationship between reduced reward-related mPFC activation and increased perceived stress might index blunted encoding of reward information following stress in MDD. This is consistent with preclinical and clinical models that emphasize the role of mPFC in stress-induced reward-related dysfunction and anhedonia. However, during acute stress, previous life stressors influence the recruitment of the mPFC in both controls and MDD individuals.

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Poster

836. Decision-Making and Stress

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Support: NINDS R01 NS 078784

McKnight Foundation

McDonnell Foundation

Title: Cognitive control predicts use of model-based reinforcement-learning

Authors: *R. OTTO[1], A. SKATOVA[3], S. MADLON-KAY[4], N. DAW[2];
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Abstract: A spate of recent work has examined how acute stress fosters reliance on automatic choice processes at the expense of controlled and/or deliberative processes in humans. One popular dual-systems theory, which posits the operation of independent, competing valuation systems in the control of choice behavior has yielded considerable insight into the question. In particular, model-free reinforcement learning (RL), which learns action preferences in a manner in accord with the "law of effect", is contrasted with the more flexible, but computationally expensive model-based RL, which explicitly represents the structure of the environment in order to prospectively evaluate actions. A recent study from our laboratory revealed how acute stress attenuates the contributions of the model-based system to choice behavior, but spares the model-free system's behavioral contributions, providing support for the dependence of the model-based system upon prefrontal-dependent, executive resources. To further examine the relationship between model-based choice and executive function, we leveraged the rich body of work examining cognitive control--which examines how people maintain higher-order representations in order to flexibly adapt behavior and direct processing in accordance with internally maintained goals--to examine if the well-documented and stable individual differences in cognitive control predict the predominance of model-based choice in behavior. We recruited 83 subjects and assessed their utilization of contextual information using established cognitive control task (AX-CPT), and subsequently had them perform a sequential RL task that affords disentanglement of model-based and model-free choice strategies. We then examined whether the temporal signatures of cognitive control in the AX-CPT predict usage of model-based reinforcement learning in a sequential choice task, finding that usage of contextual information to bias behavior

in a top-down manner (or "proactive" control) significantly correlated with usage of model-based strategies in the RL task. Interestingly, proactive control actually yielded poor performance in certain circumstances in the AX-CPT, but was associated with more model-based behavior in the RL task. The behavioral correspondence between cognitive control and model-based RL suggests that a common set of processes may underpin the two behaviors, further the elucidating the critical dependence of goal-directed choice behavior upon executive-dependent, controlled processing.

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Poster

836. Decision-Making and Stress

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Topic: F.01. Human Cognition and Behavior

Title: Anxiety impairs performance and reduces dlPFC activity during high load spatial working memory

Authors: *N. L. BALDERSTON[1,2], K. O'CONNELL[2], K. VYTAL[2], S. TORRISI[2], M. ERNST[2], C. GRILLON[2];
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Abstract: Anxiety involves both a cognitive component (i.e. worry) and a physiological component (i.e. arousal). It is currently unclear whether these components differentially impact other cognitive abilities. In previous behavioral work, we have shown that anxiety induced by the threat of shock differentially impacts verbal and spatial memory as a function of task difficulty (i.e., cognitive load). At low loads, anxiety interferes with both verbal and spatial working memory (WM) performance, while at high loads anxiety interferes only with spatial WM performance (Vytal, Cornwell, Letkiewicz, Arkin, & Grillon, 2013). These results suggest that anxiety differentially impacts verbal and spatial WM performance. However, it is unknown how the neural processes mediating WM are affected by anxiety. This study examined the effects of induced anxiety on the neural correlates of spatial WM in healthy subjects. Subjects performed a spatial n-back task during periods of threat of shock and safety from shock. The task consisted of a series of trials where an asterisk appeared in one of four quadrants on the screen. During the 1-, 2-, and 3-back blocks subjects were instructed to respond based on whether or not the position of

the asterisk was the same as 1, 2, or 3 trials back. In the control condition they indicated the whether or not the asterisk was in the top position on the current trial. As seen previously, subjects showed a threat-related impairment in performance at high WM loads (2-, 3-back). fMRI results exhibited two patterns of activation. First, consistent with the behavioral data, threat was associated with lower activation in high vs. low loads in the right dlPFC and left IPL, suggesting that anxiety interferes with WM processing. Second, threat was associated with greater activation during high vs. low loads in the right vmPFC, suggesting that cognitive load engages top-down inhibitory processes, also consistent with previously shown load-related decreases in anxiety. Taken together, our results provide evidence for the neural underpinnings of a mutual influence between cognition and anxiety.

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Poster

836. Decision-Making and Stress

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG039283

Title: Effects of acute stress on risky monetary decision-making

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Abstract: A substantial body of recent research has argued for the importance of affective factors in human decision-making. However, the precise ways in which affect interacts with decision-making is still unclear, in part because it can be notoriously difficult to dissociate the specific processes and mechanisms underlying both. A number of recent studies have manipulated acute stress, an affective response with well-characterized neurohormonal components, to examine its effect on cognitive function. The promise of such a specific manipulation can only be fully realized, however, if an equal level of specificity is applied to the choices under examination. Only by separating the valuation and decision processes that underlie choices is it possible to identify the relationships that do, and do not, exist between those

processes and the dimension of affect being manipulated. In risky decision-making, in which participants choose between options that vary in the amount that may be probabilistically won or lost, findings of the effects of stress have been mixed. Some studies have found that stress leads to risk aversion, while others have found that it leads to risk seeking. A limitation of many of these studies is that they fail to dissociate the processes that drive decision-making in risky situations, including not only risk attitudes but loss aversion and overall noisiness or consistency. Here, participants performed a standard risky monetary decision making task on each of two days. This task allows the quantification and separation of three distinct choice processes: risk attitudes (feelings about chance), loss aversion (the relative weighting of losses to gains), and choice consistency. At the beginning of each day, participants were equally likely to undergo an acute stress manipulation (the cold pressor task, or CPT, in which participants submerge their arm up to their elbow in ice water for three minutes) or a control condition (with room temperature water). We were thus able to examine participants' estimated parameter values on each day, and quantify the contribution of stress to each component of choice, within-subjects. Using this econometric model of participants' decision behavior, we found stress selectively affected risk attitudes by reducing risk aversion for gains, and did not affect loss aversion or consistency. Only by computationally separating the processes that contribute to risky monetary choice, and combining them with a well-characterized manipulation of the acute stress response, were we able to address confounds present in prior studies, and quantify the effects of stress in risky decision-making.

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Poster

836. Decision-Making and Stress

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Topic: D.04. Vision

Support: US-Japan Brain Research Cooperation Program

Title: Metacognitive biases in fearful face perception and their neuroanatomical correlates

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Abstract: Whether 'fear' is special to our brain system has long been debated. Previous studies have examined whether the processing fear-related stimuli such as faces is associated with superior behavioral performance or specialized neural circuitry. Given that fearful faces forecast upcoming danger and thus its rapid perception gives survival advantage, most studies focused on examining the processing of fearful faces at early stages. Yet, fearful faces may be uniquely processed at a later stage processing too, as such processes may be crucial for controlling complex behaviors in response to potential danger. This study examined whether fearful face biases later-stage perceptual processing, namely, criteria setting for perceptual decision-making and metacognitive reflection of one's perception (e.g., how certain one feels that a fearful face was perceived). In the experiment, participants performed three detection tasks and three discrimination tasks with fearful, angry, happy, and neutral faces. To selectively examine a later-stage processing, the perceptual sensitivity (d') was experimentally matched across the tasks. This manipulation was achieved by titrating the morphed intensity of facial expressions and the contrast of a backward mask. Under these highly controlled conditions, the results showed that participants set liberal criteria for fearful faces across the tasks. That is, participants generally made more 'fear' responses to the faces regardless of the expressed emotion when detecting fearful faces and when discriminating fearful faces against other emotions (e.g., happy), despite the fact that fear processing was not intrinsically more sensitive because of the way the stimuli were titrated. This means that while participants showed higher hit rate for fearful faces, they also showed higher rate for mislabeling other faces as fear. These rates correlated with the individual differences in gray matter volumes of prefrontal regions such as the dorsolateral and anterior prefrontal cortex as well as the anterior cingulate cortex. This suggests that prefrontal regions may prioritize perceptual decision in favor of fear-related processing. This notion is further supported by preliminary results showing that metacognition of facial expression perception is modulated by the individual differences in the prefrontal region volumes and anxiety trait. We conclude that the prefrontal cortex may contain a high-level decision mechanism by which the perception of fear-related stimuli may be prioritized and monitored in a unique fashion. Anatomical variation in these regions may reflect individual differences in anxiety and fear perception.

Disclosures: A. Koizumi: None. **H. Lau:** None.

Poster

836. Decision-Making and Stress

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Title: Electrical microstimulation of the avian 'prefrontal cortex' in interhemispheric conflict

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Abstract: Although the avian brain is structured very differently and does not possess a cerebral cortex, birds are on par with mammals concerning their cognitive abilities. Versatile evidence shows that the avian pallium is in fact homologous to that of mammals. The nidopallium caudolaterale (NCL) in the avian brain is considered to be the functional analog of the prefrontal cortex and controls executive functions. Here, we study the effect of electrical microstimulation of the NCL in an interhemispheric conflict paradigm. When one hemisphere takes control in interhemispheric conflict this is referred to as metacontrol. This has previously been studied both in human and animal models. Due to the lack of a corpus callosum, laterally placed eyes with minimal binocular overlap, and almost complete crossings of visual fibers, pigeons are ideally suited to study interhemispheric conflict. With the aid of eye caps, pigeons can be trained in one eye and one hemisphere only. Pigeons were trained on a yes/no color discrimination task with different stimuli (two colored bars) for each eye. After monocular training, conflict stimuli consisting of two stimuli, one from each eye, are presented to the pigeons binocularly. When a pigeon decides for the stimulus of one eye systematically in the conflict trials, this hemisphere performs metacontrol over the other. In half of the conflict trials, electrical microstimulation was administered to either the right or left NCL as the pigeon viewed the conflict stimulus. We observed left-sided metacontrol in a subset of pigeons. This is in line with a relative advantage of the left hemisphere in visual feature discrimination as reported previously. Left-sided microstimulation has no effect on metacontrol, whereas right-sided microstimulation seems to cancel left-sided dominance.

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Poster

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Support: JSPS Grants-in-Aid for Scientific Research A22243044

JSPS Grants-in-Aid for Scientific Research 25.13011

Title: Neural accounts of 'A-ha' experience in preference for ambiguous images

Authors: *J. STEVANOV[1], H. ASHIDA[1], M. UESAKI[1], T. A. CARLSON[2], G. C. CUPCHIK[3], A. KITAOKA[4];
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Abstract: In the absence of immediate survival relevance, the 'pleasure of comprehension' may mediate selection of plausible connections among ambiguous inputs in predicting different outcomes. Visual illusions are good examples of inherently ambiguous stimuli, as scenes and objects are perceived obviously different from their physical properties. A fundamental assumption is that the pleasure of understanding deceptive properties of illusory images can account for much of their positive appreciation. Converging evidences from behavioural studies demonstrated a pivotal role of the 'pleasure of comprehension' in preference for colour-deprived illusions with simple shapes where objective stimulus properties were insufficient to account for their preference (e.g. the 'Necker cube')(Stevanov, Markovic, Kitaoka, 2012; Stevanov, Spehar, Ashida, Kitaoka, 2012). Further studies employed brain-imaging techniques to differentiate neural substrates involved in such perceptual pleasure. A distribution of opioid receptors in the ventral visual pathway, being the densest in 'association cortex' (parahippocampal place area; PPA), implies that the activity in these brain areas is most likely associated with the release of endorphins which correlates with positive affective responses. Previous accounts implicated this region in preferential selection of stimuli (Yue at al, 2007), hence our attempt to relate activity in PPA with the occurrence of perceptual flips in ambiguous paintings of Ocampo, Arcimboldo, Del-Prete and Utagawa (Stevanov, Uesaki, Ashida, Carlson, Cupchik, Kitaoka, 2012). These images are experienced as two equally likely interchangeable percepts, challenging enough to elicit 'A-ha' experience in novice viewers. Critically, the peak response in the scene-selective brain area (PPA) was found to correlate with the occurrence of perceptual flip i.e. with the rise of a face percept. Therefore it was suggested that activity in PPA most likely reflected an affective response to the 'A-ha' moment. The subsequent study used mosaic images, where global images of objects were comprised of less conspicuous local images. We aimed at differentiating a brain response to perceptual flips from the local-to-global shift, which relates to perception of both the mosaic images and the ambiguous paintings. Stronger brain activity was found in object selective regions than in late stages of the ventral visual pathway. Different patterns of brain activity in

occipito-temporal regions obtained in two studies suggest qualitatively different contributions of perceptual flips and local-to-global changes in visual-affective processing of ambiguous images.

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Poster

837. Disorders of Executive Function

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Title: Metacognitive monitoring in severe resistant obsessive-compulsive disorder treated by deep-brain stimulation

Authors: *K. N'DIAYE[1,2,3,4], W. I. A. HAYNES[1,2,3], V. M. FACQUE[1,2], L. MALLET[2,1,3,4]; [1]Inst. Du Cerveau Et De La Moelle Epiniere, Paris, France; [2]INSERM U1127, Paris, France; [3]CNRS UMR7225, Paris, France; [4]Univ. Pierre-et-Marie Curie (UPMC), Paris, France

Abstract: Metacognition is the high-level psychological processes that enable to monitor and control one's own cognitive functioning, e.g. the confidence one has in a perceptual decision. Despite "pathological doubt" being a core feature of obsessive-compulsive disorder and repetitive checking, a common OCD symptom, being conceivable as an ill-founded attempt to accumulate evidence to restore confidence in one's decisions, little is known on metacognition in OCD and how these processes may evolve through therapeutical interventions. In the present study, we explored metacognitive monitoring of perceptual decisions in severe resistant OCD patients, both in terms of quantitative behavioral markers, and in terms of their neural bases. We capitalized on a clinical trial assessing the efficacy of high-frequency deep brain stimulation of the subthalamic nucleus and the striatum to longitudinally assess patients on their metacognitive monitoring performance as they went through a cross-over design where each target was successively stimulated over a 3-month period. For this purpose, we used a novel random-dot

motion task during which participants had to discriminate the global direction of an array of moving dots and report, on a trial-by-trial basis, their post-decisional confidence on a visual analog scale. Patients' metacognitive performance was measured through the association between the confidence ratings and the actual discrimination performance. Preliminary analyses show that metacognitive monitoring is affected by the stimulated structure. Further analyses will thus take into account stimulation-induced changes in symptom severity to determine whether individual structures within the basal ganglia may play a causal role in metacognitive monitoring and how these psychological functions might relate to OCD.

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Poster

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FRM

Title: Translational approach to study behavioral flexibility as an endophenotype of obsessive compulsive disorders

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Abstract: Behavioral flexibility is characterized by the ability of a subject to change its behavior according to contextual cues. In humans, obsessive compulsive disorders (OCD) is characterized by repetitive behavior, performed through rigid rituals. This phenomenological

observation has led to explore the idea that OCD patients may have diminished behavioral flexibility. To address this question we developed innovative translational approaches across multiple species, including human patients suffering from obsessive compulsive disorders, and rodent genetic models of OCD to provide original data in the perspective of enlightening the neurocognitive bases of compulsive behaviors. Behavioral flexibility may be challenged in experimental tasks such as reversal learning paradigms. In these tasks, the subject has to respond to either of two different visual stimuli but only one stimulus is positively rewarded while the other is not. After this first association has been learned, reward contingency are inverted, so that the previously neutral stimulus is now rewarded, while the previously rewarded stimulus is not. Performance in reversal learning is indexed by the number of perseverative errors committed when participants maintain their response towards previously reinforced stimulus in spite of negative reward. Unsurprisingly, this behavioral task has been adapted to mice using various response modalities (T-maze, lever press, nose-poke). Using animal models of compulsive behaviors give much more possibilities to study the deficient functions and their underlying neural basis that could lead to pathological repetitive behaviors. Here we present new behavioral setups that we developed in parallel in human (i.e. healthy subjects and OCD patients) and mice (i.e. controls and SAPAP3-KO mice) to study the role of the behavioral flexibility as a possible endophenotype of OCD. We observed that the subjects suffering of compulsive behaviors showed perseverative maladaptive behaviors in these tasks. By comparing the results of a similar task-design in humans and mouse models we will discuss the pertinence of such translational approach to further study the neurocognitive basis of compulsive behaviors.

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Poster

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Title: Does lateral prefrontal cortex activation during flanker task predict self-regulation impairments in ASD?

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Abstract: Self-regulation enables children to make decisions, control impulses or thoughts, and engage in pro-social behavior. Impairments in self-regulation contribute to a variety of conditions, including autism spectrum disorders (ASD). Cognitive control is the ability to flexibly manage thoughts and behaviors to achieve goals, and is thought to play a role in successful self-regulation. Flanker tasks measure interference suppression, a cognitive control process of ignoring irrelevant information. We conducted a functional MRI study to measure the neural correlates of cognitive control with a flanker task, and correlated prefrontal cortex (PFC) activation with parent ratings of self-regulation. Thirty-six children with ASD and 22 typically developing children (TDC) matched on age and IQ participated in the study. Self-regulation was measured using a composite score that averaged six emotional and behavioral subscales from the BASC-2: Hyperactivity, Inattention, Anxiety, Depression, Atypicality, and Withdrawn. The Flanker task had four conditions: Neutral, Congruent, Incongruent, and No-Go. Each trial started with a middle arrow surrounded by two flanking stimuli. On Congruent, Incongruent and Neutral trials, participants responded to the direction of the middle arrow. On No-Go trials, participants were instructed not to respond. FMRI data were analyzed with FSL; parameter estimates from the Interference Suppression contrast (Incongruent vs. Neutral) were extracted within two a priori regions of interest (ROI): left lateral and right lateral PFC. The parameter estimates were then correlated with the self-regulation composite. There were no group differences on task performance or brain activation. Both groups demonstrated robust activation of the cognitive control loop (bilateral lateral PFC, ACC, parietal regions) during the interference suppression contrast ($p<0.05$ FWE). The self-regulation composite correlated positively with activation of left lateral PFC ($r=0.42$, $p<.025$) in ASD after controlling for multiple comparisons (alpha=.05/2=.025). A modest, non-significant correlation in the same direction was found in TDC group for the left lateral PFC ($r=0.22$, $p=.33$). These findings suggest that children with ASD with more self-regulation impairments were less efficient in flanker task performance as reflected in greater left lateral PFC activation. These results establish a relationship between neural mechanisms of cognitive control and everyday self-regulation impairments in ASD.

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Poster

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Title: Deep brain stimulation of anterior thalamic nuclei modulates brain circuitries involved in emotion-attention interaction and response inhibition

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Abstract: Background: Deep Brain Stimulation (DBS) of the anterior thalamic nuclei (ANT) is used in the treatment of refractory epilepsy. Stimulating key nodes within the limbic system such as ANT disrupts epileptic activity but may also interfere with other processes that rely on the integrity of those networks. We have previously reported ANT-DBS to enhance attentional capture by threat-related stimuli and impair response inhibition as reflected in behavior. To expand our previous work, we used event related potentials (ERPs) to study the effects of ANT-DBS on brain circuitries involved in emotion-attention interaction and response inhibition. Hypothesis: We hypothesized that stimulating ANT interferes with the function of the neural circuitries involved in response inhibition and emotion-attention interaction. Stimulation was expected to modulate brain's electrical responses during response inhibition. Specifically, we expected NoGo N2-P3 amplitude to diminish due to stimulation. Furthermore, we expected stimulation to enhance the effect of emotional distractors on Go N2-P3 responses. Method: Twelve epileptic patients with ANT-DBS participated in the study. EEG was recorded while the patients performed a computer based Executive-reaction time (RT) test, i.e. a Go-NoGo visual discrimination task with emotional distractors. During the task, DBS was switched ON and OFF every few minutes. For a Go trial the task of subjects was to respond to the orientation of a

triangle (150ms). A traffic light (150ms), indicating a Go or a NoGo signal, was presented 150 ms after the triangle. A threat-related or emotionally neutral distractor was presented in the center of the traffic light. Repeated Measures Analysis of Variance was carried out for N2-P3 peak-to-peak amplitude based on factors including Stimulation (ON, OFF), Location (in ANT, outside ANT), and Emotion (neutral, threat) from 7 subjects with clearly visible ERPs. Results: ANT-DBS resulted in enhanced effect of threat related distractors on response speed. In accordance with the behavior, enhanced modulatory effect of emotional distractors on Go N2-P3 amplitude at parietal region was observed. We also found diminished right frontal NoGo N2-P3 amplitude while stimulation was ON. Conclusion: Mirroring behavioral findings we found electrophysiological evidence for ANT-DBS enhancing attention to threat and impairing response inhibition. We conclude that ANT-DBS interferes with the function of neural circuitries involved in response inhibition and emotion-attention interaction.

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Title: Hypoactivation of a fronto-striatal loop in OCD associated with goal-directed planning

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Abstract: Executive dysfunction (Chamberlain et al., 2005) and increased appetitive (Gillan et al., 2011) and aversive (Gillan et al., 2014) habit formation have been demonstrated in obsessive-compulsive disorder (OCD), consistent with the maladaptive functioning of frontostriatal neural circuits. This study investigated the hypothesis that OCD pathophysiology and its associated cognitive impairment arise from dysfunctional interactions between nodes that should work in concert rather than from damage to individual brain regions (Alexander et al., 1986; Haber and Heilbronner 2013). Thus we assessed brain activation during the attainment of a goal during planning behavior, through intermediate steps or subgoals, and associated functional connectivity between cortical and sub-cortical (specifically striatal) brain regions, as a possible neurocognitive endophenotype of OCD. Fourteen OCD patients free of comorbidities, 13 unaffected first-degree relatives of these OCD patients, and 13 matched healthy controls were tested on a functional magnetic resonance (fMRI) -optimized version of the Tower of London task. OCD patients and their relatives achieved the same number of correct responses compared with controls at the expense of longer response times. Both patients with OCD and their relatives showed hypoactivation during planning, peaking in the right precentral gyrus and extending to the dorsolateral prefrontal cortex (DLPFC). No activation differences were found between OCD patients and their relatives. Psychophysiological Interaction (PPI) analysis yielded reduced planning-related functional connectivity between the DLPFC and the putamen in OCD patients, and to a lesser degree in OCD patients' relatives, versus healthy controls. A qualitatively similar pattern of reduced functional connectivity between the DLPFC and caudate failed to reach significance. These results suggest that reduced planning-related activity in the right DLPFC is a candidate neurocognitive endophenotype for OCD. Hypoactivation of the right DLPFC and impaired functional connectivity with related subcortical brain structures might constitute an underlying neural substrate responsible for less efficient cognitive strategies in OCD and relate more generally to known deficits in goal-directed behaviour, resulting in possible bias to habit-based learning.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Efficiency of cognitive control in concurrent suppression of nicotine and alcohol stimuli

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Abstract: Rodent and human models of nicotine addiction indicate efficiency of cognitive control is a key factor in vulnerability and relapse. Dual-inhibition of addictive substances presents a particular challenge to cognitive control thus making it a likely contributor to poor outcomes associated with comorbid nicotine and alcohol addiction. Previous work in humans has considered 'ego depletion' effects but not simple cognitive capacity limits or craving on the ability to inhibit multiple types of simultaneously presented stimuli. To examine the dual-inhibition process, we developed a Dual Inhibition Paradigm (DIP) to index the cost of failure to inhibit simultaneously presented classes of stimuli. We applied the DIP to measure the inhibition of responses to alcohol and nicotine related stimuli in heavy and light drinkers across the spectrum of smokers while monitoring their nicotine craving. Results of several studies have indicated that an individual's superordinate semantic categorization of stimuli classes can dictate cognitive inhibitory capacity, which is impacted by where on the smoking spectrum the individual falls as well as their alcohol consumption category. In new experiments, we are restructuring the cognitive categorizations of stimuli in participants to circumvent the seemingly rigid capacity limit on cognitive control with the long-term view of improving intervention outcomes in comorbid nicotine and alcohol addictions.

Disclosures: J. Daffron: None. **G. Davis:** None.

Poster

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Title: Proactive control in alcohol dependence: An fMRI study of the stop signal task

Authors: *C.-S. R. LI[1], S. HU[1], R. SINHA[1], J. IDE[1,2], S. ZHANG[1];
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Abstract: Background Cognitive control is compromised in individuals with alcohol dependence (AD). Our previous work characterized altered cerebral activations during cognitive control in AD as compared to healthy control individuals (HC, Li et al., 2008 Alcoholism Clin Exp Res). Here, we employed a Bayesian model to describe trial-by-trial anticipation of the stop signal (Ide et al., 2013 J Neurosci) and modeled fMRI signals of proactive control, prediction error, and RT slowing. Our goal is to describe the behavior and neural bases of proactive control in AD. Methods24 AD and 118 age and gender matched healthy control individuals (HC) are studied with 40 minutes of fMRI of the stop signal task. Data are pre-processed and modeled using SPM. We modeled fMRI signals at fixation (F model) and go signal (G model) onset in two GLM's, with individual events parametrically modulated by estimated probability of the stop signal (or p(Stop)) and/or RT, respectively. Regional activations are identified for proactive control from the F model and for prediction error and RT slowing from the G model. ResultsCompared to HC (85/118), fewer AD (12/24) showed a significant sequential effect ($p<0.035$, chi-square test) - a correlation between p(Stop) and RT during go trials - and the magnitude of sequential effect is diminished ($p<0.006$, 2-sample t test), suggesting a deficit in Bayesian learning for cognitive control. Both HC and AD respond to p(Stop) in bilateral inferior parietal cortex, anterior pre-SMA, and right middle frontal gyrus, although the magnitude of response is diminished in AD. A direct group contrast showed greater activation in the rostral anterior cingulate cortex in AD. RT slowing engaged bilateral anterior insulae while AD also showed greater activation in the left caudate head, as compared to HC. Additional analyses are to reveal how the directional link between regional activations of p(Stop) and RT slowing is

compromised in CD. ConclusionsThe current results highlight a distinct aspect of cognitive control deficit that may serve as a circuit level marker of alcohol dependence.

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Poster

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Pearson

Title: Prefrontal activation over time during executive tasks in children with developmental coordination disorder: A NIRS study

Authors: J. K. LANGE, *A. L. SMILEY-OYEN;
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Abstract: We examined activation of the prefrontal cortex over time during executive task performance using near-infrared spectroscopy (NIRS). We tested children with Developmental Coordination Disorder (DCD) and typically developing children (TD) ages 8 to 12 years to determine if underlying activation differed from initiation to completion of task. Methods. Eight children with DCD and 10 TD children were tested for blood oxygenation levels in the prefrontal cortex during completion of the Stroop, Wisconsin Card Sort, Go/Nogo and Go/Nogo with a dual task (repeating sentences), as well as Simple Reaction Time. Behavioral variables of reaction time and errors were assessed as was oxygenation within each hemisphere, measured as oxygenated hemoglobin/total hemoglobin. The variable assessed was percent change in oxygenation from rest to each task. Results. The hypothesis that the groups would perform with similar accuracy was supported, except for the Go/Nogo task in which the DCD group exhibited more errors of commission (they moved when they should have remained on the home button). The hypothesis that the groups would show differential prefrontal activation was supported. Growth curve analysis showed TD children increased activation early in Stroop and Wisconsin Card Sort task performance followed by a subsequent decrease in brain activation, whereas children with DCD did not exhibit changes during task performance. Discussion. The behavioral

data indicate children with DCD have greater difficulty when inhibition of a motor action is required. In addition, the differential activation in Stroop and Wisconsin Card Sort supports the position that the neural underpinnings of inhibition may differ between children with DCD and TD children. This interpretation is supported by previous research that indicates increased right hemisphere activation is present in children (Querne et al. 2008) and adults (Rubia, Smith, Brammer & Taylor, 2003) during cognitive inhibition. We concluded that children with DCD exhibit differential prefrontal activation that may reflect differences during tasks that require inhibition and automation of task performance.

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Poster

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Title: Neural responses associated with Bayesian predictions of inhibitory response in occasional users predict future stimulant abuse

Authors: *K. M. HARLE[1], P. SHENOY[2], S. TAPERT[2], Y. ANGELA[2], M. PAULUS[2];
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Abstract: Bayesian ideal observer models provide a way to quantify individuals' beliefs and expectations about their environment and are a powerful analytical tool to disambiguate the neural processes that can predict important outcomes in clinical populations. Recently, we showed that occasional stimulant users show attenuated processing associated with expectations and violations of expectations during an inhibitory task. This study was aimed to determine whether this computational approach can be used to predict who progresses to problem use and who desists using three years later. A total of 157 non-dependent occasional stimulant users (OSU) were recruited from the UCSD campus (age 18-24). They completed a stop-signal task while undergoing functional magnetic resonance imaging (fMRI). They reported on average 15-20 total stimulant uses (primarily including prescription stimulants and/or cocaine. These individuals were prospectively followed for 3 years (97% follow up rate) and evaluated for stimulant use level and number of abuse and dependence symptoms. At follow-up, 37 (24%)

OSU reported at least 2 abuse or dependence symptoms, while 50 (32%) OSU had discontinued use for at least six months. These two groups were classified as problem users and desisters, respectively. Whole-brain voxel-wise logistic regression analyses suggest that stronger activations negatively correlated with Bayesian prediction errors (representing the difference between actual and expected need to stop on a given trial) increase the likelihood of becoming a problem user. This was evident in a set of frontal regions, including the right inferior frontal gyrus/anterior insula (BA47) and the mid-cingulate cortex (BA 24). These results suggest that young adults who show inadequate activations to a computational representation of their surprise to unexpected action needs are more likely to develop future stimulant abuse.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Anticipatory response indexed by contingent negative variation in persons with schizotypy and anhedonia

Authors: *S. PETROSSPOUR[1], J. NESWALD[2], S. SARKISSIANS[2], J. MORALES[2], M. SERGI[3], J. ABARA[3];
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Abstract: The purpose of this study was to examine the relationship between schizotypy, anhedonia, and CNV amplitude. Schizotypy is considered a subclinical manifestation of the same psychotic symptoms identifiable in schizophrenia-spectrum disorders and lies on a continuum. Studies of neurocognition of individuals with schizophrenia and schizotypy offer an understanding in various aspects of deficits, such as attention, working and spatial memory, emotion recognition and response inhibition. The current study examines anticipatory response in schizotypy and anhedonia population using electroencephalography (EEG) measure during an emotion recognition continuous performance task (CPT). “Psychometric schizotypes” were identified by their elevated scores on Schizotypal Personality Questionnaire-Brief. Participants also responded to questions on the Revised Physical Anhedonia Scale (RSAS) as a measure of

the individual's ability to experience pleasure from physical stimuli such as food, sex and settings, and the Revised Social Anhedonia (RSAS) as a measure of the individual's experience of pleasure from social and interpersonal events. The contingent negative variation (CNV) amplitudes were analyzed at the frontal, central and posterior leads. Analysis of the CNV amplitude showed a significant relationship between schizotypy and physical anhedonia at the frontal site (unstandardized coefficient = .542, SE = .200, $p < .05$, standardized coefficient = .566). High schizotypes with anhedonia exhibited low CNV amplitude. The pattern of an attenuated CNV for this population suggests a decrease in the neural resources allocated for orientation to stimulus and the anticipatory evaluation and response to incoming important information. These findings provide electro physiological support for the problem of emotion recognition in schizophrenia and lack of volition in anhedonia.

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Royal London Hospital

Title: Processing speed and executive functioning in preschool children with sickle cell anaemia

Authors: *M. DOWNES[1], F. KIRKHAM[2], P. TELFER[3], I. DUNDAS[3], B. KAYA[3], M. DE HAAN[1];
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Abstract: Children with sickle cell anaemia (SCA), a genetic disorder affecting red blood cells, are reported to have difficulties with executive functions, including regulating attention and organizing their behaviour to complete tasks quickly and efficiently. The frontal cortex is particularly susceptible to stroke in SCA but executive dysfunction has been observed to occur even in the absence of observable neurological damage. Thus, additional risk factors such as

sleep-disordered breathing and low oxygen levels also need to be considered. As there is a purportedly higher incidence of sleep-disordered breathing problems in children with SCA, this may be one avenue for executive function (EF) skill intervention. Sleep breathing problems are also related to poor EF skills and often emerge during the preschool years. Thus, the purpose of this study is to describe the development of processing speed and other aspects of executive functioning in preschool children from three to five years with SCA who have no observable evidence of neurological damage and to look at associations with related disease factors. Control children without SCA are matched for age, IQ, ethnicity, and socioeconomic status. All participants completed the Wechsler Preschool and Primary Scales of Intelligence, an ecologically valid task of executive functioning, and early domain NIH toolbox tasks of inhibition and processing speed. All parents completed the Behavioural Report Inventory of Executive Functioning and the Children Sleep Habits Screener. Daytime haemoglobin oxygen saturation, respiratory rate and end-tidal carbon dioxide were also measured. The results showed lower-than-average performance in both the processing speed and inhibition tasks of the NIH toolbox. There was no significant difference between the children with SCA and matched controls in achievement on the ecologically valid task of EF although there was a trend for longer completion times in the SCA group. Additionally, parents reported that almost all of the children with SCA were suspected to snore. These results suggest that deficits in EF skills may already be present in young children with SCA who have no evidence of observable neurological damage. The additional impact of sleep-disordered breathing on EF skills must also be investigated further in this cohort.

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Title: Resting state functional connectivity MRI predicts executive function in children with and without ADHD

Authors: *M. NIK-AHD[1,2], D. A. FAIR[1,3], J. T. NIGG[1,3];
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Abstract: Executive function (EF) is implicated in academic achievement, as well as health and other life outcomes. Deficits in EF are a hallmark feature of Attention Deficit Hyperactivity Disorder (ADHD). Previous studies using resting state functional connectivity MRI (rs-fcMRI) reveal that those with ADHD have atypical functional brain signatures compared to those without ADHD, suggesting a neurobiological basis for the disorder. The current study aims to determine if information from resting state functional connectivity indices can predict long-term trajectories and/or performance at a later time-point of several measures of EF. Connectivity matrices generated based on 99 predefined cortical and subcortical regions of 127 children with and without ADHD (N=58 ADHD; 7-14y) were examined at each of two time-points, separated by at least one year. We used support vector regression based multivariate pattern analysis and leave-one-out cross validation to determine whether the strongest functional connections at each subject's first time-point could predict current, future, and/or change across several EF tasks that assess Working Memory (e.g., spatial span backward & forward), Response Inhibition (stop-signal reaction time, SSRT), Arousal/Activation (D-prime), and Temporal Information Processing (tapping task). We show functional connectivity measurements were capable of predicting current EF outcomes for spatial span forward (predicted EF values as a function of true EF values; $p<0.0001$; $r^2 = 0.14$), changes in spatial span backward ($p<0.0001$; $r^2 = 0.18$), and stop-signal reaction time ($p<0.0001$; $r^2 = 0.11$). Regions of the brain with connections most predictive of spatial span forward findings included the default, dorsal attention, and ventral attention networks, while those for spatial span backward additionally included frontoparietal and cinguloopercular networks. Regions of the brain with connections most predictive of SSRT included the default, frontoparietal, ventral attention, dorsal attention, and cinguloopercular networks. We show that rs-fcMRI can be used to predict performance on EF tasks, particularly for measures of response inhibition and working memory. This work holds great promise in identifying children at high-risk of deficits in EF and thus associated life outcomes such as academic achievement.

Disclosures: **M. Nik-Ahd:** None. **D.A. Fair:** None. **J.T. Nigg:** None.

Poster

837. Disorders of Executive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 837.13/RR5

Topic: F.01. Human Cognition and Behavior

Support: The City College of New York City Seeds Grant

Title: Racial/ethnic differences in attentional bias and cue reactivity among individuals with marijuana use disorders

Authors: *L. M. RUGLASS, A. SHEVORYKIN, R. MELARA;
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Abstract: Marijuana is the most widely used illicit substance in the United States, with 4.5 million individuals manifesting a marijuana use disorder (MUD) in 2012. Studies indicate racial/ethnic minorities (REM) exhibit higher rates of MUD and experience greater social and medical consequences due to their addictions compared to Whites. Understanding more about the triggers of use in REM marijuana users is essential for intervention efforts. Substance cue reactivity -- the physiological and psychological reactions to drug-related stimuli -- is considered an essential component of the addiction process associated with drug-seeking and drug-using behaviors. The goal of this ongoing pilot study is to examine racial/ethnic differences in attentional bias, marijuana-cue reactivity and related disparities. Fifteen individuals with MUD and 12 healthy controls completed a modified version of the Eriksen flanker task while psychophysiological indicators of cognitive processing and behavioral measures (reaction time and accuracy) were collected. Participants were shown a series of three lines and asked to respond to the orientation (vertical or horizontal) of the second (target) line. The flankers (first and last lines) were identical to each other and either matched (congruent trial) or did not match (incongruent trial) the target in orientation. The targets were superimposed on neutral, negative, positive, or marijuana-related images. Preliminary results revealed that, compared with healthy controls, marijuana users demonstrated reduced inhibitory control as evidenced by slower reaction times across all images ($p<.05$), suggesting a general breakdown in the ability to inhibit distraction. Results revealed no statistically significant racial/ethnic differences between Blacks ($n = 12$), Whites ($n = 6$), or Hispanics ($n = 9$) in accuracy rates or processing speed when responding to marijuana cues; however, there was a main effect of race/ethnicity on reaction time during a focused attention task, that approached significance, $F(2,24) = 2.681$, $p = .09$. Post-hoc mean comparisons revealed that Blacks responded slower during congruent trials compared to Whites ($M = 644.85$ ms versus $M = 492.47$ ms, respectively, $p<.05$), when distracted by a marijuana image. This finding suggests Blacks were not able to benefit from facilitation when a marijuana cue was present. The attentional deficits and reduced inhibitory control we see among marijuana users may be a consequence of their chronic marijuana use or a pre-existing condition that influences the development of addiction. Treatments that target these cognitive deficits may lead to better outcomes for marijuana users.

Disclosures: **L.M. Ruglass:** None. **A. Shevorykin:** None. **R. Melara:** None.

Poster

837. Disorders of Executive Function

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Program#/Poster#: 837.14/RR6

Topic: F.01. Human Cognition and Behavior

Title: Prefrontal cortical function during treatment for alcohol dependence

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Abstract: Recent studies suggest that advances in neuroimaging methodology may offer treatment programs evidence-based measures for assessment and prognosis for patients with alcohol dependence. Of the numerous brain regions implicated in addiction, the prefrontal cortex has received considerable attention, due to its involvement in response inhibition and salience attribution (Goldstein and Volkow 2011). The current study employs functional near-infrared spectroscopy (fNIRS), a low cost and clinically applicable brain imaging device (Bunce et al. 2006), to monitor prefrontal cortical function in alcohol-dependent patients. The purpose of this study is to use a clinically applicable neuroimaging technology to determine whether prefrontal cortical function subserving response inhibition (measured by a color-word Stroop task) and salience attribution (measured by an alcohol visual cue reactivity paradigm) predicts three-month posttreatment outcome in alcohol-dependent patients. Alcohol-dependent participants (n=12) are imaged 21-28 days after entry into supervised residential treatment and, after discharge, are followed weekly via internet reporting to determine sobriety for the subsequent 90 days. Normal controls (n=8) undergo identical tasks as patients except for weekly internet reporting. We hypothesize that, in contrast to controls, alcohol-dependent patients will exhibit greater activity in the dorsolateral prefrontal cortex (DLPFC) during both the interference condition of the Stroop task and alcohol cues in the visual cue reactivity paradigm. Preliminary findings show that alcohol-dependent patients exhibited increased response to alcohol stimuli in the left DLPFC ($p = 0.092$). Furthermore, response to alcohol stimuli correlated with Severity of Alcohol Dependence Questionnaire (SADQ) scores ($r = .44$, $p = 0.054$), supporting the relationship between PFC response to alcohol cues and severity of dependence. Additional analysis will examine the relationship between PFC response to alcohol cues and natural reward cues, as well as PFC activity during the Stroop word-color interference task, with three-month posttreatment

outcome. This investigation may demonstrate the feasibility of implementing cost-effective brain imaging technology in the alcoholism treatment environment. References Bunce, et al. (2006). Functional near-infrared spectroscopy. Engineering in Medicine and Biology Magazine, IEEE, 25, 54-62. Goldstein and Volkow (2011). Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. Nature Reviews Neuroscience, 12, 652-669.

Disclosures: J.D. Harris: None. **R.E. Meyer:** None. **S.C. Bunce:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); fNIR Devices LLC.

Poster

837. Disorders of Executive Function

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Program#/Poster#: 837.15/RR7

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust grant (089589/Z/09/Z)

Cambridge Behavioural and Clinical Neuroscience Institute (BCNI) funded by a joint award from the Medical Research Council and the Wellcome Trust (G0001354)

Title: A neurocognitive and white matter profile of behavioral flexibility in obsessive-compulsive disorder

Authors: *A. M. APERGIS-SCHOUTE[1,3], F. E. VAN DER FLIER[3,4], K. J. WHITAKER[1,3], M. M. VAGHI[3,2], M. KASER[1], A. SULE[1,3,5], N. A. FINEBERG[3,6,7], T. W. ROBBINS[3,2], B. J. SAHAKIAN[1,3];
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Abstract: Obsessive-compulsive disorder (OCD) affects approximately 50 million people worldwide and is characterized by intrusive thoughts and repetitive behaviors (Franklin and Foa, 2011). Impaired cognitive flexibility is central to the neuropsychopathology of the disorder (Chamberlain et al., 2005). In line with this, aberrant orbitofrontal activation has been found

during reversal learning, a process dependent on flexible executive functioning (Chamberlain, et al., 2008). However no studies to date have found a behavioral deficit in reversal learning, possibly due to the low cognitive and emotional load of the tasks used, as everyday flexible responding is often required during stressful and demanding situations. In order to address this issue, we developed a novel reversal learning task which combined a high cognitive load with time pressure, reward and punishment. In this task participants had to respond quickly with the correct finger to a corresponding target on the screen. A colored border around the screen signaled which was the correct hand to use and this color association was occasionally reversed. In order to gain insight into hypothesized differences in the flexibility of cognitive control between OCD patients and controls, we employed diffusion tensor imaging (DTI) to investigate correlates with white matter integrity. Our novel reversal learning task showed a significant interaction between feedback condition and accuracy where controls performed better under reward and punishment compared to neutral trials, while OCD patients' performance was negatively affected by punishment compared to neutral and reward trials. Previous research from our group showed a similar selective impairment in OCD patients under punishment conditions in a go/no-go task (Morein-Zamir, et al., 2013), which emphasizes a failure of cognitive control in OCD, particularly under negative motivational contingencies. Our overall aim is to systematically address the issue of behavioral flexibility with the use of several well-established cognitive tests measuring inhibition, set-shifting, planning and memory. The addition of our novel reversal learning task could elucidate the crucial role of punishment in the inflexible behavior typically seen in OCD. By combining an extensive battery of cognitive tests with DTI we aim to further our understanding of the role of white matter tract alterations, specifically within the cortico-striato-thalamo-cortical circuitry, and its behavioral significance in OCD.

Disclosures: **A.M. Apergis-Schoute:** None. **F.E. van der Flier:** None. **K.J. Whitaker:** None. **M.M. Vaghi:** None. **M. Kaser:** None. **A. Sule:** None. **N.A. Fineberg:** None. **T.W. Robbins:** None. **B.J. Sahakian:** None.

Poster

837. Disorders of Executive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 837.16/RR8

Topic: F.01. Human Cognition and Behavior

Title: Interference between working and semantic memory cognitive domains on voluntary balance control : Effect of age and stroke

Authors: *S. SUBRAMANIAM[1], T. BHATT[2];
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Abstract: ***Purpose***: The purpose of this study was to examine the extent of cognitive motor interference (CMI) of working and semantic memory cognitive domains on voluntary balance control across stroke, old and young adults. ***Hypothesis***: We postulated that young adults would not have any CMI under both the cognitive domain based tasks. Working memory task would negatively impact balance in old, while both cognitive tasks would affect stroke, along with a significantly higher impact on cognition with working memory task in chronic stroke adults. ***Methods:*** Individuals with hemiparetic stroke (N=10), age matched healthy older (N=10) and young adults (N=10), performed, the LOS balance test in the backward (BWD) direction, under single (ST) and under two DT conditions which involved two different cognitive tasks, word list generation (WLG) and counting backwards (CB). The WLG and CB tasks were also performed in sitting (ST) for the same duration as the balance tests. Cognitive ability was recorded for all the conditions by the number of words (WLG) and digits counted correctly (CB). For the LOS, the self-initiated center of pressure (COP) response time (RT) was obtained on the ST and both DT conditions. The "balance cost" ([ST-DT]/ST)*100 was computed and compared for reaction time (RT), movement velocity (MV) and maximum excursion (MXE) for both the cognitive tasks. The "cognitive cost" was also similarly computed and compared using the number of responses counted. ***Results*:** Across groups the balance cost was significantly higher for stroke in the CB condition than older adults ($p < 0.05$) but was similar between these two groups for the WLG task. Across groups the cognitive cost was significantly higher in stroke in comparison with old adults for both the CB and WLG task ($p < 0.05$). Stroke survivors had significant increase in balance and cognitive cost under both the CB and WLG cognitive task in comparison to young adults ($p < 0.01$). While old adults had significant increase in MXE and MV cost for the balance cost comprised to young adults for only the CB tasks ($p < 0.05$), with no significant difference in cognitive cost for both tasks. ***Conclusion***: The results confirm the hypothesis and suggest that under healthy conditions people can allocate the desired resources for each task allowing optimal dual-task performance in young adults. Greater interference under working memory cognitive task in old adults, suggest limited availability of resources with aging. Furthermore, the CMI seen across both the cognitive domains after stroke and more so in the working memory task indicates possible decreased capacity of processing resources, due to the stroke-induced cortical lesion.

Disclosures: S. Subramaniam: None. **T. Bhatt:** None.

Poster

837. Disorders of Executive Function

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Program#/Poster#: 837.17/RR9

Topic: F.01. Human Cognition and Behavior

Support: European Research Council

Délégation Générale de l'Armement

Title: Impulsivity as a marker of cognitive fatigue: An fMRI study

Authors: ***B. BLAIN**[1,2], S. BOURET[1], G. HOLLARD[2], M. PESSIGLIONE[1];
[1]Brain & Spine Inst. (ICM), Paris, France; [2]Ctr. of Econ. of Sorbonne, Panthéon-Sorbonne university, France

Abstract: Previous studies in experimental psychology have suggested that exerting self-control enhances impulsivity in subsequent choices. Here, we compare the effects of cognitive and motor efforts on inter-temporal choices, at both the behavioral and neural levels. In total, 81 healthy adults participated in the study, divided into four groups. FMRI data were acquired in a subset of 59 subjects, while they were performing cognitive control tasks, intermingled with inter-temporal choices (between a small immediate payoff and a larger delayed payoff). There were two fMRI sessions, at the beginning and at the end of the experiment. In between, group 1 (n=29) performed a series of hard cognitive tasks (3-back and contextual control tasks), group 2 (n=24) exerted intense physical effort (on a home bike), group 3 (n=12) performed easy cognitive tasks (1-back and sensorimotor control), and group 4 (n=16) read newspapers or played video games. Behavioral data showed an increase in impulsive choices (preference for immediate rewards), after hard cognitive work (in group 1), specifically. At the neural level, only group 1 exhibited a decrease in the activity of the prefronto-parietal network involved in both cognitive control and inter-temporal choices. These findings are consistent with the theory that cognitive control resources might be depleted after intensive use, releasing impulsive tendency to go for immediate gratification.

Disclosures: **B. Blain:** None. **S. Bouret:** None. **G. Hollard:** None. **M. Pessiglione:** None.

Poster

837. Disorders of Executive Function

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Program#/Poster#: 837.18/RR10

Topic: F.01. Human Cognition and Behavior

Support: This work was funded by the Department of Defense (Center for Neuroscience and Regenerative Medicine) and the NIH Intramural Research Program.

Title: The relationship between neuropsychological functioning, white matter integrity, and brain volume in traumatic brain injury

Authors: S. MCNALLY[1], K. C. LOPEZ[2], S. LEVY[2], D. PHAM[2], Y.-Y. CHOU[2], J. MCENTEE[2], J. BUTMAN[1], *J. DSURNEY[2], L. CHAN[1];
[1]NIH, Bethesda, MD; [2]Rehabil. Med., Ctr. For Neurosci. and Regenerative Medicine-, Bethesda, MD

Abstract: Introduction: Advanced Magnetic Resonance Imaging (MRI) techniques, such as Diffusion Tensor Imaging (DTI) and automated analysis of brain volumes, are new tools that may have some benefit in the assessment and treatment of traumatic brain injury (TBI). Some investigators have established correlations between various brain structures and specific cognitive tasks (Kantarci et. al., 2011), but data in this area is very limited. In the present study, we sought to examine the relationship between DTI and brain volume data with performance on standardized neuropsychological tests. Methods: T1-weighted images were collected on a Siemens 3T MRI scanner. Volumes were analyzed using FreeSurfer's automated segmentation, and mean fractional anisotropy (FA) was calculated for several regions found to be implicated in executive function. FA values were computed from diffusion-weighted images using the SINAPS software package that includes motion and distortion correction, as well as white matter tract segmentation (Bennett et. al., 2013). Neuropsychological assessments used to assess executive function were the Booklet Category Test, Trail Making Test B, Wisconsin Card Sorting Test, Controlled Word Association Test, and the Wechsler Adult Intelligence Scale Similarities subtest. Results: A total of 37 patients with TBI (mean age: 42 ± 16) categorized as mild (n=11), moderate (n=20), and severe (n=6) were evaluated between six months and one year following injury. There were a number of correlations found between tests of executive function and imaging measures. Notably, volumetric analysis showed significant positive correlations between several measures of executive function and volume of the right caudate nucleus, right thalamus, and left insula. Analysis of DTI measures showed significant positive correlations between mean FA and tests of executive function in the anterior thalamic radiation, and the body and tapetum of the corpus callosum. Limitations: Focal parenchymal lesions were evident in two patients. Conclusion: The present study suggests that findings from more advanced MRI techniques may be associated with clinical deficits in patients with TBI.

Disclosures: S. McNally: None. **K.C. Lopez:** None. **S. Levy:** None. **D. Pham:** None. **Y. Chou:** None. **J. McEntee:** None. **J. Butman:** None. **J. Dsurney:** None. **L. Chan:** None.

Poster

837. Disorders of Executive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 837.19/RR11

Topic: G.06. Computation, Modeling, and Simulation

Title: A population-based feature extraction technique to identify functional biomarkers in the brain of alcoholic subjects

Authors: N. KARAMZADEH[1], M. KELLMAN[1], Y. ARDESHIRPOUR[1], F. CHOWDHRY[1], A. ANDERSON[2], E. WEGMAN[3], *A. GANDJBAKHCHE[4];
[1]Natl. Inst. of Child Hlth. and Human Develop., [2]Natl. Inst. of Hlth., BETHESDA, MD; [3]George Mason Univ., Fairfax, VA; [4]Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD

Abstract: We present a novel feature extraction technique, Relative Brain Signature (RBS) that enables signifying differences between a finite numbers of populations. To evaluate our technique, we have used a set the EEG dataset of the "UCI Machine Learning Repository" of 77 alcoholics and 43 control subjects. For every subject, one RBS vector with respect to alcoholic and control populations were computed. An RBS vector denotes the relationship of a subject to one of the control or alcoholic populations (Figure 1). We employ the extracted RBS vectors to identify functional biomarkers over the cortical area of the alcoholics that had manifested distinct functional behavior in comparison to the control subjects (Figure 2). Moreover, we have evaluated the efficacy of the RBS vectors in correctly categorizing the subjects with respect to their original populations. To achieve this goal, a machine-learning algorithm to classify the subjects was employed. Subjects were correctly classified into alcoholic and nonalcoholic populations with accuracy up to 85%.

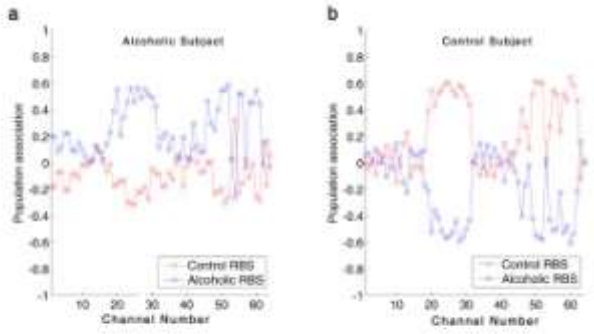

Figure 1 | RBS vectors for an alcoholic and a control subject are illustrated. Two RBS vectors for an alcoholic subject (a) and a control subject (b) are shown. The component values of the RBS vectors, quantifies the strength of the relation between an ERP waveform and the alcoholic population (shown in blue) or to the control population (shown in red). A positive value closer to 1, for a component of the RBS vectors indicates a stronger association to a certain population whereas smaller positive values and the negative values suggest the corresponding ERP data belongs less to a population.

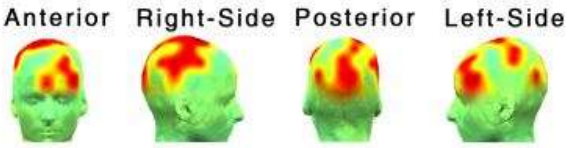

Figure 2 | The top 25 functionally distinct cortical areas between alcoholic and control subjects, which are obtained and depicted using the RBS vector.

Disclosures: N. Karamzadeh: None. **M. Kellman:** None. **Y. Ardeshirpour:** None. **F. Chowdhry:** None. **A. Anderson:** None. **E. Wegman:** None. **A. Gandjbakhche:** None.

Poster

837. Disorders of Executive Function

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 837.20/RR12

Topic: G.06. Computation, Modeling, and Simulation

Title: The pursuit of DLPFC: Comparison of non-neuronavigated methods to target the left dorsolateral prefrontal cortex with transcranial Direct Current Stimulation (tDCS)

Authors: *O. SEIBT;
Biomed. Engin., The City Col. of The City Univ. New York, New York, NY

Abstract: Background: The dose of transcranial direct current stimulation (tDCS) is defined by electrode montage and current, while the resulting brain current flow is more complex and varies across individuals. The left dorsolateral prefrontal cortex (lDLPFC) is a common target in

neuropsychology and neuropsychiatry applications, with varied approaches used to experimentally position electrodes on subjects. Objective: To predict brain current flow intensity and distribution using four conventional bicephalic frontal 1 x 1 electrode montages that nominally targeting lDLPFC. Localization for montage was based on individual anatomical landmarks. Methods: MRI-scans were segmented in seven tissue compartments each in order to assign isotropic material properties. The EEG10-20, the Beam F3-System, the 5cm-Rule and the novel OLE-System were scrutinized as electrode positioning methods for 5 x 5 cm2 rectangular sponge-pad electrodes over F3-F4 with high-resolution computation models. Results: The induced peak electric field in lDLPFC, for the same positioning method, decreases with an increase in overall head volume. Enlarging the electrode distance on the skin reduces scalp shunting and, additionally, an electrode displacement towards thinner skull structures leads to the augmentation of cortically injected current. For the different montages, results from the OLE-system and the EEG10-20 were qualitatively similar, and superior to other montages. Conclusion: Cortical electric field distribution fluctuations, for a given dose, are a function of inter-individual differences and pose the need for subject specific tDCS therapy. We present a novel, reproducible, ease-to-use electrode positioning method that uses anatomical landmarks in combination with standardized placement to optimize lDLPFC targeting

Disclosures: O. Seibt: None.

Poster

838. Executive Function II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 838.01/RR13

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1106400

Title: There is no “best” brain parcellation for complex brain networks--one parcellation is not sufficient

Authors: *M. BERTOLERO, M. D'ESPOSITO;
Univ. of California, Berkeley, Berkeley, CA

Abstract: To analyze the brain as a complex network using graph theory, we must first choose how to parcel the brain into distinct regions--the graph’s nodes. The method for deriving the nodes is called a parcellation method. Choosing between different parcellation methods is a

challenging problem, as different ways in which the brain is parceled into nodes drastically impacts the conclusions drawn from graph theoretic analyses. We used resting-state fMRI to define hubs based on the nodes' within-module degrees and participation coefficients, and the BrainMap database to define functionally flexible regions. We also examined the impact of damage to these regions. We found that the results from these analyses change when the parcellation method is changed, even when each parcellation method generates a similar numbers of nodes, and only differ concerning the boundaries (e.g., anatomical boundaries versus clustering voxels into a node based on functional homogeneity). Moreover, we found that, even if one uses the same boundary definition with different resolutions (i.e., number of nodes), both the rank order of subjects' global metrics and the location of the hubs vary across the resolutions. Thus, both the resolution and boundaries of nodes impact graph theoretic analyses. We propose two solutions to these problems that are not parcellation-dependent. First, we propose a new parcellation method, which we call the Random Average Parcellation Method. This method avoids making assumptions about the resolution and the boundaries. This method samples across a large set of resolutions or random boundaries (or both), computing node-to-node correlations, but retaining the voxel-to-voxel correlation matrix and averaging across the matrices to produce a voxel-level graph that reflects the strongest voxel-to-voxel connections. Thus, this method does not depend on any parcellation method assumptions about where the boundaries should be or the resolution, as it averages across these parameters. We present three graphs--one from sampling different resolutions, one from sampling different boundaries, and one from sampling both. Second, if one only wants to observe the anatomical location of nodal properties (for example, where the high and low degree nodes are), one can average the results from the most frequently published parcellations or random parcellations to produce a voxel-level map of where, for example, the high and low degree voxels are. We present these maps for degree, participation coefficients, within-module degree, and current-flow betweenness centrality. We also present how these results differ from standard parcellation techniques.

Disclosures: **M. Bertolero:** None. **M. D'Esposito:** None.

Poster

838. Executive Function II

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Program#/Poster#: 838.02/RR14

Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP to Riddle, J

Title: Investigation of network connectivity with simultaneous TMS-fMRI

Authors: *J. RIDDLE[1], I. CAMERON[2], D. RAHNEV[1], M. D'ESPOSITO[1];
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Abstract: Single pulse transcranial magnetic stimulation (sTMS) can be used to activate or perturb a focal region of the neocortex. While sTMS effects are observed directly under the stimulated site, there are also perturbations found in regions distant from the stimulated site. Concurrent TMS-fMRI can quantify TMS induced changes in the network organization of the brain. The current study applied sTMS during resting state fMRI to explore connectivity changes in the well-known oculomotor brain network, which consists of the frontal eye fields (FEF), the parietal eye fields (PEF), supplementary eye fields (SEF), V5/MT, and the dorsolateral prefrontal cortex. We targeted right FEF with TMS, which was anatomically localized as the junction of the superior frontal sulcus and the precentral sulcus. For a control TMS site, a second region just lateral to rFEF along the precentral sulcus was targeted. Each subject participated in two counterbalanced sessions on separate days, TMS to rFEF or control. On each day, 30 minutes of resting state fMRI was collected with sTMS every 4-8 seconds. The oculomotor network was functionally localized using a separate, no TMS, resting state session. We used beta series correlational analyses to quantify changes in connectivity within the oculomotor network during rFEF TMS versus control site TMS. Each TMS pulse was modeled as a separate event and the responses to these events across the network were treated as a time series for the correlational analysis. A decreased correlation between the site of stimulation, rFEF, and the rest of the network was observed, which was still present with exclusion of rFEF activity from the analyses. These changes in the oculomotor network were not observed following TMS to the control site. These results suggest that TMS effects extend beyond the site of stimulation propagating along established brain networks.

Disclosures: J. Riddle: None. **D. Rahnev:** None. **I. Cameron:** None. **M. D'Esposito:** None.

Poster

838. Executive Function II

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Program#/Poster#: 838.03/RR15

Topic: F.01. Human Cognition and Behavior

Support: NDSEG to CLG

NIH Grant AG034642

Title: Task-based reorganization of brain networks in healthy aging

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Abstract: Healthy aging is accompanied by decline in executive functions and alterations in underlying brain structure and function. As executive processes are thought to rely on integration between frontal cortex and distributed brain regions, their integrity may be reflected in the brain's network architecture. While recent evidence suggests that more globally integrated networks support effortful cognition, it is not yet clear how network properties are modulated by task performance in older adults. Here, we investigated the relationship between executive functioning and the flexibility of network organization during task performance in aging. We created networks from fMRI data collected during a resting-state scan and performance of a working memory task with varying levels of difficulty for 38 older (ages 60-80) and 18 young (ages 18-26) adults. Metrics derived from these graphs allowed us to assess intrinsic brain network properties and their modulation during task, respectively. Specifically, graphs were generated from functional connectivity matrices between AAL atlas nodes and were then partitioned into communities. Mutual information was used to compare the nodal composition of communities during rest and task. Further, the participation coefficient (PC) of lateral frontal regions was used to quantify the distribution of connections across communities. Finally, we examined the relationship between these functional metrics and the fractional anisotropy of the superior longitudinal fasciculus (SLF), a core tract connecting frontal and posterior brain regions. Older adults performed worse than young adults on the task (i.e., reaction time and accuracy), particularly at higher levels of difficulty. Older adults had lower mutual information between rest and task, suggesting that they exhibit greater task-based reorganization of community structure than young adults. Older adults showed increased PC of lateral frontal regions at all levels of task difficulty, while young adults did not show this pattern until the hardest task condition. The increase in lateral frontal PC from rest to task in older adults was related to faster task performance and greater SLF integrity. These results suggest that older adults adopt a more integrated brain network organization to support task performance, even during less demanding conditions. In particular, frontal regions showed an increased distribution of connections during task by participating in many communities. This flexibility of network architecture in older adults appears to be beneficial, in that is supported by greater white matter greater integrity and allows for increased task performance.

Disclosures: C.L. Gallen: None. **G.R. Turner:** None. **A. Adnan:** None. **M. D'Esposito:** None.

Poster

838. Executive Function II

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Title: The role of parietal cortex in the representation of task-reward-associations

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Abstract: Behaviors that are rewarding today may not be so in the future. So our cognitive system needs to be able to adapt to changes in the association between behaviors (e.g. performing your job) and rewards (e.g. your salary). Most previous work has focused on the association of simple actions with rewards, not more of abstract task-sets with rewards. This difference is important because humans also attach value to complex tasks (e.g. jobs), not only to simple actions (e.g. hand movements). Tasks are also more abstract and their implementation therefore does not depend on the current environmental conditions as much as simple actions do. This makes tasks more robust against changes in the environment. Previous work on the component parts of task-reward-associations, i.e. task and rewards, highlighted the role of parietal and prefrontal regions in task representation and striatal and orbitofrontal regions in reward representation. Those regions might therefore also play a role in representing task-

reward-associations directly. Here, we used a simple cued intention task to investigate task-reward mappings. Subjects underwent fMRI while performing the task. We used time-resolved multivariate pattern recognition analysis (MVPA) in order to determine which brain areas encode information about task reward mappings, and to assess the evolution of information across time in these brain areas. Importantly, we were able to distinguish the neural signals of task-reward associations from task preparation and reward expectation processes. We found that the inferior parietal cortex plays a key role in representing task-reward associations, while also representing their component parts, task-sets and rewards. Using time-resolved MVPA we were able to show that the parietal cortex flexibly switches between representing task-reward associations and their component parts within trials, always representing the information that is currently relevant to the subject. This finding extends our knowledge of the function of the parietal cortex in reward-guided behavior and further highlights its flexibility in rapidly changing its content of representation.

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IARPA D10PC20023

Indiana METACyt Initiative

Title: Medial prefrontal cortex signals prediction errors across multiple domains of pain and cognitive control

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Abstract: A recent account of medial prefrontal cortex (mPFC) function, the PRO model, hypothesizes that activity within the mPFC is driven primarily by prediction errors, or the

discrepancy between what was predicted and the outcome actually received, regardless of valence (Alexander & Brown, 2011). In addition, recent research has suggested that the dorsal Anterior Cingulate Cortex (dACC) is responsive to a wide variety of modalities, including negative affect, pain, and cognitive control (Shackman et al, 2011; Etkin et al, 2011). Based on these empirical findings and the predictions of the PRO model, here we use FMRI to test whether prediction effects and prediction error effects depend on the modality eliciting the prediction error and the valence of the outcome. To test this, we used a factorial design consisting of cues signaling the probability of receiving either aversive or non-aversive levels of electrical stimulation, and cues signaling the probability of receiving a congruent or incongruent spatial Stroop. Our results show overlapping activations in the dACC for prediction errors regarding upcoming levels of pain and upcoming levels of required cognitive control. Furthermore, post-hoc analyses revealed that the observed effects for both surprising pain and surprising spatial Stroop stimuli were driven by outcomes that were worse than expected as well as by outcomes that were better than expected, in contrast to predictions from Reinforcement Learning theory (Holroyd & Coles, 2002) and conflict monitoring theory, but instead consistent with predictions of the PRO model (Alexander & Brown, 2011).

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Poster

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Program#/Poster#: 838.06/RR18

Topic: D.04. Vision

Title: Electrophysiological evidence for modulation of emotional appearance by spatial attention

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Abstract: Past decade has seen an increased number of studies on investigating the effect of attention on perceptual awareness. Carrasco and colleagues have shown that spatial attention influences phenomenal appearance based on simple perceptual attributes like color, contrast, and spatial frequency as well as second order perceptual features. However, it is not clear whether and how spatial attention influences higher order perceptual information like emotions. We investigated whether spatial attention alters emotion appearance and if it does then where and when such an effect takes place. We manipulated exogenous attention in a comparative judgment

task using non-informative peripheral cues preceding the emotional faces differing in emotional intensity. A set of emotional faces (happy and sad) that differed in equal amounts of emotional intensity were obtained based on psychophysical ratings and scaling. Twelve volunteers participated in the psychophysical study. They reported position of the face that appeared to be happier or sadder. The result showed that spatial exogenous attention does influence emotional appearance with happy attended faces being perceived as happier than unattended happy faces. Similar change in appearance was also present for sad faces. We followed the psychophysical study with an ERP study to investigate the neural mechanisms underlying this effect. Nine participants performed the same task under similar conditions for happy faces. As expected, the behavioral data showed that attention enhances the appearance of happy faces (makes it happier). The ERP data revealed that attention influences early components (P1 and N1) and this was evident mainly in electrodes in the inferior temporal cortex. Given the evidence for specialized face processing in the temporal areas, the results indicate that spatial attention influences emotion appearance by modifying processes fairly early in the inferior temporal cortex. The study for the first time provides behavioral and electrophysiological evidence for the effect of spatial attention on the phenomenology of emotion perception. Further studies will be directed towards understanding the phenomena with sad faces to see whether the neural changes underlying the attentional effects on conscious emotional appearance for sad faces are similar or different with those we have found with happy faces, given possible differences in processing of negative and positive emotional information found in earlier studies. Future studies would explore the effect not only with respect to other emotions but also with differences in the way attention is manipulated.

Disclosures: **M.V. Mishra:** None. **N. Srinivasan:** A. Employment/Salary (full or part-time):; University of Allahabad.

Poster

838. Executive Function II

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Research Activity start-up from MEXT (24800023)

Grant from the Uehara Memorial Foundation

Title: Temporal changes in brain-behavior correlation during response inhibition

Authors: *T. OSADA[1], K. JIMURA[2], S. HIROSE[1], A. KUNIMATSU[3], K. OHTOMO[3], Y. KOIKE[2], S. KONISHI[1];
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Abstract: Previous neuroimaging studies of response inhibition have examined correlations between brain activity and behavioral efficiency to explore brain regions associated with efficient response inhibition. The present study investigated the temporal stability of the brain-behavior correlations. Healthy human participants performed a standard stop-signal task that required inhibition of manual responses in trials where a signal indicating to make a manual response was changed, after a short delay, to a signal to stop the response. The efficiency of response inhibition can be quantified as stop-signal reaction time (SSRT), with more efficient inhibition reflected in a shorter SSRT. An across-subject correlation analysis was first performed based on the experimental session where the SSRT was stable. Negative correlations were observed between SSRT and brain activity during response inhibition in multiple brain regions, reflecting greater activity in more efficient inhibition, consistent with prior studies. Then we temporally divided the stable experimental session into two phases, the first and second halves of the session. Although SSRT did not change between the first and halves, the negative correlation between SSRT and brain activity was observed dominantly in the second half. In a cerebellar region showing the strongest negative correlation in the second half, the brain activity increased relative to the first half in efficient performers, whereas the brain activity decreased in poor performers. These results indicate that, even though SSRT is stable, robust brain-behavior correlations can more effectively be detected in a later part of the experimental session.

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Poster

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Support: Grant-in-Aid for Scientific Research B (22300134) to S.K.

Title: Cerebro-cerebellar network associated with learning of response inhibition as revealed by functional connectivity analysis

Authors: *S. HIROSE[1,2], K. JIMURA[1,2,4], A. KUNIMATSU[3], O. ABE[3], K. OHTOMO[3], S. KONISHI[1,2];
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Abstract: Brain regions in the cerebral cortex involved in response inhibition have been well studied, but the cerebellar involvement in response inhibition is less understood. In this functional MRI study, we conducted a psycho-physiological interaction (PPI) analysis to investigate the cerebro-cerebellar interaction during response inhibition in two separate days of go/no-go task performance. The efficiency index, which we previously devised to evaluate efficiency of performance of the go/no-go task, was calculated separately for each of the two days. The subjects performed the task more efficiently on the second day than on the first day. The PPI analysis revealed across-day decrease in the functional interaction from the right inferior frontal cortex to the cerebellum (lobule VII or VI). It was also revealed that the functional interaction increased across days from the same cerebellar region to the primary motor area. These results suggest that the detected cerebellar region is involved in response inhibition, and raise the possibility that the changes in the cerebro-cerebellar interaction support performance improvement in response inhibition.

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Poster

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Title: Meta-analytic parcellation of the lateral prefrontal cortex delineates the inferior frontal junction area

Authors: *P. S. MUHLE-KARBE[1], J. DERRFUSS[2], M. BRASS[1], P. T. FOX[3], S. B. EICKHOFF[4,5];
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Abstract: The inferior frontal junction area (IFJ) is located in the posterior lateral prefrontal cortex (LPFC), at the intersection of the inferior precentral and the inferior frontal sulci. It has been suggested that this area plays a prominent role in cognitive control processes (e.g., Brass et al., 2005; Asplund et al., 2010). Recent studies have aimed at distinguishing the IFJ anatomically and functionally from adjacent areas in the LPFC (e.g., Derrfuss et al., 2011), revealing relatively clear borders between the IFJ and more caudal and ventral areas (e.g., Broca's region, or the inferior frontal eye-field). However, little is known about the transition from the IFJ to more rostral and dorsal regions in the LPFC (often referred to as "mid-DLPFC") that are also implicated in cognitive control. In the present study, we employed a meta-analytic parcellation of the left LPFC to examine whether the IFJ can be identified and dissociated from neighboring areas on the basis of whole-brain co-activation patterns (see Eickhoff et al., 2011). We defined an anatomical seed region that was oriented along the left inferior frontal sulcus and encompassed the adjacent parts of the inferior frontal, middle frontal, and precentral gyri (MNI y-coordinates between -6 and +36). Accessing activation foci documented in the BrainMap database (Fox & Lancaster, 2002), we computed connectivity profiles for each seed voxel reflecting the co-activation probability with every other voxel in the brain-wide grey matter. Based on the similarity of connectivity profiles, the seed region was divided into clusters using the K-means cluster method. Topological and information-theoretic criteria consistently identified a six-cluster solution as the optimal parcellation of the seed region. One of the ensuing clusters closely corresponded to previous definitions of the IFJ (MNI center of gravity at -37, 5, 31), and could be dissociated from a more caudal and lateral cluster on the precentral gyrus (-52, 2, 39), a more dorsal cluster on the posterior middle frontal gyrus (-43, 14, 39), a more ventral cluster that extended on the inferior frontal gyrus (-51, 25, 21), and two rostral clusters in the "mid-DLPFC" (-36, 25, 19; and -40, 31, 28). Each cluster was moreover characterized by specific patterns of whole-brain co-activation, and by the functional profiles of the contributing experiments. Together, our results highlight that the IFJ can be distinguished from neighboring areas on the basis of whole-brain co-activation patterns. These findings support the idea of functional specialization in the frontal lobe and illustrate the usefulness of meta-analytic techniques in the delineation of cortical modules.

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Poster

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Support: Wellcome Trust grant to MH

Title: Role of orbitofrontal cortex in reward sensitivity: Evidence from human lesions

Authors: *S. G. MANOHAR[1,2], M. HUSAIN[1,2];
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Abstract: Medial prefrontal cortex has been implicated in representation of reward, both in functional imaging and single-cell recording studies. Lesions to orbitofrontal cortex in animals lead to perseveration, disinhibition, emotional disturbances and altered reward preferences. In contrast, lesions to dorsomedial prefrontal cortex lead to reduced post-error performance monitoring and deficits in pairing actions with reward values. In *humans*, orbitofrontal cortex damage produces disorders of reward-related learning and risk-taking, but this constitutes only circumstantial evidence that reward representations are damaged in these individuals. Here we directly test for sensitivity to incentives using a saccadic paradigm, in a group of 20 patients with medial frontal lesions. Participants were tested 1 to 4 years after rupture of an anterior communicating artery aneurysm. Their lesions were distributed over the medial frontal wall and included supplementary motor cortex, anterior cingulate, ventromedial prefrontal cortex and medial orbitofrontal cortex. Our task involved a saccade to a target while avoiding an early onset distractor. Saccades were rewarded based on speed, but critically, prior to each trial participants heard a spoken reward cue that informed them the maximum reward available on the subsequent saccade_thus we were able to manipulate the incentive. In healthy people, peak saccadic velocity was modulated by the incentive, allowing a parametric measure of reward sensitivity. Lesions were mapped and then coregistered to a template. We used voxel-based regression to find areas that significantly correlated with reward sensitivity. We constructed a t-statistical map thresholded at $p<0.05$. Two regions were found in which lesions correlated negatively with reward sensitivity, bilaterally in posterior medial orbitofrontal cortex, and a small region of right pre-SMA. We conclude that damage to these areas attenuates patients' ability to dynamically modulate their movement speed according to current incentives. Our results may provide a central link between various facets of the deficits previously described in medial frontal patients, several of which might be manifestations of an attenuated representation of reward value.

Disclosures: **S.G. Manohar:** None. **M. Husain:** None.

Poster

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Support: DFG Grant BE4045/10-1

Title: Investigating of the roles of dopamine and GABA for human action control by means of genetics and MR spectroscopy

Authors: ***A.-K. STOCK**[1], C. QUETSCHER[2], J. T. EPPLEN[3], U. DYDAK[4,5], C. BESTE[1]; [1]Dept. of Child and Adolescent Psychiatry, TU Dresden, Dresden, Germany; [2]Biopsychology, [3]Dept. of Human Genetics, Med. Fac., Ruhr-University Bochum, Bochum, Germany; [4]Sch. of Hlth. Sci., Purdue Univ., West Lafayette, IN; [5]Sch. of Med., Indiana Univ., Indianapolis, IN

Abstract: Action control and action cascading are complex cognitive endeavors which can be accomplished using different processing strategies. From studies of action control deficits, we know that dopamine and its modulation of striatal GABAergic signalling play a key role in action control. Yet, very little is known about how variations in these two transmitter systems influence cognitive and behavioral performance in healthy humans. In order to close this gap, we investigated the effectiveness of serial vs. parallel action cascading strategies by means of a stop-change paradigm. To investigate whether action control is differentially modulated by dopamine D1 and D2 receptors, we assessed DRD1 (rs4531) and DRD2 (rs6277) receptor polymorphisms in n=162 healthy individuals. In order to assess the neurophysiological characteristics of serial vs. parallel processing strategies, we recorded an EEG while the participants performed the task. We found that signaling via D1 and D2 receptors seems to shift processing strategies into opposing directions on a serial-to-parallel continuum. Homozygous DRD1 G allele carriers, who are assumed to have a higher D1 receptor efficiency than carriers of the A allele, showed a more serial and more effective action cascading strategy (reflected by behavioral results and a smaller frontocentral P3 component). By contrast, homozygous DRD2 T allele carriers, who have a higher striatal density of D2 receptors than C allele carriers, seem to apply a less effective and more parallel action cascading strategy (reflected by behavioral results and a larger frontocentral P3 component). For the investigation of the role of GABA, we used a different approach since signaling within the striatum, which mainly depends on GABA, cannot be properly mapped

using EEG methods. N=20 healthy participants underwent Magnetic Resonance Spectroscopy (MRS) of the striatal GABAergic system. Our results indicate that striatal GABA and glutamate + glutamine concentrations drive high performance in more serial action cascading strategies, probably via stronger attentional gating. Altogether, our findings provide insight into the behavioral, neurophysiological and neurobiochemical mechanisms that drive cognitive processes *in situ*ations requiring a cascading of actions.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: DFG Grant BE4045/10-1

Title: Striatal GABA levels predict response inhibition performance and its cortical electrophysiological correlates

Authors: ***C. QUETSCHER**[1,2], C. BESTE[2,1], A. YILDIZ[1], S. DHARMADHIKARI[3,4], B. GLAUBITZ[5], T. SCHMIDT-WILCKE5[5], U. DYDAK[3,4];
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Abstract: Response inhibition processes play an important role in cognitive control and are mediated via a network that consists of different cortical areas and basal ganglia nuclei. At the basal ganglia level, striatal GABAergic medium spiny neurons are known to be important for response selection. While there is extensive evidence for the role of the nigrostriatal dopamine pathway in response inhibition, quite little of known about the relevance of the striatal GABAergic system it innervates. We therefore examined the relevance of the striatal GABAergic system for response inhibition processes using a novel combination of magnetic

resonance spectroscopy (MRS), EEG, and behavioral data of n=40 participants performing a GoNogo task. Our results show that striatal GABA levels predict the efficacy of response inhibition processes with higher striatal GABA levels being related to better response inhibition performance. Moreover, striatal GABA levels show a strong positive correlation with the degree of phase-locking in the Nogo-N2 time range, a frontal midline component that reflects pre-motor processes like conflict monitoring or updating of the response program. This shows that striatal GABA affects specific sub-processes of response inhibition through the modulation of the reliability of neuronal synchronization processes. Specifically, pre-motor inhibitory processes are affected while mechanisms reflecting the evaluative processing of the successful outcome of inhibition remain rather unchanged. To the best of our knowledge, this is the first study providing direct evidence for the relevance of the striatal GABAergic system for response inhibition functions and their cortical electrophysiological correlates in humans.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Spaced cognitive training promotes transfer

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Abstract: Cognitive training studies yield wildly inconsistent results. One dimension on which studies vary is the scheduling of training sessions (Morrison & Chein, 2011). In this study, we systematically address whether or not spacing of practice influences training and transfer. We randomly assigned 115 participants to an active control group or one of four training groups who received working memory training based on a “running span” task (Zhao, Wang, Liu and Zhou, 2011). All groups received the same total amount of training: 20 sessions of training with 60 trials for an average of 20 minutes per session. The training was spread across 2 days, 5 days, 10 days, or 20 days. The active control group received 20-minute sessions of math instruction for 20 sessions. Before and after training participants in all five groups performed a single transfer test

that assessed fluid intelligence, the Raven's Progressive Matrices Test. Overall, participants in all four training groups improved significantly on the training task, as reflected by increased processing speed. More importantly, the only training group to show significant improvement on the Raven's was the group who had the greatest amount of spacing (20 days group) during training and improvement in this group was significantly higher than that of the control group.

Disclosures: P. Shah: None. **Z. Wang:** None. **R. Zhou:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Socioeconomic status as a moderator of improvements in executive function following cognitive training in adolescents

Authors: *B. KATZ, P. SHAH;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Socioeconomic status (SES) has been correlated with cognitive functioning in a wide variety of domains throughout the lifespan. Working memory and attentional capacity, key elements of executive function, have both been demonstrated to be lower in individuals from lower socio economic backgrounds. While this connection has been well established, more detailed neurocognitive effects of SES have yet to be determined. Little is known regarding how SES might impact cognitive plasticity throughout adolescence. An online cognitive training program consisting of 20 different games targeting executive function was created as well as a pre- and post- assessment, also administrated online. Students from over 60 English-speaking schools were then recruited to participate in the training program; demographic information as well as school-level SES data was collected. A linear mixed model revealed significant interactions between particular demographic factors, suggesting that socioeconomic status may be an important factor in determining outcomes from cognitive training interventions.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Enhancing executive function and stress management skills in college students

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Abstract: Evidence suggests that there are two avenues to reduce the pervasive adverse effects of acute and chronic stress on mental and physical health-directly by improving skills to cope with stress and indirectly by improving cortically-mediated executive functions that support skills needed to cope with stress. Further, there may be synergistic effects of combining these two approaches to increase resilience to stress. This submission describes an innovative "top-down bottom-up" approach to preventing mental health problems among college students by simultaneously targeting stress management and executive functioning skills. College students present an important target for preventive interventions because they experience high rates of mental health problems (including depression and substance abuse) yet typically do not access available mental health services. 74 university undergraduates (Mage = 19.6 years, SD= 1.1; 86% female) were randomly assigned to one of three 6-week intervention conditions: 90-minutes per week of a computerized cognitive intervention (Lumosity) targeting executive functions (working memory, attentional control, planning/inhibition; n=27); a 90-minute weekly group stress management program teaching coping skills (n=28); or combined Lumosity training and group stress management (180 minutes per week; n=19). Eligible participants were lifetime free of autism spectrum disorder, bipolar disorder, and schizophrenia; and currently did not meet Patient Health Questionnaire (PHQ) criteria for depression, anxiety, or eating disorders. Data were collected at baseline, 6-week, and 12-week follow-up via online and in-person lab assessments. Repeated measures ANOVA indicated that all study participants improved over time in their self-reported executive functioning and use of adaptive coping strategies. Furthermore, significant group x time interactions indicated that those assigned to the Lumosity-only and combined stress management + Lumosity conditions showed significantly greater improvements on the BRIEF Global Executive Composite than those assigned to the stress-management only condition. These findings support the use of a computerized cognitive intervention for enhancing executive function skills and effects for the Lumosity program were stronger as compared with an active condition that involved teaching coping skills. Further

analyses will examine group differences in improvement in executive function skills as assessed with subtests from the DKEFS and CogState computerized battery; and in symptoms of anxiety, depression, and perceived stress as assessed with the PHQ and Perceived Stress Scale.

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Poster

838. Executive Function II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 838.16/RR28

Topic: F.01. Human Cognition and Behavior

Support: NIH R01DA023248

NIH K02DA026990

Title: Proactive control in cocaine dependence: An fMRI study of the stop signal task

Authors: *J. S. IDE[1], S. HU[2], S. ZHANG[2], C.-S. R. LI[2];
[1]Dept. of Sci. and Technol., Univ. Federal De Sao Paulo (UNIFESP), Sao Jose Dos Campos, Brazil; [2]Yale Univ., New Haven, CT

Abstract: Background: Cognitive control is compromised in individuals with cocaine dependence (CD). Our recent work characterized impairment of cognitive control and how these deficits predicted relapse in CD (Luo et al., 2013 Brain). Here, we employed a Bayesian model to describe trial-by-trial anticipation of the stop signal (Ide et al., 2013 J Neurosci) and modeled fMRI signals of proactive control, prediction error, and RT slowing. Our goal is to describe the behavior and neural bases of proactive control in CD. Methods: 60 CD and 66 age and gender matched healthy control individuals (HC) are studied with 40 minutes of fMRI of the stop signal task. Data are pre-processed and modeled using SPM. We modeled fMRI signals at fixation (F model) and go signal (G model) onset in two GLM's, with individual events parametrically modulated by estimated probability of the stop signal (or p(Stop)) and/or RT, respectively. Regional activations are identified for proactive control from the F model and for prediction error and RT slowing from the G model. Results: Compared to HC, CD showed a diminished sequential effect - a correlation between p(Stop) and RT during go trials, suggesting a deficit in Bayesian learning for cognitive control. Both HC and CD showed responses to p(Stop) in

bilateral inferior parietal cortex, anterior pre-SMA, right middle frontal gyrus, and left cerebellum, although the magnitude of response is diminished in CD. CD also showed greater deactivation in the left somatomotor cortices. Unsigned prediction error engaged a wide range of cortical and subcortical structures in HC but less significantly so in CD. Likewise, CD participants demonstrated altered activation during RT slowing, as compared to HC. Additional analyses are to reveal the directional link between regional activations of p(Stop) and RT slowing and how this directional process is compromised in CD. Conclusions: The current results highlight a distinct aspect of cognitive control deficit that may serve as a circuit level marker of cocaine dependence.

Disclosures: J.S. Ide: None. **S. Hu:** None. **S. Zhang:** None. **C.R. Li:** None.

Poster

838. Executive Function II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 838.17/RR29

Topic: F.01. Human Cognition and Behavior

Title: Performance on an online neuropsychological assessment is correlated with performance on standardized academic assessments in middle school students

Authors: *C. SIMONE, N. F. NG, F. FARZIN, J. L. HARDY, M. D. SCANLON;
Lumos Labs, Inc, San Francisco, CA

Abstract: Cognitive abilities such as working memory and attention have been shown to be important moderating factors for educational achievement. While there are studies investigating the relationship between cognitive ability and academic performance, relatively few studies have compared online measures of cognitive ability with online measures of academic achievement. In this study, we sought to determine the correlation between performance on Lumosity's Brain Performance Test (BPT) and performance on Northwest Evaluation Association's Measures of Academic Progress (MAP) in a group of middle school students (grades 6-8). The BPT is a reliable (r=0.70), online battery of brief cognitive assessments adapted from classic pen-and-paper neuropsychological tests. A sample of 687 (376 male) middle school students ages 10-14 took the MAP and BPT during the 2013-2014 academic school year. The students were from 12 public or private charter schools across the United States. The BPT consisted of computerized versions of 7 neuropsychological tasks: Trail-Making parts A and B, Arithmetic Reasoning, Digit Symbol Coding, Forward and Reverse Spatial Memory Span and Progressive Matrices, and

required about 20 minutes to complete. In order to compare performance across subtests, we percentile rank normalized raw scores from each subtest. An overall index score for the BPT was calculated by percentile rank normalizing the sum of a student's normalized scores from all 7 subtasks of the BPT. The NWEA MAP Math and Reading assessments are a reliable (r=0.83 and r=0.77 respectively), widely-used measure of academic proficiency. They are online, computer-adaptive assessments that take about 50 minutes each to complete and scores are reported using a normalized Rasch Unit scale. We calculated Pearson correlations between the normalized BPT overall and subtest scores and MAP Mathematics and Reading overall and subtest scores. We found that the overall score on MAP Mathematics test significantly correlated with the normalized overall score BPT with r=0.58, and MAP Reading test correlated with the BPT with r=0.47. The shared variance for the BPT and MAP test reported in this study can be interpreted as representing the shared factors underlying performance on both cognitive and academic assessments. These findings provide evidence supporting the theory of cognitive ability as an important factor underlying academic achievement.

Disclosures: **C. Simone:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **N.F. Ng:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **F. Farzin:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **J.L. Hardy:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **M.D. Scanlon:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc.

Poster

838. Executive Function II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 838.18/RR30

Topic: F.01. Human Cognition and Behavior

Title: Cingulate cortex in mechanisms of motor control: Evidence from causal modeling of fMRI signals

Authors: *A. ASEMI[1], S. BRESSLER[1], V. DIWADKAR[2];
[1]Behavoiral Science,bldg 12, Florida Atlantic Univ., Boca Raton, FL; [2]Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: Control is a central organizing principal underlying brain network interactions and a ubiquitous element of higher human function. Motor control, a specific class of control mechanisms, subserves multiple complex behaviors including the integration of motor responses with cognitive decisions. Evidence and theoretical models suggest that both the dorsal Anterior Cingulate Cortex(dACC) and the dorsal prefrontal cortex (dPFC) participates in control processes, yet the relative contributions of dACC and dPFC in simple motor control are not understood. Our goal here was to investigate the neural basis of motor control with emphasis on the network control function of heteromodal frontal regions. Using a simple unimanual visuomotor integration task, we demonstrate that the dACC (but not dPFC)sends task-specific directed signals to the Supplementary Motor Area(SMA), suggesting a primary for the dACC in simple motor control. 11 adolescent subjects responded to a flashing white stimulus by tapping the right forefinger, and fixated on a cross-hair in the center of the field of vision at rest. Each ROI's eigenvariate time series was extracted for task-involved voxels by an 'effects of interest' contrast, and estimated in a 5 mm sphere centered on the peak of F-contrast significance over a sequence of images. A multivariate autoregressive model (order 1) for each subject was estimated for each condition (Tapping vs. Rest). Each estimated model coefficient indicated the strength of influence from 1 ROI to another. 2-way, repeated-measures, within-subject, ANOVAs tested for influence differences. Post-hoc paired t-tests were performed when ANOVA showed significant main effects or interactions. The positive t-value indicates that the DACC SMA influence is greater for Tapping than Rest. Thus the Tapping and Rest conditions significantly differed for dACC to SMA but not for dPFC to SMA. These results suggest a highly specialized role for the dACC in motor control, and support the proposal that the dACC is involved in motor control in this task. Our analyses clearly demonstrate different directional and behavioral specificity for the dPFC and dACC in their relations with the SMA.

Disclosures: A. Asemi: None. **S. Bressler:** None. **V. Diwadkar:** None.

Poster

838. Executive Function II

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Program#/Poster#: 838.19/RR31

Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant-in-Aid #24240041

Title: Higher affinity state allele variants in DRD4 and CHRNA4 lead to increased executive attention and related brain activations

Authors: *Y. UEDA, Y. KIKUNO, H. YAMAMOTO, J. SAIKI;
Kyoto Univ., Kyoto-shi, Japan

Abstract: Executive attention plays an important role in focusing on relevant information while preventing interference from distracting stimuli. The dorsal anterior cingulate cortex (dACC) and dorsolateral prefrontal cortex (DLPFC) are mainly implicated in this function, although it is unknown how individual differences in executive function develop. Activation of dACC and DLPFC occurs via dopaminergic neurons in the mesocortical pathway. Moreover, dopamine release in midbrain is due to the activation of nicotinic acetylcholine receptors. In this study, we investigated executive attention using a behavioral performance measurement and brain activity with fMRI as well as single nucleotide polymorphisms in the dopamine receptor D4 gene (DRD4 rs1800955) and acetylcholine receptor subunit α-4 gene (CHRNA4 rs1044396). To measure executive attention, we used the multi-source interference task paradigm (MSIT), where participants were presented three digits and asked to identify the mismatch ("target") among them via a button press. In control trials, the target was always placed congruently with the button box response positions and distractors constituted a number that was irrelevant to participant's response. In interference trials, the target number was placed incongruently with the button box response positions and the distractors included other numbers located on the button box, creating an attentional conflict. The dACC and DLPFC were intensely activated in the interference condition when compared with the control condition. Moreover, participants with the TT and CC genotype in DRD4 and CHRNA4, respectively, showed reduced interference from distractors and significantly increased brain activity in dACC, DLPFC, and midbrain when compared with other genotyped participants. Considering that the TT and CC genotypes are associated with higher neurotransmitter affinity on dopaminergic and cholinergic neurons, respectively, these data suggest that their effect on executive attention depends upon the ease of neurotransmitter uptake in the mesocortical pathway.

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Poster

838. Executive Function II

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DA026452

Title: Motor slowing following unexpected events: Individual roles and functional connectivity of the pre-supplementary motor area and the right inferior frontal cortex

Authors: *J. R. WESSEL[1], V. G. BABOYAN[2], N. TANDON[2], A. R. ARON[1];
[1]Psychology, UC San Diego, San Diego, CA; [2]Univ. of Texas Med. Sch. at Houston, Houston, TX

Abstract: Unexpected environmental events cause a slowing of ongoing motor behavior (Parmentier et al. 2012). Using scalp encephalography (EEG), we recently showed that unexpected events recruit the same human brain network as outright action stopping (Wessel & Aron, 2013). As outright action-stopping is known to engage pre-supplementary motor area (preSMA) and the right inferior frontal cortex (rIFC), here we tested the hypothesis that preSMA and rIFC would also be recruited by unexpected events, and that activity in these structures would explain surprise-induced motor slowing. As scalp EEG has relatively poor spatial resolution and weak single-trial signal-to-noise ratio, we studied a patient with stereotactically implanted depth electrodes in these areas and recorded the intracranial EEG (iEEG). The patient performed two motor tasks. First, a stop-signal task was used to identify electrodes within both preSMA and rIFC that showed a well-established iEEG signature of action-stopping in the beta- (13-30Hz) and gamma-bands (>30Hz). In a second task, the patient responded verbally to stimuli that were preceded by expected (80%) or unexpected tones (20%). As shown before, unexpected tones induced motor slowing, indicated by increased verbal reaction times. Crucially, both preSMA and rIFC exhibited gamma-band increases following unexpected compared to expected tones. Using a single-trial general linear model, we investigated whether neural activity in each area reflected surprise (Bayesian relative entropy), motor slowing, or their interaction (motor slowing induced by surprise). We found that gamma-activity in both preSMA and rIFC reflected the processing of surprise. In addition, preliminary analyses suggest that activity within the rIFC is also predictive of the amount of motor slowing induced by surprise. Furthermore, after unexpected tones, low-frequency (4Hz) coherence between preSMA and rIFC was significantly increased, indicating functional connectivity between these nodes of the stopping-network following unexpected events. Taken together, these findings confirm that activity within two frontal cortical nodes for action-stopping relates to motoric slowing following unexpected events. They furthermore reveal that while both preSMA and rIFC are sensitive to the amount of surprise associated with an unexpected event, activity within the rIFC is also specifically related

to surprise-induced motor slowing. This is consistent with the theory that the rIFC harbors an inhibitory control system that is triggered by surprising / salient events.

Disclosures: **J.R. Wessel:** None. **V.G. Baboyan:** None. **N. Tandon:** None. **A.R. Aron:** None.

Poster

838. Executive Function II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 838.21/RR33

Topic: F.01. Human Cognition and Behavior

Title: Brain eigen-frequency scanning technique and its applications

Authors: ***Q. MENG**[1], M. ISLAM[1], E. HONG[2], F.-S. CHOA[1];
[1]UMBC, Baltimore, MD; [2]UMB, Baltimore, MD

Abstract: In a computer network there are distinct data channels and control channels where massive amount of visual information are transported through data channels, but the information streams are routed and controlled by intelligent algorithm through “control channels”. Recent studies on cognition and consciousness have shown that the brain control channels are closely related to the brainwave beta (14-40 Hz) and alpha (7-13 Hz) oscillations. The high-beta wave is used by brain to synchronize local neural activities and the alpha oscillation is working for desynchronization. In this work we demonstrated that by scanning a person’s brain using binaural beats technique, we were able to identify the individual’s preferred control channels. And those control channels were also proved to be reproducible within a period of at least 6 months. By comparing human brain’s executive functions under control channels and those without binaural beats sent into the subjects’ ears, we demonstrated that control channels could be used to generate positive influence on subjects’ brain executive functions. In the experiment, with 5 male subjects, a 16-channel EEG measurement system was used to record and identify subject’s control channels. Frequencies were scanned from 5Hz to 35Hz and brain signals were recorded for 10 seconds with binaural beats on. During the brain scanning, subjects were asked to close their eyes and with no movements. These channels were identified when EEG signal was peaking at certain binaural beating frequencies (Eigen frequencies of each individual's brain). After the EEG recording system was off the head, subjects were asked to do Stroop tests under the influence of binaural beats running at these control-channel frequencies. We found that the high-beta signal indeed speeded up all subjects' executive function performance and reduced the time to complete incongruent tests, while the alpha signal didn’t seem to be able to slow down

the executive function performance. This led us to think that the desynchronization function had to be initiated from the subjects themselves and could not be controlled externally. Furthermore, it is possible that neural signal propagation speed slows down related to aging and mental illness could cause a red shift of the Eigen-frequencies. We would like to observe such phenomenon the future.

Disclosures: Q. Meng: None. **M. Islam:** None. **E. Hong:** None. **F. Choa:** None.

Poster

839. Human Cognition I

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.01/RR34

Topic: F.01. Human Cognition and Behavior

Support: Evelyn F. McKnight Brain Research Foundation

Arizona Alzheimer's Consortium

Title: Comparing regional activations between older and younger adults on an fMRI task-switching and memory updating paradigm

Authors: *K. KAWA[1], J. A. CARDOZA[1], A. M. STICKEL[1], M. B. SCHMIT[2], M. S. LOZANO[1], E. L. GLISKY[1], L. RYAN[1];
[1]Psychology, [2]Neurosci. and Cognitive Sci., Univ. of Arizona, TUCSON, AZ

Abstract: Although executive functions tend to decline in older adults, there is a wide range of variability in performance. Research in our laboratory has shown that some older adults perform as well or better than younger adults on measures of executive functions. We investigated two measures of executive functions, updating and shifting, using running span (updating) and task-switching (shifting) fMRI paradigms. In the running span task, participants were presented with series of numbers and asked to continually remember the last three numbers. The length of the series of numbers varied randomly from 3 to 5 to 7, in order to test for effects of task difficulty. In the task-switching session, participants were presented with letters of the alphabet in either a square shape or diamond shape. If the letter was presented in a square, they responded whether the letter was printed in upper case or lower case. If the letter was presented in a diamond, they responded whether the letter was a consonant or a vowel. We found that older adults showed greater activations in medial frontal and lateral temporal regions with increasing difficulty on the

running span task, while younger adults showed relatively little increases in activation in response to difficulty. Regarding task-switching, the two groups showed quite different networks of activation - older adults engaging basal ganglia and parietal regions, while younger adults engaged medial and lateral prefrontal regions. This suggests that the two groups may be engaging different cortical and subcortical networks during these two executive functioning tasks.

Disclosures: **K. Kawa:** None. **J.A. Cardoza:** None. **A.M. Stickel:** None. **M.B. Schmit:** None. **M.S. Lozano:** None. **E.L. Glisky:** None. **L. Ryan:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.02/RR35

Topic: F.01. Human Cognition and Behavior

Title: Neurocognitive changes in late adulthood: A dynamic field theory approach

Authors: *G. J. DEGIROLAMO, A. R. SCHUTTE;
Psychology, Univ. of Nebraska- Lincoln, Lincoln, NE

Abstract: As the number of senior citizens continues to increase both in the United States and internationally, it is becoming increasingly important to examine the neurocognitive changes that are associated with late adulthood. Some of the neurological changes that occur naturally with age include atrophy in the medial temporal lobe (Golomb et al., 1993; Jack et al. 1997) and declining hippcampal activation on recollection tasks (Daselaar et al., 2006). Additionally, there is a significant level of atrophy in the pre-frontal cortex (Aizenstein et al., 2006) and striatum (Raz et al, 2003). The brain attempts to compensate for these changes through a postior-to-anterior shift in activation and a reduction in hemispheric specialization through increasing levels of bi-lateral activation (Dennis & Cabeza, 2008). When it comes to cognitive performance, some components are preserved into late adulthood while others decline. For example, Iachini et al. (2005) found that spatial perception ability was preserved while visuospatial working memory declined. Additionally, as task complexity increases, seniors begin to perform significantly worse than young adults on a memory task (Bo and Seidler, 2010, Kessels et al., 2010). There are several factors that can influence the degree to which these neurocognitive changes occur, such as exercise (Bugg and Head, 2011; Colcombe and Kramer, 2003), stress (McEwan, 2002), cardiovascular health (Haley et al., 2008), and genetics (McClearn et al., 1997). These changes

can lead to clinical outcomes (such as dementia or Parkinson's Disease) in some instances and healthy, non-clinical outcomes in other instances. Given the wide variety of factors that influence age-related cognitive changes and the variety of potential outcomes, this raises the question of how to best describe neurocognitive changes associated with late adulthood. Arguably, the best approach would be to apply dynamic systems theory (DST) to cognitive aging. DST argues that development is context-dependent, time-dependant, and occurs through the destabilization of a system that leads to a shift toward a new behavior. In order to apply DST to cognition, a sub-theory known as Dynamic Field Theory can be used. Dynamic Field Theory uses dynamic neural fields to computationally model how information is mentally represented and the influence of these representations on behavior. The purpose of this poster is to present a dynamic neural field model explaining how neurocognitive changes in late adulthood influence spatial cognition.

Disclosures: G.J. Degirolamo: None. **A.R. Schutte:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.03/RR36

Topic: F.01. Human Cognition and Behavior

Title: The Relationship between resting state functional connectivity of executive control network and cognitive decline in aging : A retrospective longitudinal study

Authors: *B. H. LEE[1,2], K. S. DO[2], J. CHA[3], J.-H. KIM[1], J.-M. LEE[3], G. H. KIM[4], J. CHIN[1], M. K. SUH[1], Y. NOH[5], S. W. SEO[1], D. L. NA[1];
[1]Neurol., Samsung Med. Ctr., Seoul, Korea, Republic of; [2]Psychology, Sungkyunkwan university, Seoul, Korea, Republic of; [3]Dept. of Biomed. Engin., Hanyang Univ., Seoul, Korea, Republic of; [4]Neurol., Ewha Womans University, Mokdong Hosp., Seoul, Korea, Republic of; [5]Gachon Univ. Gil Med. Ctr., Incheon, Korea, Republic of

Abstract: It has been reported that normal cognitive aging may be accompanied by change in the functional brain networks. Aging brain may shows either more increased or decreased functional connectivity compared to the younger brain revealing decline or compensation mechanism of brain function. However, it has not been fully elucidated whether the change of functional connectivity in aging brain is related to the future change of cognitive function since there have been hardly any longitudinal studies. This study aimed to investigate how the baseline functional connectivity would relate to cognitive changes by examining the baseline resting state

fMRI data and the longitudinal follow-up neuropsychological tests retrospectively. We examined data from 284 people with normal cognition who completed neuropsychological tests and resting state fMRI procedure using 3T MRI at the baseline investigation. Among them only 69 people who were followed up cognitive tests at least once or more through the 1-4 years after (mean of 24 months). Degrees of functional connectivity of four resting state networks (default mode network, executive control network (ECN), dorsal attention network, salience network) and brain volumes were calculated. The difference score of cognitive performance in the baseline and the follow up were calculated with the z score based on the age and education norm. The differences in baseline demographic and brain characteristics were compared between decliner and nondecliner groups and analyzed through the multiple regression, efforts have been made to see which of the factors were related the cognitive changes (the number of declines in performances of the cognitive subtests). The results demonstrated that the nondecliner group (n=48) had less brain atrophy (more gray matter volume and less CSF volume) and tended to have less ECN connectivity in baseline compared to the decliner group (n=21). Multiple regression analysis showed that the number of cognitive domains that declined among seven tasks was predicted by ECN connectivity independent of the baseline brain volume and demographic variables where increased ECN connectivity in baseline was positively related to the number of decline in cognitive subtests after 1-4 years. The results showed that increased ECN in baseline could be a predictor of cognitive decline in later years. In contrast, the brain volume was negatively correlated with the cognitive decline, being a protective factor for cognitive decline. The increased ECN (task related network, frontal network) connectivity, therefore, might be considered as a risk factor that can predict cognitive decline of older people in the future.

Disclosures: **B.H. Lee:** None. **K.S. Do:** None. **J. Cha:** None. **J. Kim:** None. **J. Lee:** None. **G.H. Kim:** None. **J. Chin:** None. **M.K. Suh:** None. **Y. Noh:** None. **S.W. Seo:** None. **D.L. Na:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.04/RR37

Topic: F.01. Human Cognition and Behavior

Support: Irish Research Council

Title: An event-related potential study of perceptual decision-making and aging

Authors: A. M. HAYES[1], S. P. KELLY[2], *R. G. O'CONNELL[1];
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Abstract: Aging is associated with slower performance on a range of perceptual tasks. Computational models propose that this originates from a strategically higher and more cautious criterion setting in older age but neurophysiological evidence has been lacking. Recently a decision signal has been identified in the human EEG that integrates information to a criterion level at which time the decision is effected (O'Connell, Dockree and Kelly, 2012; Kelly & O'Connell, 2013). The aim of the current study was to investigate the effect of healthy aging on this neural decision signal. Participants comprised 36 older (aged 66-85) and 40 younger (aged 18-38) adults. Two tasks were undertaken: a stimulus with intermittent gradual contrast decrease was presented in the first and a continuous version of the two-alternative random dot motion discrimination task was presented in the second. In both tasks, participants responded with a speeded button press. There was no effect of age on accuracy or speed of performance for detection of contrast change while older adults were significantly less accurate and responded slower for motion discrimination. Analysis of the neural decision variable signal revealed that, contrary to the simple boundary elevation predicted by mathematical models, elderly performance differences were associated with a number of neural adjustments foremost among which was a diminished evidence accumulation rate.

Disclosures: A.M. Hayes: None. **S.P. Kelly:** None. **R.G. O'Connell:** None.

Poster

839. Human Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: NIH NIA R01 AG034138

Angier B. Duke Scholarship

Duke University: Bass Connections

Title: Does aging affect multi-sensory integration processes in the brain?

Authors: E. A. YALCINBAS[1], M. A. JOHNSON[1], J. M. GROH[2], *M. T. DIAZ[1,3];
[1]Brain Imaging and Analysis Ctr., Duke Univ., DURHAM, NC; [2]Dept. of Psychology and Neuroscience, Ctr. for Cognitive Neuroscience, Dept. of Neurobio., Duke Univ., Durham, NC; [3]Dept. of Psychology; Social, Life, & Engin. Sci. Imaging Ctr., Pennsylvania State Univ., University Park, PA

Abstract: Speech perception relies heavily on multisensory integration. It has been hypothesized that our sensory modalities process quantifiable relationships between auditory and visual components of natural speech; thus, when we are presented with a mismatched audiovisual speech stimulus, a fusion or combination effect can occur (Jiang & Bernstein, 2011). The McGurk effect is an example of the former possibility, and it emerges when the visual information we receive as we look at a speaker's face contradicts the auditory information we receive as the speaker makes a sound (McGurk & MacDonald, 1976). Typically, a video of a person saying the syllable "ga" dubbed with a voice recording of the syllable "ba," produces the unique percept "da." At the same time, it is known that sensory organs decline with age and that the functionality of brain circuits devoted to sensory processing are increasingly compromised. However, previous studies suggest that although older adults' sensory perception in a single modality tends to deteriorate, they may benefit from multimodal sensory integration to a greater extent than younger cohorts, a phenomenon that has been termed inverse effectiveness (Laurienti, et al., 2006). In this event-related, fMRI experiment 20 healthy younger and older participants identified what phoneme they perceived. Auditory and visual information was either consistent (i.e., representing the same phoneme) or inconsistent (i.e., eliciting the McGurk effect). Behavioral analyses revealed a main effect of trial type: reaction times for consistent trials were significantly faster than those for the inconsistent trials, and an Age x Condition interaction: older adults were significantly slower on inconsistent, but not consistent trials compared with younger adults. fMRI results showed a main effect of Age in which older participants had greater bilateral activation in lateral occipital cortices, motor cortex, and superior parietal lobes compared to younger participants, while younger participants had greater activation in orbital frontal and insular cortices, and superior temporal gyri compared to older participants. These findings do not support an inverse effectiveness in multimodal perception for older adults.

Disclosures: E.A. Yalcinbas: None. **J.M. Groh:** None. **M.A. Johnson:** None. **M.T. Diaz:** None.

Poster

839. Human Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: Velux-Stiftung (project No. 369)

SNF Doc.Mobility Fellowship

Title: Relation between behavioral measures and structural and functional connectivity of the cingulum bundle in normal aging

Authors: *S. HIRSIGER[1,2], V. KOPPELMANS[5], S. MÉRILLAT[3,2], F. LIEM[4], B. ERDENIZ[5], R. SIDLER[5,6,7], L. JÄNCKE[4,3,2];
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Abstract: Introduction: Normal aging is associated with behavioral deterioration and declines in brain structure and function. Age-related behavioral declines may be the result of deterioration of white matter tracts, causing structural differences, besides alterations in co-activation between brain areas (functional connectivity (FC)). Therefore, we aimed to investigate the relationship between SC and FC in the cingulum bundle in a homogenous well-educated, cognitively healthy sample of 165 older participants (mean age=70.15±4.50) using multi-modal imaging. Additionally, we investigated if the cingulum's SC and FC are related to cognitive and motor performance. Methods: Diffusion weighted images (DWI), structural MRI (T1) and resting-state functional MRI (rsfMRI) images were obtained for all participants. For SC, FMRIB's Diffusion Toolbox was used to obtain DTI metrics (Fractional Anisotropy (FA), Mean, Axial, and Radial Diffusivity (MD, AD, RD) and probabilistic tractography. For FC, functional connectivity maps were obtained using the Data Processing Assistant for rsfMRI (DPARSF) software package and the rs-fMRI Data Analysis Toolkit (REST). The MNI coordinates determining the posterior cingulate cortex (pC) were the same for our tractography and rsfMRI analyses. For tractography, the frontal lobe ROI was based on Wakana et al. (2007) while for rsfMRI the peak correlation coordinate between the pC ROI and the medial prefrontal cortex (mPFC) was chosen. Connectivity strength was analyzed by exploring the DTI metrics within the cingulum and the functional correlation between the pC and mPFC. In addition, all participants were assessed with cognitive tests measuring processing speed (PS), memory, and executive functions (EF), and motor tests of fine motor skills. Analyses were adjusted for age, gender, education, blood pressure and atrophy. Results: MD: r(163)=.32; AD: r(162)=.27; and RD: r(163)=.22, (p range: <.001-.005) were associated with age. No relationship between SC and FC was found. All behavioral measures were significantly negatively associated with age (r range: -.21 - -.40).

Brain-behavioral associations were only found for FC. FC was associated with PS (p=.011), semantic memory (p=.050), and fine motor skills (p=.001). Discussion: We found that SC of the cingulum tract was associated with age whereas FC was related to behavioral measures. Interestingly, only behavioral measures comprising a PS component showed associations with FC. Based on our results we conclude that age may differentially affect SC and FC in the cingulum tract, and that individual differences in FC seem to be a predictor for behavioral outcome measures.

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Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.07/RR40

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 44041

Title: Changes in resting-state functional connectivity of the human brain with age

Authors: ***S. HRYBOUSKI**[1], F. OLSEN[1], R. CARTER[2], P. SERES[3], N. MALYKHIN[3]; [2]Psychiatry, [3]Biomed. Engin., [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Objective: Neuroimaging is a powerful non-invasive tool that can be used to differentiate pathological from non-pathological aging. Independent Component Analalysis (ICA), when applied to functional Magnetic Resonance Imaging (fMRI) timeseries without an explicit task, detects factors with a high degree of temporal coherence, while maximizing spatial independence. These factors, termed as resting-state networks, can be used to study changes in functional organization of the brain with age. The predominant theory of aging suggests that association cortices are most affected by the aging process, while primary visual, somatosensory, motor and auditory systems of the brain are not. The goal of the present study was to examine changes in resting-state functional connectivity across the entire adult human lifespan. In the current study we tested the hypothesis that functional networks, which are localized to frontal, temporal, and parietal association cortices, show reduction in functional connectivity with age, while primary sensory and motor networks remain preserved. Methods: A total of 108 healthy volunteers (18-85 years old) were recruited for this study. Images were acquired using T2*-

weighted Gradient Echo Planar Imaging, 2 Gradient Echo, and T1-weighted 3D MPRAGE sequences images on a 1.5T Siemens Sonata system. All functional volumes were corrected for geometric distortions, slice acquisition delay and in-scanner head motion using SPM12b. Following normalization to the template space, functional data was smoothed with the 8 mm FWHM gaussian kernel. Network analyses were performed in GIFT (v3.0) toolbox for MATLAB. The number of components for the spatial ICA analysis was determined using Kullback Information Criterion approach, and component reliability was assessed using ICASSO bootsrapping procedure. Results: Based on previous literature we identified visual, sensorimotor, default-mode, and frontal-parietal components. Results showed general reduction in functional connectivity across all these components. Furthermore, the default-mode component showed greater connectivity with age in the right postcentral and supramarginal gyri, and sensorimotor component showed increased connectivity with age in bilateral lingual and fusiform gyri. These areas of increase in functional connectivity are not typically associated with the canonical nodes of these networks and likely represent compensatory mechanisms to account for loss of connectivity with the predominant nodes. Conclusions: In summary, our findings demonstrate broad changes in functional connectivity with age that are not restricted to the association cortices.

Disclosures: S. Hrybouski: None. **P. Seres:** None. **R. Carter:** None. **F. Olsen:** None. **N. Malykhin:** None.

Poster

839. Human Cognition I

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Program#/Poster#: 839.08/RR41

Topic: F.01. Human Cognition and Behavior

Support: McKnight Brain Institute, University of Arizona

Arizona Alzheimer's Research Consortium

Title: fMRI correlates of successful encoding and retrieval in response to increasing difficulty during an episodic memory task

Authors: E. BAENA[1], *L. RYAN[2];
[2]Evelyn F. McKnight Brain Inst., [1]Univ. of Arizona, Tucson, AZ

Abstract: The aim of the present study was to delineate the neural substrates engaged in successful memory encoding (subsequent memory effect) and successful retrieval (recognition effect) in an episodic memory task, as task difficulty increases. The present study evaluated patterns of activation in younger (ages 18-24) and older (ages 62-83) adults. During encoding, subjects judged whether the words in a pair were synonyms or antonyms without any expectation of a memory test. Memory for the word pairs was tested with yes/no recognition judgments. Difficulty was manipulated by increasing word frequency, such that task difficulty increases as word frequency increases. Behavioral results indicated that younger adults were more accurate than older adults. In an fMRI analysis of successful encoding (subtracting forgotten items from remembered items), younger and older adults both activated left-lateralized regions in the inferior prefrontal, middle temporal, and middle frontal/anterior cingulate gyrus. When comparing age-related activations, consistent with previous research, older adults showed less activation than young adults in bilateral parahippocampal gyri and more activation than young adults in the middle frontal cortex. Additionally, older adults showed increased activation in the left posterior parahippocampal gyrus and left inferior parietal lobule. During successful retrieval, both young and older adults recruited similar areas for all successfully retrieved items. However, bilateral increases in activation due to difficulty were observed in frontal and parietal regions for the older adults, whereas young adults showed increases in posterior and medial regions. Our results support the hypothesis that increased prefrontal activations in older adults during successful retrieval are compensatory, because of an effort to maintain performance in the face of increasing cognitive difficulty and also because of decreases in medial-temporal activations during encoding compared to younger adults.

Disclosures: E. Baena: None. **L. Ryan:** None.

Poster

839. Human Cognition I

Location: Halls A-C

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Program#/Poster#: 839.09/RR42

Topic: F.01. Human Cognition and Behavior

Support: NSERC

Title: Age-related differences in cortical activity during a tactile discrimination task following an acute bout of moderate intensity aerobic exercise

Authors: C. POPOVICH, *R. STAINES;
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Abstract: Aging studies have shown age-related behavioural deficits particularly during higher-order executive function tasks requiring selective attention. Neuroimaging data in older adults has shown that atrophy of frontal lobe regions is disproportionately greater relative to other brain areas. A growing body of neuroimaging research has revealed that acute bouts of moderate intensity aerobic exercise can enhance cortical activity specifically in frontal lobe areas including the prefrontal cortex (PFC) during a variety of executive functioning tasks including: inhibitory control, conflict resolution, and selective attention. The PFC has an important role in selective attention by modulating modality-specific cortical regions in multiple sensory modalities. Previous work in our lab using electroencephalography (EEG) and a somatosensory oddball task has shown enhanced attentional modulation in modality-specific areas in healthy young adults following a 20 minute bout of moderate intensity aerobic exercise. Here we used EEG to examine if exercise-induced increases in PFC activity would enhance attention-based modulation of tactile event-related potentials (ERPs) generated at early stages of cortical somatosensory processing in healthy older adults. We hypothesized that exercise preceding performance of the odd-ball task would increase PFC activity thereby enhancing ERPs to attended tactile stimuli and suppressing those to unattended stimuli. We recorded somatosensory ERPs while participants performed a tactile discrimination odd-ball task before and immediately after completing a 20-minute bout of moderate intensity aerobic exercise on a cycle ergometer. During the task, participants received vibrotactile stimulation to the second and fifth digit on the left hand and were instructed to detect discrete changes in vibratory stimuli applied to one digit only. ERP amplitudes for the P50, P100, N140, P300, and long latency positivity (LLP) were quantified for attended and non-attended trials at frontocentral and centroparietal electrodes. Preliminary results showed an increase in the amplitude of the P100 and the LLP to attended versus unattended stimuli at centroparietal and frontocentral sites respectively post-exercise. The LLP has multiple cortical generators while the somatosensory P100 is likely generated in higher-order somatosensory regions. However, both of these ERPs are modulated by attentional processes. These preliminary results suggest that exercise may enhance attentional modulation of early modality-specific ERPs in a target detection task.

Disclosures: C. Popovich: None. **R. Staines:** None.

Poster

839. Human Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: UNAM DGAPA PAPIIT ID300312

Title: Effects of aging on the neural correlates of source memory retrieval across the adult life span

Authors: *S. CANSINO[1], P. TREJO-MORALES[1], C. ESTRADA-MANILLA[1,2], E. H. PASAYE-ALCARAZ[3,4], E. AGUILAR-CASTAÑEDA[4], P. SALGADO-LUJAMBIO[5], A. L. SOSA-ORTIZ[6];
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Abstract: The ability to retrieve the spatial and temporal details of our personal past experiences is referred to as source memory. Behavioral experiments provided evidence that source accuracy for spatial context decreases linearly with advancing age. However, the neural correlates associated to source memory decline across adulthood are little known because the majority of the studies only compared individuals from extreme age groups. To identify brain activity variations across the adult life span during source memory retrieval, young, middle-aged and old adults were scanned with functional magnetic resonance imaging (fMRI). Twelve healthy individuals from both sexes participated in each age group. During encoding, images of common objects were randomly presented in one of the quadrants of the screen while the participants judged whether they represented natural or artificial objects. At retrieval, the images presented at encoding were randomly mixed with new ones and displayed at the center of the screen. Participants judged whether each image was new or old and, if old, they were instructed to indicate in which quadrant of the screen the image was presented in the encoding session. The contrast between items attracting correct versus incorrect source judgments revealed that young adults recruited prefrontal and parietal regions, whereas older adults showed greater activity in occipital and fusiform gyri. Brain activity in association with correct source judgments diminished significantly between young and old adults, whereas that from the middle-aged group did not differ significantly from the other two groups. The results suggest that by middle-age the neural correlates of successful source memory retrieval, detectable by fMRI, are still preserved.

Disclosures: S. Cansino: None. **P. Trejo-Morales:** None. **C. Estrada-Manilla:** None. **E.H. Pasaye-Alcaraz:** None. **E. Aguilar-Castañeda:** None. **P. Salgado-Lujambio:** None. **A.L. Sosa-Ortiz:** None.

Poster

839. Human Cognition I

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.11/RR44

Topic: F.01. Human Cognition and Behavior

Title: Changes in white matter microstructure and perceptual speed are associated in very old age

Authors: *M. LÖVDÉN;
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Abstract: The integrity of the brain's white matter is important for neural processing and displays age-related differences, but the contribution of changes in white matter to cognitive aging is unclear. We used latent change modeling to investigate this issue in a sample of very old adults (aged 81-103 years) assessed twice with a retest interval of 2.3 years. Using diffusion-tensor imaging, we probed white matter microstructure by quantifying the mean fractional anisotropy and mean diffusivity of six major white matter tracts. Measures of perceptual speed, episodic memory, letter fluency, category fluency, and semantic memory ability were collected. Results showed mean change of fractional anisotropy and mean diffusivity in the major white matter tracts over time. Between-person differences in change of fractional anisotropy were significant for five of the examined six tracts. For mean diffusivity, such differences were significant for three of the tracts. The individual differences in change of fractional anisotropy were associated across tracts. In contrast, the changes of mean diffusivity were weakly associated among tracts, and generally also correlated weakly with changes in fractional anisotropy. Mean longitudinal decline was also observed for the investigated cognitive abilities. Importantly, decreases of perceptual speed were significantly associated with changes in both fractional anisotropy and mean diffusivity in the corticospinal tract. We conclude that white matter microstructure is a potent correlate of aging-related changes in sensorimotor aspects of behavior, but that it is unclear whether its impact extends to higher-order cognition.

Disclosures: M. Lövdén: None.

Poster

839. Human Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant KL2 TR000095

Title: Cognitive and neural effects of a computerized cognitive training in older adults with mild cognitive impairment (mci): A feasibility study

Authors: *F. LIN[1], D. TADIN[2], K. HEFFNER[1], G. SCHIFITTO[1], M. MAPSTONE[1];
[1]Univ. of Rochester Med. Ctr., Rochester, NY; [2]Univ. of Rochester, Rochester, NY

Abstract: Aims: NIH emphasizes the importance of behavioral interventions in slowing the progression from MCI to dementia. Computerized vision-based speed of processing (VSOP) training is one of the most widely implemented behavioral interventions for dementia-free Americans. In the present study, we tested the feasibility of implementing VSOP training in elders with MCI, and the preliminary training effect on cognitive and neural function. Methods: Ten participants with MCI were randomly assigned to 6-week VSOP training (n = 5) or computerized leisure activity (LA, n = 5). Assessments were conducted at baseline and post-training when interviewers were blinded to group assignment. Assessments included UFOV (assessing processing speed, divided attention, and selective attention), and EXAMINER (working memory and cognitive control). Resting state functional connectivity (rsFC) of brain networks that are theoretically related to VSOP training, including Central Executive Network, Frontoparietal Network, and Default Mode Network, were collected using functional MRI in VSOP group only. Results: All participants completed 6-week training. Two groups were similar in demographic characteristics and baseline cognitive performance. There were improvements in selective attention following VSOP training ($p = .043$) but not in the LA group. VSOP training group had significantly greater training effects on selective attention, divided attention, and working memory than LA group did (Cohen's d: 0.98 to 2.25). Furthermore, there was preliminarily significant difference in several regions within the three brain networks before and after VSOP training using uncorrected p value < .01. VSOP training seemed to correct these pathologically disrupted connections and improve the efficiency of neural networks. Increased connectivity between amygdale and middle frontal gyrus after VSOP training was significantly associated with greater increase in selective attention ($\rho = .90$, $p = .037$). Conclusion: Implementing computerized VSOP training is feasible in older adults with MCI. A larger sample size is necessary to further assess whether VSOP training may improve cognitive performance, reflecting neural changes measurable via rsFC.

Disclosures: **F. Lin:** None. **D. Tadin:** None. **K. Heffner:** None. **G. Schifitto:** None. **M. Mapstone:** None.

Poster

839. Human Cognition I

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Program#/Poster#: 839.13/RR46

Topic: F.01. Human Cognition and Behavior

Title: Impact of aging on fronto-striatal reward processing

Authors: ***M. VINK**, I. KLEEREKOOPER, R. S. KAHN;
Dept Psych, UMC, Utrecht, Netherlands

Abstract: Introduction With advancing age, performance declines on various cognitive measures, such as decision making, learning and attentional control. Underlying this decline may be an age-related attenuation in the ability to learn the association between cues and outcome, specifically cues predicting reward (stimulus-reward coupling). This ability is crucial for goal-directed behavior. Here, we explore the effect of aging on reward processing in a group of healthy adults aged 40 to 70 years. We hypothesize that advancing age impairs the shift from reward outcome to anticipation in reward processing. Specifically, we hypothesize that across age, activation during the anticipation of reward will decline, whereas activation during the receipt of reward will increase. Methods Forty-nine healthy subjects aged 40 to 70 years (mean age 54.89 y; SD 6.96; 23 males) performed a modified version of the Monetary Incentive Delay task while being scanned with fMRI (2D-EPI, TR = 1600ms, TE = 23ms, 372 scans). Age-related changes in activation are analyzed using a whole brain approach and in two predefined anatomical Regions of Interest (ROIs) which are all involved in reward processing: the bilateral ventral striatum, dorsal caudate, putamen, insula, cingulate cortex, supplementary motor area and orbitofrontal cortex. Results There was no effect of age on behavior. Activation during Reward Anticipation showed a linear decline across age in the ventral striatum, indicating a decline in the contrast between the processing of reward cues and neutral cues. In contrast, activation during Reward Outcome increased with age. Activation in the orbitofrontal cortex was not related to age. Discussion This is the first study to show a gradual decline in front-striatal reward processing between the ages 40 to 70. These findings suggest a shift from reward anticipation towards receiving reward with increasing age, possibly due to a diminished ability to differentially encode reward cues in the ventral striatum. This shift is in direct opposition to the

shift we observed during adolescent development (Hoogendam et al., 2013). Remarkably, we observed this decline in ventral striatum functioning in healthy subjects, and we did not find an effect of age on task performance. Hoogendam, J. M., Kahn, R. S., Hillegers, M. H. J., van Buuren, M., & Vink, M. (2013). Different developmental trajectories for anticipation and receipt of reward during adolescence. Developmental cognitive neuroscience, 6, 113-24.

Disclosures: **M. Vink:** None. **I. Kleerekooper:** None. **R.S. Kahn:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.14/RR47

Topic: F.01. Human Cognition and Behavior

Title: Glucose facilitation of cognition; is this dependent on age?

Authors: ***H. MACPHERSON**[1], B. ROBERTSON[2], A. SCHOLEY[1];
[1]Swinburne Univ., Melbourne, Australia; [2]Lancaster Univ., Lancaster, United Kingdom

Abstract: Glucose is the primary energy source of the brain and the administration of a 25 or 50g glucose containing drink has been consistently demonstrated to improve cognitive performance. Acute glucose administration has been shown to preferentially enhance cognition under conditions of high cognitive demand and also for tasks which possess an episodic memory component, possibly due to effects on the hippocampus. However, the extent to which this glucose facilitation effect is uniform across the lifespan is uncertain. In this study it was hypothesized that glucose would only enhance episodic memory during performance of a demanding secondary task, and this effect would be most prominent in older adults. This study used a repeated measures, cross-over trial design to assess the cognitive effects of glucose in younger and older adults. Participants were 24 healthy younger (average age 20.6 years) and 24 healthy older adults (average age 72.5 years). During each testing session participants completed a verbal recognition memory task in the presence and absence of a secondary visual tracking task. Both the single and dual task paradigms were completed on two occasions, once after consuming a drink containing 25g glucose and once after consuming a placebo drink. The results demonstrated that the addition of the secondary tracking task significantly reduced the number of words recognized in the memory task and slowed response time. This impairment in recognition memory was greater in older than younger adults, and older adults also demonstrated poorer tracking accuracy on the secondary task. In terms of the effects of glucose administration, the

glucose drink improved speed of recognition memory response to a greater extent in in older than younger adults and improved tracking accuracy on the secondary task in older adults. In the older group, glucose enhanced episodic memory regardless of whether the secondary task was performed. These findings, together with the improvement in tracking accuracy, suggest that in older people, who may not be performing at an optimum level, acute glucose administration can exert general enhancements to cognition regardless of the level of cognitive demand.

Disclosures: H. Macpherson: None. **B. Robertson:** None. **A. Scholey:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: FCOMP-01-0124-FEDER-021145 (PTDC/SAU-ENB/118383/2010)

Title: Theta and alpha neurofeedback protocol for age-related memory deficits

Authors: *N. DIAS[1], A. SILVA[2], J. REIS[2], J. CERQUEIRA[2], N. SOUSA[2];
[1]ICVS/3Bs Associate Lab., Braga, Portugal; [2]ICVS/3Bs Univ. of Minho, Braga, Portugal

Abstract: With the growing life expectancy, the number of elderly people is increasing tremendously worldwide. The progressive decrease of synaptic plasticity and neuronal inter-connectivity in the ageing brain, concomitant with alterations in key cognitive abilities such as working memory and attention, may be delayed, stopped or reversed by neurorehabilitation. Hence, current approaches used to modify cognitive capabilities are of utmost importance to contemporary society and often divided into behavioral training procedures and techniques for direct modulation of neural mechanisms such as neurostimulation. Neurofeedback, which is based on electroencephalogram signals, is used to train individuals on learning how to influence brain function by modulating their own brain rhythms. However, the potential effects of rehabilitation through behavioral training, neuromodulation and even a combined methodology are poorly understood. In the present study, we examine the effects of a protocol with neurocognitive tasks and neurofeedback training. For this purpose a protocol for neurorehabilitation covering the two proposed methodologies was developed. It supports Alpha and Theta neurofeedback up-training and as well as neurocognitive tasks, namely the n-Back Task and the Corsi Block-Tapping Task. Thirty four participants (16 males and 18 females) with

age above 55 years-old, were intervened in a twelve-day protocol with either a neurocognitive and neurofeedback combined protocol, a neurocognitive single protocol, a neurofeedback single protocol or a sham neurofeedback single protocol. In general, both neurofeedback and neurocognitive practice appear to induce an enhancement of Alpha and Theta activities as well an enhancement in working memory overall state. Considering the promising results found in this study, we look forward to evaluate the rehabilitative impact of protocol length, other EEG rhythms and their brain sources, strategies for transference of neuromodulation skills to real life scenarios and refined behavior evaluation tools.

Disclosures: **N. Dias:** None. **A. Silva:** None. **J. Reis:** None. **J. Cerqueira:** None. **N. Sousa:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG034570

Title: Effects of age and β-amyloid deposition on the neural systems for gist and visual detail memory encoding in cognitively normal older adults

Authors: ***H. OH**[1], J. ELMAN[2], S. BAKER[2], C. MADISON[1], J. W. VOGEL[1], S. CROWLEY[2], W. J. JAGUST[1,2];
[1]Helen Wills Neurosci. Inst., Univ. of California-Berkeley, Berkeley, CA; [2]Life Sci. Div., Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Although successful visual memory encoding can involve gist memory of studied scenes, the precise visual details, or both, changes in neural systems supporting these cognitive operations due to normal and pathological aging remain to be elucidated. Using functional magnetic resonance imaging (fMRI) and positron emission tomography with the radiotracer [11C] Pittsburgh compound B (PIB-PET), we examined how regional brain activity and functional connectivity underlying gist and visual detail memory change with age and β-amyloid (Aβ) deposition, a pathological hallmark of Alzheimer's disease, in cognitively normal older adults. Twenty-two young (mean age=23.6, 11 males) and 49 cognitively normal older adults (mean age=76.5, 20 males) performed an episodic encoding task of visual scenes during fMRI

scans, followed by a postscan recognition task where subjects made an old/new judgment with confidence ratings based on written descriptions of the gist of visual scenes and a true/false judgment on 6 written visual details of the scenes. 150 encoding trials were sorted based on gist accuracy and the number of details correctly recognized. Using PIB-PET scans, older adults were dichotomized by presence (PIB+) or absence (PIB-) of Aβ deposition. Psychophysiological interaction was assessed using an individually defined right parahippocampal gyrus (rPHG) seed region to determine functional connectivity between rPHG and other brain regions. Compared to PIB- older subjects, young subjects showed increased activation in visual association areas (VA) and hippocampus for successful gist memory (high confidence hit vs. miss), but PIB- older adults, compared to young subjects, showed stronger connectivity between rPHG and lateral frontal cortex for gist memory. With respect to memory for visual details, young subjects showed both higher regional activity and stronger functional connectivity in the medial temporal lobes, VA, and posterior cingulate/precuneus than PIB- older subjects. Compared to PIB- older adults, PIB+ older adults showed both reduced regional activation across multiple brain regions and reduced connectivity between rPHG and hippocampus for gist memory, but increased regional activation in VA and precuneus and stronger connectivity between rPHG and lateral frontal and parietal cortex for remembering more visual details. These findings suggest that with age, visual episodic encoding is achieved by gist-based processing as well as enhanced connectivity of associated neural systems while older adults with Aβ recruit more neural resources to process visual details potentially to compensate for the Aβ-related hippocampal dysfunction.

Disclosures: **H. Oh:** None. **J. Elman:** None. **S. Baker:** None. **C. Madison:** None. **J.W. Vogel:** None. **S. Crowley:** None. **W.J. Jagust:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIA U01 AG22376

NHLBI 3U01AG022376-05A2S

NIA 1P30 AG028740

Title: Greater intensity and time of non-sedentary activity is associated with higher cognitive performance in sedentary older adults

Authors: J. R. NOCERA[1,2], D. G. HIRE[3], D. R. COOK[3], *K. MCGREGOR[1], J. KATULA[3], S. R. RAPP[3], K. M. SINK[3], J. JENNINGS[3], R. A. FIELDING[4], K. REID[4], A. KRAMER[5], J. VERGHESE[6], A. C. KING[7], T. M. MANINI[8], T. W. BUFORD[8], S. ANTON[8], N. NADKARNI[9], M. A. ESPELAND[3];
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Abstract: Higher levels of physical activity have been associated with improved cognition in older adults (OA). However, among sedentary OA it is unclear the degree to which the intensity of activity during non-sedentary behavior impacts cognition. The objective of our analysis was to examine the cross-sectional association between objectively measured intensity of non-sedentary activity and cognition in a randomized clinical trial of sedentary OA (The LIFE Study). Activity data were collected by hip-worn accelerometers. Intensity of non-sedentary activity was quantified as average activity count >100 counts/min. Unadjusted and adjusted linear regression were used to model the relationship between accelerometry data and cognitive domains [working memory (n-back), task switching, response inhibition, immediate and delayed recall (Hopkins Verbal Learning Test-Revised; HVLT-R) and processing speed (Digit Symbol Coding; DSC]. Adjusted models included demographic confounders and health parameters. The final population included 1274 participants (78.7±5.3 years; 66.7% female). In the unadjusted model, higher intensity activity during non-sedentary activity was associated with better performance on the 1-back (accuracy) (β =0.08; 95% CI:0.02,0.13), task switching cost (β=0.09; 95% CI:0.03,0.14), HVLT-R immediate (β=0.13; 95% CI:0.08,0.19), HVLT-R delayed (β=0.13; 95% CI:0.07,0.18), and the DSC (β=0.18; 95% CI:0.16,0.23) (Standardized scoring with positive scores reflecting better performance; all *p*'s<0.01). Greater average minutes of non-sedentary activity was also associated with better performance on the above outcomes (*p*'s<0.01), excluding task switching cost. All associations remained significant after adjustment. This work suggests that intensity during activity and time in non-sedentary activity is related to cognitive performance in several domains in sedentary OA.

Disclosures: J.R. Nocera: None. **D.G. Hire:** None. **D.R. Cook:** None. **K. McGregor:** None. **J. Katula:** None. **S.R. Rapp:** None. **K.M. Sink:** None. **J. Jennings:** None. **R.A. Fielding:** None. **K. Reid:** None. **A. Kramer:** None. **J. Verghese:** None. **A.C. King:** None. **T.M. Manini:** None. **T.W. Buford:** None. **S. Anton:** None. **N. Nadkarni:** None. **M.A. Espeland:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.18/SS1

Topic: F.01. Human Cognition and Behavior

Support: NHMRC Grant

Title: Age-related compensation during motor imagery: A multimodal study

Authors: *H. BURIANOVA[1], L. MARSTALLER[2], P. SOWMAN[3], A. RICH[3], M. WILLIAMS[3], B. JOHNSON[3], G. SAVAGE[3];
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Abstract: Motor imagery (MI) is an active process during which the representation of simple or complex motor movements is internally reproduced without any overt physical action. The neural correlates of MI overlap greatly with those of motor execution, and thus MI has been utilized in rehabilitative settings to optimize motor function after stroke or injury. As older adults are more prone to impairments in motor function, understanding age-related effects on MI is important. In a multimodal study, young and older adults took part in the Finger Configuration Task (FCT), either executing or imagining sequences of finger movements, whilst their brain activity was measured by magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI). The results show that MI ability does not diminish with age; however, the quality of the underlying neural signal changes. In contrast to young adults, during MI older adults show increased activity in somatosensory areas and in additional areas outside the motor network. This activity correlates positively with both vividness and FCT accuracy scores, and is also associated with stronger beta desynchronization. In contrast to older adults, young adults show increased activity in areas implicated in executive control of attention. Activity in these areas does not correlate with either vividness or accuracy scores. The converging fMRI, MEG, and behavioural results yield evidence of age-related compensation in areas involved in bottom-up processing of somatosensory information, and weaker top-down control of attention. Our findings have important implications for the utilization of MI as a rehabilitative strategy in older adults.

Disclosures: H. Burianova: None. **L. Marstaller:** None. **P. Sowman:** None. **A. Rich:** None. **M. Williams:** None. **B. Johnson:** None. **G. Savage:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.19/SS2

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 AG034613

NIH P50 AG016573

Title: Stability of age-related deficits in the Mnemonic Similarity Task across task variations

Authors: S. M. STARK[1], R. STEVENSON[1], *C. E. STARK[2];
[1]Neurobio. & Behavior, [2]Univ. CA, Irvine, Irvine, CA

Abstract: Previously, our lab developed a task that is sensitive to age-related declines in mnemonic discrimination utilizing a recognition memory paradigm consisting of repeated items, similar lures, and novel foils (Stark et al, 2013 Neuropsychologia). In this Mnemonic Similarity Task, we reported a decline across the aging spectrum for identifying lures as "similar", with no corresponding decrease in recognition for identifying repeat items as "old". We interpreted these results as consistent with deficits in pattern separation that may result from age-related changes in the dentate gyrus subfield of the hippocampus. Previous studies from our lab have reported a correlation with this mnemonic discrimination deficit and perforant path integrity (the connection between the entorhinal cortex and the dentate gyrus) as well as age-related changes in functional MRI activity in the CA3/DG (Yassa et al., 2011 PNAS). Here, we addressed the possibility that other non-mnemonic cognitive deficits may be contributing to this effect. Perhaps older adults are more "efficient" learners, only encoding the gist and not the details that are subsequently tested. Therefore, we instructed participants during the study task that they would later be tested on these images using an old, similar, new test. Despite these overt instructions to study details, we replicated the age-related deficit in mnemonic discrimination and no age-related change in recognition of repeated items. Next, we examined if limits in the processing speed in older adults might bias them towards using the old response instead of the similar response. Again, using a self-paced version of the task, we replicated the age-related deficit in mnemonic discrimination. We adapted a continuous recognition design to evaluate the effect of lag and the role of proactive interference on later mnemonic discrimination. While there was an effect of lag (number of intervening items between the first presentation and the subsequent lure or repeat), it was consistent across young and older adults, replicating the age-related deficit in mnemonic discrimination. Finally, we evaluated the role of the decision-threshold by modifying

the task to an old/new response only in order to calculate a d-prime measure of recognition memory performance. Consistent with our predictions, older adults shift in d-prime scores indicate a that lure representations are closer to target representations than in young adults. Together, these findings demonstrate the robust nature of this age-related deficit in mnemonic discrimination as measured by the mnemonic similarity task, despite variations in task design.

Disclosures: S.M. Stark: None. **C.E. Stark:** None. **R. Stevenson:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.20/SS3

Topic: F.01. Human Cognition and Behavior

Support: Medical Practice Plan American University of Beirut

Title: Vitamin D level, inflammatory pattern and cognitive performance in adults and older adults

Authors: *H. J. DARWISH;
Nursing, American Univ. of Beirut, Beirut, Lebanon

Abstract: The incidence of Vitamin D deficiency or insufficiency is prevalent in most of the countries. In Lebanon, low vitamin D level is prevalent in older adults; yet, vitamin D effects on age-related cognitive performance have not been fully explored clinically. Thus, the current study aimed to investigate the vitamin D level and cognitive function of adults (> 30 years) and older adults (> 60 years), and to examine in collected blood samples the following cytokine levels: IL 1ra, IL1β, IL6 and IL10 using the 'BioRad' multiplex assay kit. This is a cross sectional descriptive study on 254 adults and older adults recruited from the American University of Beirut-Medical Center Outpatient clinics and the major Elderly Nursing Homes in the greater Beirut and outside of Beirut over a period of 2 years (2010-2012). Health history using the brief risk factor surveillance system (CDC, 2009) was collected. Blood samples collected and Serum 25-OHD measured in the core laboratory at the American University of Beirut using DiaSorin RIA (Diasorin, Incstar, Sallugia, Italy). Health history using the brief risk factor surveillance system (CDC, 2009), and lifestyle such as hours in the sun, exercise, smoking, smoking, sunscreen, work, vitamin D intake, multivitamins, Calcium, veiling and years of education were collected. Subjects were screened for anxiety and depression using Hopkins

Symptoms Checklist (HSCL-25)-Arabic . Cognitive testing was done using the Montreal Cognitive Assessment (MoCA) tool-Arabic, Rey Complex Figure Test and Recognition Trial Test (ROCF) and Symbol Digit Modalities (SDM). The results showed that vitamin D was a significant predictor of performance on ROCF Immediate Recall (X2=27.9283, DF= 7, p=0.0002) and Delayed Recall (X2=23.6718, DF= 7, p=0.0013) in older adults. Years of education were also a significant predictor of performance on MoCA, ROCF Immediate and delayed recall, recognition and SDM in older adults. Increased Vitamin D abnormality among Lebanese adults and older adults was found. Cognitive function and vitamin D level were positively correlated in older adults and low level of vitamin D associated with greater risk of cognitive impairment in older adults. The analysis of the inflammatory and anti-inflammatory cytokine is ongoing. In conclusion, vitamin D level seems to be significant predictor of cognitive performance in older adults.

Disclosures: H.J. Darwish: None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.21/SS4

Topic: F.01. Human Cognition and Behavior

Support: NIA/NIH Grant R01 AG028466 (SDM)

NIA/NIH Intramural Research Program

Title: The effects of long-term cortisol exposure on virtual spatial navigation in middle-aged and older adults

Authors: *U. SAELZLER[1], S. RESNICK[2], S. MOFFAT[1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Natl. Inst. on Aging, Baltimore, MD

Abstract: Normal cognitive aging is associated with self-reported and empirically observed losses in the ability to successfully navigate through unfamiliar environments. In addition, there are large individual differences in navigational skill across all ages and it has been hypothesized that variation in cortisol exposure may be one factor that affects cognitive and neural outcomes in older animals and humans. Numerous behavioral and neuroimaging studies posit that spatial navigation depends, in part, on the hippocampus and pre-frontal cortex and both of these regions

contain high densities of corticosteroid receptors, making these regions particularly sensitive to the potential effects of cortisol. The objective of the present study was to determine whether longitudinal measures of cortisol predict middle-aged and older adult performance on two virtual environment (VE) spatial navigation tasks. Participants were recruited from the Baltimore Longitudinal Study of Aging (BLSA) to complete one of two VE navigation tasks in addition to a battery of neuropsychological tests administered during biannual visits. One hundred fifty-seven BLSA participants (mean age = 67.8 years) received both VE navigation testing and had one or more measures of 24 h urinary free cortisol (UFC) at the time of, or prior to, the time of the administration of the VE tasks. The VE tasks consisted of a virtual route learning task (N = 83; mean age = 67.5) and virtual place learning task (N = 74; mean age = 68.2). Participants had a mean of three (range 1- 7) urine samples over an average interval of six (range 0 - 22.4) years. Cortisol variables in the present study included the within-individual mean of the multiple UFC measures (UFC) and the rate of change in UFC over time (slope). Results indicated that elevated UFC predicted poorer navigational performance, although chronically depressed UFC was also associated with impaired performance on the route learning task. There was no association between UFC slope and navigation performance on either task. These results provide evidence that chronically elevated cortisol may be detrimental to navigation performance. Results will be discussed within a framework of corticosteroid effects on hippocampal and pre-frontal cortex anatomy and function.

Disclosures: U. Saelzler: None. **S. Resnick:** None. **S. Moffat:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.22/SS5

Topic: F.01. Human Cognition and Behavior

Support: NIH grant AG038465

NIH grant AG026158

Title: White matter integrity mediates age-related effects on cognitive abilities

Authors: *Y. GAZES, J. STEFFENER, Q. R. RAZLIGHI, D. J. BARULLI, Y. STERN; Taub Inst., Columbia Univ., New York, NY

Abstract: Age-related changes in cognitive abilities were related to changes in the integrity of 18 white matter tracts in an exploratory study. Four cognitive abilities were examined: processing speed, memory, vocabulary, and reasoning, each of which was derived as the mean of z-scores for three different tasks. Processing speed ability was approximated by performance on Digit Symbol, Letter Comparison, and Pattern Comparison. Memory ability was approximated by Logical Memory, List Learning, and Word Order. Vocabulary ability was measured by Synonym, Antonym, and Picture naming. Lastly, reasoning ability was measured by performance on Matrix Reasoning, Paper folding, and Letter Set. White matter integrity was calculated for 18 white matter tracts using Tracts Constrained by Underlying Anatomy (TRACULA; FreeSurfer 5.2). There were 158 participants, ranged 20 to 78 years old, in the analysis with one participant excluded (n=157) for analyses involving Processing Speed and Reasoning due to missing data. After determining that age was significantly correlated with all four abilities, mediation analysis was performed using Hayes' PROCESS macro in SPSS (v21). The indirect effect of age on cognitive ability through white matter integrity was examined one tract at a time for each of the four cognitive abilities. The significance of a medication is indicated by the exclusion of zero in the 95% confidence interval for the indirect effect. For Processing Speed, posterior corpus callosum was a significant mediator (Indirect effect 95% CI: 0.0003 to 0.0039). For Memory, the right inferior longitudinal fasciculus was a significant mediator (Indirect effect 95% CI: -0.0052 to -0.0001). And for Reasoning, the left inferior longitudinal fasciculus was a significant mediator (Indirect effect 95% CI: -0.0075 to -0.0001). None of the white matter tracts mediated the age effect on the Vocabulary ability. While pairwise relationships among age, cognition, and white matter integrity have been shown in numerous studies, our study provided evidence that age-related changes in processing speed, memory, and reasoning can be explained by age-related changes in white matter integrity of specific tracts. Therefore, the changes in cognitive abilities that were found to decline with age were attributable to decline in white matter integrity. In contrast, vocabulary, an ability that is maintained throughout aging, was not mediated by changes in any of the tracts.

Disclosures: Y. Gazes: None. **J. Steffener:** None. **Q.R. Razlighi:** None. **D.J. Barulli:** None. **Y. Stern:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.23/SS6

Topic: F.01. Human Cognition and Behavior

Support: Arizona Alzheimer's Consortium

Evelyn F. McKnight Brain Institute

Title: Age-related declines in a hippocampal-mediated visual associative memory task: Integration or segmentation?

Authors: *M. B. MEMEL, L. RYAN;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Older adults demonstrate an age-related decline in associative memory – the ability to bind and remember two entities as a related unit – which has been shown to be hippocampally dependent (Meltzer and Constable, 2005). Age-related declines in associative memory have been observed for object-color pairs (Chalfonte & Johnson, 1996), word pairs (Castel & Craik, 2003), and picture pairs (Naveh-Benjamin, Hussain, Guez, & Bar-On, 2003). Naveh Benjamin (2000) attributes this decline to a difficulty in binding the components of an item during encoding. Additionally, older adults exhibit "hyper-binding" during verbal associative memory (Campbell et al., 2013), whereby older adults are more likely to falsely recombine words that were presented close together in the study list as opposed to far apart, suggesting an impairment in item segmentation. In order to further assess older adults' ability to engage in integrative binding of object-scene pairs and pattern separation that segments an item from its neighbors, we presented two types of visual stimuli in a single experiment. Common household objects were either visually integrated in semantically-related backgrounds or distinctly separated from the background by a bold, red outline. First, we hypothesized an improvement in older adult's performance with visually integrated object-scenes as compared to the non-integrated object-scenes, as visual integration may aid binding processes. Second, we hypothesized that older adults would be more susceptible to near re-pairings than middle or far re-pairings (separated by 1, 12, or 24 pairs respectively), as a result of poor pattern separation. The results demonstrate that both older and younger adults recognize previously viewed object-scene pairs similarly, regardless of the level of visual integration. However, older adults are more likely to incorrectly recognize recombined pairs as previously viewed, compared to younger adults. Interestingly, contrary to Campbell et al. (2013), younger adults are *more* susceptible to near recombinations as compared to middle or far, whereas older adults show no difference across conditions. These findings suggest that older adults engage hippocampally-mediated intra-item binding to the same degree as younger adults, even when the stimuli are not visually integrated. However, they are less likely than younger adults to engage in pattern separation processes that segment one pair from another in the list.

Disclosures: M.B. Memel: None. **L. Ryan:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.24/SS7

Topic: F.01. Human Cognition and Behavior

Support: SENACYT

Melo Brain Project

Title: Sociodemographic and health-related factors associated with cognitive function in the oldest old in Panama

Authors: *G. B. BRITTON[1], A. E. VILLARREAL[1], S. A. GRAJALES[1], V. VASQUEZ[2], A. MONTALVAN[2], .-. PANAMA AGING RESEARCH INITIATIVE[3];
[1]INDICASAT AIP, Panama, Panama; [2]Psychiatry, Social Security Hosp., Panama, Panama; [3]PARI, Panama, Panama

Abstract: Low- and middle-income countries (LMICs) are experiencing rapid population aging, yet current data about cognitive function in developing regions is limited. Estimates for Panama indicate that the number of aged people over 60 years will increase from 7% in 2012 to 17% by 2050. The Panama Aging Research Initiative (PARI) seeks to address the challenges of an aging population by building capacity in aging research in Panama. Here we report the factors associated with cognitive function in study participants aged 80+ years. The Mini-Mental State Examination (MMSE) was used to assess cognitive status, with a cut-off score of ≥24 considered cognitively normal. Only participants who completed the MMSE (N=144; 71.3% of persons 80+ years of age) were included in the analysis. Of these, 47.9% (N=69) had normal cognition and 52.1% (N=75) were cognitively impaired. Cognitively normal adults were significantly younger (mean=84.6 years) than cognitively impaired adults (mean=86.8 years). Both groups reported positive social support and did not differ in number of depressive symptoms. Results of multivariate analyses showed that low education, low body mass index, physical inactivity, impairments in activities of daily living and lower grip strength were associated with a higher risk of cognitive impairment. Roughly half of the oldest old in our sample had normal cognitive function. Importantly, less than 8% reported poor social or family support and 12.9% (N=18) met Geriatric Depression Scale criteria for depression. Some factors associated with cognitive impairment, such as physical inactivity, are modifiable behaviors, suggesting that increasing physical activity could help decrease the risk of cognitive impairment. Hispanics are notably underrepresented in U.S. aging studies, and moreover, are usually classified as a single ethnic

group by researchers although they represent a wide variety of racial and ethnic groups. PARI investigators are currently conducting longitudinal studies that may provide novel insights into successful cognitive aging in this under-served population.

Disclosures: G.B. Britton: None. **A.E. Villarreal:** None. **S.A. Grajales:** None. **V. Vasquez:** None. **A. Montalvan:** None. **-. Panama Aging Research Initiative:** None.

Poster

839. Human Cognition I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 839.25/SS8

Topic: F.01. Human Cognition and Behavior

Support: Intramural Research Program of the NIH, National Institute on Aging

Title: Reliability of diffusion tensor imaging measures within and across scanners

Authors: *C. E. GONZALEZ[1], V. K. VENKATRAMAN[1], B. A. LANDMAN[2], J. O. GOH[3], S. M. RESNICK[1];
[1]Natl. Inst. On Aging, Natl. Inst. O, Baltimore, MD; [2]Electrical Engin., Vanderbilt Univ., Brentwood, TN; [3]Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Diffusion tensor imaging (DTI) measures are used as imaging markers to show individual differences in relation to behavioral measures (Xu et al 2011). However, the ability to detect reliable associations is limited by the reliability of the diffusion measures. Several studies have examined reliability and reproducibility of diffusion measures within and across scanners with mixed results (Fox et al 2012; Pagani et al 2010). We use DTI MRI scans from the Baltimore Longitudinal Study of Aging to examine the within-subject reliability of FA and MD for white and gray matter regions within and across field strengths (between-scan intervals less than 2.25 years). All participants were cognitively normal and the data was acquired on 1.5T Intera and 3T Achieva scanners. For each between-scan comparison, the numbers of subjects (mean age and between-scan interval) were as follows: 1.5T-1.5T n=16 (82.1 ± 5.7 years, 1.2 years), 1.5T-3T n=29 (78.9 ± 6.7 years, 1.7 years) and 3T-3T n=109 (78.1 ± 8.5 years, 1.7 years). DTI processing followed standard practice for tensor fitting and quality assessment (Lauzon et al 2013). BrainColor labels for gray matter regions and EVE labels for white matter regions provided regional measures of MD and FA. We calculated the Pearson correlation coefficient, intra-class coefficient adjusted for age and interval, and coefficient of variance (COV) for each

of the between-scanner comparisons. These indices provided lower-bound measures of reliability given the varying times between scans. The results showed that cross-scanner reliability is generally lower than within-scanner reliability. Within 1.5T, MD showed better COV and ICC (R= 0.847 for hippocampus) compared to FA for the gray matter regions and no obvious gray/white matter distinction in MD correlation levels. Uncinate fasciculus showed higher COV (MD = 8.158, FA = 13.572) for FA and MD than other regions. Across field strengths, FA showed higher COV for gray matter consistently and MD was reliable in certain regions such as right hippocampus (R=0.834; COV=4.68) and was poor in amygdala (R= 0.5; COV=16.98). The across field strengths reliability measures in white matter regions showed consistently lower values compared to within scanner reliability measures using 1.5T; it had higher COV in uncinate and inferior fronto-occipital fasciculus for FA and MD.Within 3T, the reliability measures were improved compared to 1.5T. While some regions had consistently poor reliability, such as uncinate fasciculus, the FA and MD values are generally reliable. However, any investigation of diffusion measures must account for the variation in reliability across the different ROIs and field strengths.

Disclosures: C.E. Gonzalez: None. **V.K. Venkatraman:** None. **B.A. Landman:** None. **J.O. Goh:** None. **S.M. Resnick:** None.

Poster

840. Human Cognition II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 840.01/SS9

Topic: F.01. Human Cognition and Behavior

Support: Volkswagen Foundation: II 83/177

Title: Don’t lose your brain at work - work task mobility is associated with greater brain volume in frontal and striatal regions

Authors: *B. GODDE[1], J. OLTMANNS[1], U. M. STAUDINGER[2];
[1]Jacobs Ctr. Lifelong Learning, Jacobs Univ., Bremen, Germany; [2]Columbia Aging Ctr., Columbia Univ., New York, NY

Abstract: Despite age-related declines in some facets, cognitive functioning retains the potential for enhancement throughout the lifespan. Characteristics of the work context such as job complexity have been positively related to adult cognitive functioning (Schooler, 2007) and

it has been assumed that particularly the cognitive challenge in confrontation with unknown problems and novel situations facilitates positive plasticity in adult development (Bowen, Noack & Staudinger, 2011; Lövdén, Bäckman, Lindenberger, Schaefer & Schmiedek, 2010). To date, the (neurophysiological and neuropsychological) mechanisms underlying these associations are not well understood. We examined the effect of repeated work-related task changes (=work task mobility; WTM) on cognitive flexibility and brain structure in adults across the working life span. To control for the influence of education and SES, we investigated the effect of repeated WTM at a low to medium level of education. Of 3.500 assembly line workers from a production company in northern Germany who had been full-time employed with that company over the last 16 years, 179 persons returned a screening questionnaire. This allowed us to identify 10 (n=20) pairs of participants who differed in WTM (high/low) but were optimally matched for age, sex, job complexity as well as academic performance, openness to new experience and leisure time activity in young adulthood. In order to investigate long-term effects of WTM on a structural brain level, we collected anatomical MR images and used voxel-based-morphometry (VBM) to assess cerebral volume differences between high and low mobile participants. First results revealed that high as compared to low WTM was significantly related to more volume in prefrontal and striatal regions. Two areas that are typically related to age-related cognitive decline but seem to be particularly important when it comes to cognitive flexibility and learning. Within the limitations of the matching process, these initial results highlight the importance of cumulative effects of work on brain aging. Referernces Bowen, C. E., Noack, C. M. G., & Staudinger, U. M. (2010). Aging in the work context. In K. W. Schaie & S. Willis (Eds.), Handbook of the psychology of aging (7th ed., pp. 263-277). San Diego, CA: Elsevier Academic Press. Lövdén, M., Backman, L., Lindenberger, U., Schaefer, S., & Schmiedek, F. (2010). A theoretical framework for the study of adult cognitive plasticity. Psychological Bulletin, 136, 659-676. Schooler, C. (2007) Use it_and keep it, longer, probably: A reply to Salthouse (2006). Perspectives on Psychological Science, 2, 24-29.

Disclosures: **B. Godde:** None. **J. Oltmanns:** None. **U.M. Staudinger:** None.

Poster

840. Human Cognition II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 840.02/SS10

Topic: F.01. Human Cognition and Behavior

Title: Human cognition research on a large scale: The human cognition project

Authors: *K. KERLAN, F. FARZIN, D. A. STERNBERG, K. KATOVICH, N. NG, C. SIMONE, A. KALUSZKA, J. L. HARDY, M. SCANLON;
Lumos Labs, Inc., San Francisco, CA

Abstract: Our growing understanding of human cognition has been impeded by conventional research methods involving many small-scale studies with local convenience samples at individual university laboratories utilizing incomparable task designs. These methods make cognition research slow, costly and difficult to replicate. The Human Cognition Project (HCP) is a large-scale, novel, online open science project aimed to complement traditional laboratory-based experimental approaches used in the cognitive sciences. Inspired by Lumosity's web-based cognitive training platform that includes a suite of games, assessments, surveys, and tracking metrics, HCP advances studies of cognitive training by supporting world-wide collaborations and offering researchers open access to the largest database of human cognitive performance, with data from over 60 million individuals to date. HCP is guided by the hypothesis that bringing together a broad network of academic scientists and clinicians will accelerate investigations of cognitive functions such as processing speed, working memory, visual attention, and flexibility, thereby advancing the field of human cognition. Since the project formally began in 2011, HCP has resulted in seven peer-reviewed publications demonstrating the efficacy of Lumosity training. Ongoing studies include investigations of population trends in cognitive changes across the lifespan, neuroplasticity associated with targeted cognitive processes, and effectiveness of cognitive training as a neurotherapeutic tool for individuals affected by mild cognitive impairment, Multiple Sclerosis, stroke, ADHD, cancer, and TBI. Here we present an overview of the HCP science model as well as several primary findings from published studies that have resulted from the project.

Disclosures: **K. Kerlan:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **F. Farzin:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **D.A. Sternberg:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **K. Katovich:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **N. Ng:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **C. Simone:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **A. Kaluszka:** A. Employment/Salary (full or part-time):; Lumos Labs,

Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **J.L. Hardy:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **M. Scanlon:** A. Employment/Salary (full or part-time):; Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc..

Poster

840. Human Cognition II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 840.03/SS11

Topic: F.01. Human Cognition and Behavior

Title: The brain's compensatory response to cognitive fatigue

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Abstract: Prolonged performance of a demanding cognitive task induces cognitive fatigue. Past studies have noted that cognitive fatigue and cognitive aging share common features, including response slowing, increase of response variability, increase of low frequency oscillations, and decline of cognitive control. These similarities raise the intriguing possibility that some aspects of the cognitive aging process could be studied with a cognitive fatigue model. In cognitive aging, it is well-established that the brain develops compensatory mechanisms to offset age-induced impairments. Whether or not the brain also employs such a mechanism during the development of cognitive fatigue remains unknown. To address this question, 16 healthy young adults were recruited to perform a 3-hour continuous cued color-word Stroop task while brain activity was measured using high-density electroencephalography (EEG). The event-related-potential (ERP) technique was applied to study the time-on-task effects in different brain regions. Dividing the 3-hour period in 40-minute periods, we observed that the midline frontal and parietal ERP amplitude within 250-450 ms relative to cue onset linearly declined over the 3-hour performance period, whereas the anterior frontal ERP amplitude within 700-1100 ms relative to cue onset demonstrated a time-on-task profile of an inverted-U shape, reaching its maximum during the period between 60 and 100 minutes into the task. To test whether this increased anterior frontal ERP reflected the brain's compensatory response to fatigue induced impairments,

we analyzed the relationship between the ERP amplitude and behavioral performance, and found that larger anterior frontal ERP amplitude was associated with faster reaction time only for the time-on-task period between 60 and 100 minutes. No such relationship was found for the early and later periods of task performance. These findings suggest that the anterior frontal regions, which are not part of the primary task-related network, are recruited to compensate for the cognitive-fatigue-induced impairments in the primary task-related network, and this compensatory mechanism terminates as cognitive fatigue worsens. Our study is the first to demonstrate that cognitive compensation is engaged to cope with fatigue induced cognitive impairments and this engagement is transient over the course of 3 hours.

Disclosures: C. Wang: None. **A. Trongnetrpunya:** None. **B.M. Kluger:** None. **M. Ding:** None.

Poster

840. Human Cognition II

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Topic: F.01. Human Cognition and Behavior

Support: NIH 2R01 AG021476

NIH UL1TR000445

NIH M01-00109

Title: Estrogen improves hippocampally-mediated cognition in women who report postmenopausal cognitive impairment

Authors: *P. A. NEWHOUSE[1], C. VALIQUETTE[1], K. ALBERT[1], R. ASTUR[3], E. EISENBERG[2], B. MCDONALD[4], M. NAYLOR[5], A. SAYKIN[4], J. DUMAS[5]; [1]Vanderbilt Psychiatric Hosp., [2]Obstetrics and Gynecology, Vanderbilt Univ. Sch. of Med., Nashville, TN; [3]Psychology, Univ. of Connecticut, Storrs, CT; [4]Indiana Univ. Sch. of Med., Indianapolis, IN; [5]Psychiatry, Univ. of Vermont, Burlington, VT

Abstract: Background: Epidemiologic studies suggest that exposure to estrogen (E2) in the early years after menopause is associated with reduced risk of being diagnosed with dementia/Alzheimer's disease in later life. However, interventional studies such as the Women's Health Initiative show that administration of estrogen/progestins non-selectively to older

postmenopausal women may increase the risk of dementia. Subjective cognitive complaints in later life are associated with an increased risk of dementia, but the question of whether women who report cognitive problems might benefit from E2 supplementation in the early years after menopause (during the "critical period") has not been answered. We decided to examine the effects of E2 on cognitive functioning in a group of women who report significant cognitive changes after menopause. Methods: Forty two normal early postmenopausal women were cognitively and behaviorally screened and classified as cognitive complainers (CC; n = 21, Age: 56.3 ± 2.9) if they endorsed more than 20% of cognitive symptom items in an extensive self-report battery validated in a study of subjective cognitive impairment, or non-complainers (NC; n = 21, Age: 55.5 ± 3.1) otherwise. Both groups exhibited normal psychometric performance for age. Subjects were scanned (structural and fMRI), cognitively tested at baseline, and then administered 1 mg of oral 17-β estradiol or placebo daily for 3 months. Follow-up scanning and testing then took place, followed by anti-cholinergic drug challenges (reported elsewhere). Subjects were tested with the Selective Reminding Task (SRT) for verbal episodic memory, and spatial navigation utilizing the computerized Virtual Morris Water Maze (VMWM) task. Results: On the SRT, CC women's total immediate recall was significantly improved after E2 treatment compared to NC women (p = .018), whose total recall declined after treatment. Recall failure and recall consistency were unchanged. The CC women also showed significantly improved long-term verbal recall after E2 treatment, while NC women did not (p = .028). In the VMWM task, E2 treatment improved platform latency performance during the learning phase in the CC group (p = .025) compared to the NC group. Conclusions: This study provides evidence that E2 may enhance hippocampally-mediated cognitive performance in women who note postmenopausal changes in cognition but not in women without cognitive complaints. E2 may thus have promise for maintenance/improvement of cognitive functioning after menopause in a subgroup of potentially higher-risk women.

Disclosures: P.A. Newhouse: None. **C. Valiquette:** None. **K. Albert:** None. **R. Astur:** None. **E. Eisenberg:** None. **B. McDonald:** None. **M. Naylor:** None. **A. Saykin:** None. **J. Dumas:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIA RO1 AG021476

NIA K01 AG030380

UL1TR000445

M01-00109

Title: Estrogen improved working memory performance for postmenopausal women with no subjective cognitive complaints

Authors: *J. A. DUMAS[1], C. VALIQUETTE[2], K. ALBERT[2], B. MCDONALD[4], E. EISENBERG[3], M. NAYLOR[1], A. SAYKIN[4], P. NEWHOUSE[2];
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Abstract: Older adults who report subjective cognitive complaints but perform normally on neuropsychological tests are at increased risk for pathological aging. We have previously shown that middle-aged postmenopausal women who reported subjective cognitive complaints had differences in brain functioning compared to women with no complaints (Dumas et al. 2013). In the current study we examined the effects of 3 months of estrogen treatment on cognition in these women. In addition we examined the ability of estrogen to reverse the temporary cognitive impairment from anticholinergic medications to examine the neurochemistry underlying estrogen's effects on cognition. Thirty-five early postmenopausal women were classified as cognitive complainers (n=19, aged 56.3 ± 2.9 years) if they endorsed more than 20% of the items in an extensive self-report battery (Saykin et al. 2006) or non-complainers (n=16, aged 55.5 ± 3.1 years). Participants were administered 1 mg oral 17-β estradiol or placebo for 3 months. After the hormone treatment phase, women participated in 4 anticholinergic medication challenge sessions. The medications included 20 mg of the oral nicotinic antagonist mecamylamine (MECA), 2.5 µg/kg IV of the muscarinic antagonist scopolamine (SCOP), a combined dose of 10 mg MECA and 2.5 µg/kg SCOP, and matching placebos. Women performed an extensive cognitive battery and we present data from the sensitivity measure from the Nback test of working memory. The overall model of hormone treatment, complainer status, cholinergic drug, and working memory load showed the expected main effects of working memory load ($F(3,93)=143.62$, $p<.0001$) and challenge drug ($F(3,93)=7.94$, $p<.0001$). Sensitivity decreased as the working memory load increased and sensitivity decreased for the challenge sessions compared to placebo. In addition, there was an interaction of hormone treatment and complainer status ($F(1,31)=5.36$, $p=.02$) that showed 3 months of estradiol improved working memory performance for the women with no cognitive complaints and impaired performance for women with cognitive complaints. These data suggest that working memory as measured by the Nback was not sensitive to anticholinergic medication but was sensitive to estradiol treatment and the presence of subjective cognitive complaints. This data pattern is in direct contrast to our data in

the same sample of women presented at this meeting (Newhouse et al. 2014, SFN) showing that estradiol improved episodic memory performance for women with cognitive complaints. Thus, estrogen treatment appears to affect working memory and episodic memory differently in women with and without cognitive complaints.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIA RO1 AG021476

NIA K01 AG030380

UL1TR000445

M01-00109

Title: Estrogen treatment reduces brain functional connectivity in post-menopausal women

Authors: *J. N. VEGA[1], L. ZURKOVSKY[1], B. D. BOYD[1], J. A. DUMAS[2], N. D. WOODWARD[1], M. NAYLOR[2], E. EISENBERG[1], P. NEWHOUSE[1];
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Abstract: Background: Loss of estrogen during the postmenopausal period in women can produce measurable and significant impairment of performance on certain cognitive tasks. It has been suggested that administration of estradiol may enhance or preserve certain types of cognitive functioning in post-menopausal women (Sherwin, 2003, *Endo Rev, 24*,133-51). We have shown that increased functional connectivity of the frontal cortex and decreased functional connectivity of the medial temporal lobe is associated with higher rates of subjective memory complaints after menopause (Zurkovsky et al., SfN 2013, 666.17). These data suggest that the etiology of changes in post-menopausal women may be related to changes in brain connectivity. Methods: To explore how estrogen treatment in post-menopausal women affects connectivity patterns within specific brain networks, this study examined functional connectivity during

resting-state fMRI in 23 healthy post-menopausal women (mean age 55.74, SD ± 2.88), after 3 months of treatment with 1 mg of oral 17-β estradiol (n=11) or placebo (n=12). Seed regions were chosen for Dorsal Attention Network (DAN), Frontoparietal/Executive control network, and Default Mode Network (DMN) based on prior literature (Vincent et al., 2008, *J Neurophysiol, 100,* 3328-42; Woodward et al., 2011, *Schizo Res, 130*, 86-93; Seeley et al., 2007, *J Neurosci, 27*, 2349-56). Differences in connectivity between estradiol and placebo treated groups were evaluated using independent samples *t*-tests. Group contrasts for a given seed region were restricted to the appropriate resting-state network mask (Yeo et al., 2011, *J Neurophysiol, 106,* 1125-65). Results: Greater connectivity was observed in the placebo treated group between bilateral SPL seed regions and regions in DAN (p=0.01, k=38) and between a posterior cingulate (PCC) seed region and regions in DMN (p=0.01, k=46) compared to the estrogen treated group. Specifically, differences were observed in bilateral superior parietal lobes (SPLs), left inferior parietal lobe (IPL) for DAN, and in bilateral middle temporal gyri and superior frontal gyrus for DMN. Conclusions: Results indicate that estrogen treatment in post-menopausal women reduces functional connectivity within DAN and DMN. These data add to findings of increased baseline connectivity in post-menopausal women with high levels of subjective cognitive complaints, and suggest that estrogen benefits cognition is tied to decreased connectivity within DAN and DMN.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH grant AG038465

NIH grant AG026158

Title: Making cognitive latent variables manifest: Distinct neural networks for fluid ability and processing speed

Authors: *C. G. HABECK, J. STEFFENER, D. BARULLI, Y. GAZES, D. SHAKED, Q. RAZLIGHI, Y. STERN;
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Abstract: Cognitive psychologists posit several specific cognitive abilities that are measured with sets of cognitive tasks. Tasks that purportedly tap a specific underlying cognitive ability are strongly correlated with one another while performances on tasks that tap different cognitive abilities are less strongly correlated. For these reasons, latent variables are often considered optimal for describing individual differences in cognitive abilities. Although latent variables cannot be directly observed, we reasoned that all tasks representing a specific latent ability should have a common neural underpinning. We tested this idea in an fMRI study by attempting to demonstrate that tasks representing one ability (i.e., either processing speed or fluid reasoning) had a pattern of neural activation distinct from that for tasks in the other ability. 107 participants between the ages of 20 and 77 were imaged in an fMRI scanner while performing 6 tasks, 3 representing each cognitive ability. Consistent with prior research, performance of these 6 tasks clustered into the two abilities based on their patterns of individual differences in the sense that tasks postulated to represent one ability showed higher similarity across individuals than tasks postulated to represent a different ability. This finding was extended in the current report to the spatial resemblance of the task-related mean activation patterns as the topographic similarity of the mean activation maps for tasks postulated to reflect the same reference ability was higher than for tasks postulated to reflect a different reference ability. These findings suggest that a major reason for differences in the strengths of correlations between various cognitive tasks may be the degree of overlap in the neural structures that are active when the tasks are being performed. In other words, rather than having to postulate a hypothetical construct to account for correlations at behavioral level, they may be a consequence of topographic similarities in the neural activation across different brain regions. Moreover, for any pairing of tasks it holds that the similarity in terms of behavioral performance is strongly associated with the topographic similarity of the underlying neural networks. This latter relationship, while intuitively obvious, to our knowledge, has not been demonstrated previously.

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Poster

840. Human Cognition II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: FCT Grant: SFRH/SINTD /60129/2009

FCT Grant: TDC/SAU-NSC/111814/2009

Title: Brain correlates of indecisiveness and sensitivity to negative outcomes in Obsessive Compulsive Disorder: an fMRI study with a risk-based decision making task

Authors: *P. MORGADO, P. MARQUES, J. M. SOARES, N. SOUSA, J. J. CERQUEIRA; Life and Hlth. Sci. Res. Inst. (ICVS), Univ. of Minho, Braga, Portugal

Abstract: Decision-making processes are affected in obsessive-compulsive disorder (OCD). Previous studies have shown decision-making impairments in tasks with implicit rules, but not in those in which explicit and stable rules are provided. 20 OCD patients and 20 healthy controls, matched for gender, age and educational level were enrolled in this study and performed a risk-based decision-making task in a functional magnetic resonance imaging study. Data revealed that patients with OCD showed higher levels of indecisiveness as assessed by longer times to decide and decreased differential reaction times throughout the experimental paradigm; interestingly, this pattern of altered temporal dynamics in decision-making was not associated with differences in choice preferences between OCD patients and controls. Noticeably, when compared with controls, OCD subjects displayed an inverse pattern of amygdalar activation: on one hand, there was a significant deactivation of the amygdala before high-risk choices and on the other hand, an increased activation of this brain region before low-risk choices. Moreover, in the decision phase of the paradigm there was lower activity on the caudate nucleus in OCD patients. Finally, upon receiving a negative outcome, OCD patients showed an increased activation of (orbito)fronto-striatal regions and the anterior cingulate cortex. These results contribute for the comprehension of decision-making impairments among OCD patients, although more studies are needed to detail the brain circuits involved.

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Poster

840. Human Cognition II

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Program#/Poster#: 840.09/SS17

Topic: F.01. Human Cognition and Behavior

Title: A continuous recognition task paradigm implemented on-line for measurement of memory function

Authors: *J. W. ASHFORD, JR;
Psychiatry & Behavioral Sciences, Stanford, Stanford / VA Aging Clin. Res. Ctr., Redwood City, CA

Abstract: MemTrax is a short cognitive test for memory measurement. It is a "continuous recognition task" (CRT) paradigm, a type of assessment widely used in advanced research on memory mechanisms. CRTs are especially sensitive for early detection of memory problems, such as those caused by dementia and Alzheimer's disease. CRTs are also useful for detecting changes that occur with head injury, altered levels of consciousness, and a variety of other brain illnesses or injuries that cause memory changes. MemTrax provides an efficient on-line assessment tool for measuring memory health as well as attention and recognition reaction time, within a period of less than three minutes. MemTrax users are provided with a set of images on any video screen, which they either look at and remember, or indicate that they recognize a repeated image, by a specific movement response, within the shortest period of time possible. MemTrax is implemented as a test with 25 unique images and 25 repeats (5 of the repeats being second repeats). The pictures are scenes or objects occurring in 5 sets of 5 images (e.g., water scenes, mountains, clothing, vehicles, etc.). True-positives and correct rejections are recorded, as well as reaction time for true positives. In a prior study, the test was administered in an audience setting, allowing audience members 5 seconds to indicate on a sheet of paper whether they had seen a picture before, and testing of over 1,000 individuals indicated an age effect on a transformation of percent correct (d'). In examining 18,435 individuals who provided ages, 21 - 99, took the test for the first time on-line, and performed better than random chance, age only explained 2% of the variance in reaction time and 1.5% of the variance in d'. However, when individual years were averaged, age explained 94% of the variance in reaction time between 21 and 85 years and 80% of the variance in d'. Specific standard deviations for reaction time and d' at each age allows an estimation of performance impairment at any level of specificity chosen. In conclusion, MemTrax is a quick, fun, widely accessible memory assessment tool providing information that can be analyzed to determine whether MemTrax can screen effectively and efficiently for a variety of brain dysfunctions.

Disclosures: J.W. Ashford: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MemTrax, LLC owned by family member.

Poster

840. Human Cognition II

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Topic: F.01. Human Cognition and Behavior

Support: Pilot Research Grant Program, Office of the VP for Research at UT Austin

Chief of the Army – Grant to West Point's Network Science Center

Title: Hippocampal activity mediates the relationship between sleep and memory monitoring accuracy

Authors: *S. M. SHERMAN, J. D. MCNEELY, D. M. SCHNYER;
Psychology, Univ. of Texas At Austin, Austin, TX

Abstract: Previous work suggests that older adults experience parallel declines in sleep, episodic memory, and memory monitoring. These age-related changes appear to be linked such that sleep disruptions contribute to deficits in memory. However, little is known about the neural mechanism underlying the relationship between sleep and memory functioning. The purpose of this study was to investigate whether neural activity during an associative memory task mediates the relationship between sleep and memory monitoring accuracy in elderly adults. Thirty-two healthy older adults between the ages of 60 and 77 years old completed a neuropsychological assessment to ensure they were functioning within the normal range for their age. All participants were in good general health and had no sleep problems. Participants wore an actigraph continuously for 10 days to record sleep patterns under normal environmental conditions and filled out online daily sleep logs. They returned to the lab and underwent structural and functional magnetic resonance imaging (fMRI) while performing a new associative learning (AL) task. The memory task involved learning a list of unrelated word pairs and then being tested on whether newly presented pairs were previously seen together. Participants made a confidence judgment after each memory response. Behavioral results from the associative memory task demonstrated that average daily sleep across the 10 days was positively associated with memory monitoring accuracy, as measured by the gamma statistic ($B = .41$, $t(30)= 2.44$, $p = .02$). This relationship was not explained by sleep the night before testing. The fMRI analysis examined the BOLD signal associated with successful associative memory retrieval (correct memory for intact vs. rearranged pairs). Region of interest analyses indicated that left hippocampal activity during successful memory retrieval was positively correlated with monitoring accuracy ($B = .58$, $t(30)= 3.87$, $p = .0005$) and average daily sleep across the 10-day period ($B = .38$, $t(30) = 2.12$, $p = .04$). Additionally, linear regression analyses revealed that sleep no longer predicted memory monitoring when controlling for left hippocampal activity ($B = .23$, $t(29) = 1.46$, $p = .16$, n.s.). A formal mediation analysis demonstrated that the link between sleep and memory monitoring was mediated by left hippocampal activity. These findings indicate that more sleep relates to successful memory retrieval monitoring partially as a result of greater hippocampal activity in older adults.

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Poster

840. Human Cognition II

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Program#/Poster#: 840.11/SS19

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH RO1 MH069942

Stony Brook Research Foundation

Title: Neural correlates of selective visual processing during working memory in individuals with major depressive disorder

Authors: ***T. M. LE**, A. KUJAWA, D. KLEIN, W. DEAN, G. BAYLON, H.-C. LEUNG; Stony Brook Univ., Stony Brook, NY

Abstract: Various forms of working memory deficits have been observed in individuals with major depressive disorder (MDD). However, neuroimaging findings on working memory deficits in MDD remain inconclusive, likely due to differences in task designs, stimulus characteristics, performance variability, and the heterogeneity of samples (e.g., age, medication status, severity, and state of illness) across studies. Since previous research has primarily focused on emotion processing and frontal lobe dysfunctions, it was unclear whether changes to basic face and/or non-face visual processing contributed to the observed downstream differences. The present fMRI study examined brain activity during selective processing and maintenance of visual representations (neural faces and scenes) in medication-free young adults. We used a visual working memory updating paradigm and examined frontal and material-sensitive visual association regions such as the fusiform face area (FFA) and the parahippocampal place area (PPA). It was predicted that the FFA and PPA would show increased activation during selective processing of faces and scenes, respectively, and that this process would be modulated by prefrontal areas. Functional and anatomical images were acquired from 32 subjects (17 MDD, 15 age-matched controls) using a Siemens Trio 3 T system. On each trial, two visual stimuli (a face and a scene) were presented consecutively. After a 2-s delay, a cue stimulus ("face", "scene" or "both") indicated which one or both studied items would be tested on later. After a 9-s delay, a probe stimulus was presented for the participants to judge whether it matched the to-be-remembered item(s). All cues were fully informative. Our data showed that while load effects in

the left middle frontal gyrus varied with the number of relevant items held in memory in healthy controls, the same pattern was not significant in the MDD group. Rumination, a key feature of MDD, was positively correlated with activity in the right FFA during the Remember Scene (Ignore Face) condition across MDD subjects, suggesting that the no-longer-relevant representation persisted with higher levels of rumination. Significantly longer response time in the same condition was observed for the MDD group compared to the controls. These findings were not caused by reduced visual specialization in the MDD group as activity in the FFA and PPA regions, though more variable, was comparable to the control group. Together, our findings not only provide evidence of working memory deficits in medication-free MDD subjects but also demonstrate potential alterations in selective visual representation in this population.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R01MH093535

Title: Anorexia nervosa and body dysmorphic disorder display differences in processing of emotional stimuli

Authors: K. LAWRENCE, T. MOODY, S. KHALSA, M. STROBER, S. BOOKHEIMER, *J. D. FEUSNER;
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Abstract: Anorexia nervosa (AN) and body dysmorphic disorder (BDD) are related disorders that negatively affect body image. Individuals with AN believe they are overweight and take extreme measures to lose weight, while those with BDD are preoccupied with perceived flaws in their appearance (often of their face) and perform repetitive behaviors to check or fix their appearance. Prior research suggests that AN is characterized by high premorbid levels of trait anxiety and those with BDD often experience high degrees of anxiety. However, the neural correlates of anxiety in these disorders are poorly understood. Additionally, despite similarities in phenomenology and high rates of comorbidity between AN and BDD, no previous research

has directly compared anxiety or fear processing between the two. We used functional magnetic resonance imaging to compare neural activity in response to fearful faces in weight-restored individuals with AN (n=26), those with BDD (n=35) and healthy controls (n=37). The three groups did not significantly differ in age, sex, or years of education, and all were unmedicated. Participants passively viewed blocks of fearful and neutral faces. Examining the differences between fearful and neutral faces among groups ($Z>2.0$, corrected cluster significance threshold of $p=0.05$), we found that individuals with AN displayed greater activity to fearful faces in the right frontal pole compared to healthy controls; within group analyses revealed significant right frontal pole activity in AN but not healthy controls. The AN group had increased activation in the precentral and postcentral gyri relative to the BDD group. In comparison to healthy controls, individuals with BDD displayed decreased activity in the insula and increased precuneus activity. Within group analyses suggested these differences were due to relative decreases in insula activity in BDD and decreases in precuneus activity in controls. Results suggest that individuals with AN and those with BDD both show abnormal neural activity for processing of emotional stimuli, although the patterns between them differed. Surprisingly, there was greater limbic activation in the BDD group for neutral compared with fearful faces; this could be related to a previously found tendency in BDD for misinterpretation of neutral faces as angry.

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Poster

840. Human Cognition II

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Program#/Poster#: 840.13/SS21

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: KAKENHI(No.26350874)

Title: A novel method for predicting mental health disorders based on the handwriting features while writing numbers

Authors: *S. SAKURABA, H. KAWAGUCHI;
Dept. of Life Sci., Toyo Univ., Itakura, Japan

Abstract: The number of patients with mental health disorders has recently increased. Because the recurrence of mental health disorders in such patients is high, there is a social need for a

method that predicts the onset of mental health disorders. Therefore, we investigated whether the Uchida-Kraepelin test, one of the tests used to examine responses to stressful situations, could serve as a method for predicting mental health disorders using the handwriting of participants. We analyzed the participants' handwriting using a "digital pen," which can digitize handwriting with a spatial resolution of 0.3 mm and a temporal resolution of 13 ms. During this test, participants were asked to perform two sets of single-digit additions for 15 min each (with a 5-min rest period between each set). In total, 151 students (who were aged 18-19 years in the first year) were recruited for a follow-up cohort study conducted over 4 years (once per year in early April). The participants voluntarily completed the Uchida-Kraepelin test and answered a mental health questionnaire (General Health Questionnaire [GHQ30], 30 items). We analyzed the time intervals between the first and second stroke of a number (4, 5, and 7; mean time interval: t1) and those between the completion of writing a number and initiation of writing the next number (mean time interval: t2). The ratio of the mean time interval (t2/t1) for people with mental health disorders was significantly higher than that for healthy individuals. In addition, a correlation was observed between the t2/t1 ratio and social dysfunction scores on GHQ30 ($p < 0.05$ for the first year; $p < 0.05$ for the third year). Furthermore, the dropout rate of the high-risk group (the group with $t2/t1 \geqq 11$) was ten times higher than that of the low-risk group (the group with $t2/t1 < 11$; $p < 0.01$). These results suggest that it may be possible to predict mental health disorders using the t2/t1 ratio. The protocols used in this study were approved by the Ethics Committee at Toyo University. This work was supported by KAKENHI (No. 26350874).

Disclosures: **S. Sakuraba:** None. **H. Kawaguchi:** None.

Poster

840. Human Cognition II

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NARSAD Young Investigator Award

OCAST Grant HR10-141

NIMH Grant K01MH096175-01

Title: Resting-state fMRI demonstrates that illness severity in anorexia nervosa is associated a lack of differentiation between sensory and fronto-parietal neural networks

Authors: *K. L. KERR[1,2], S. E. MOSEMAN[3,2], S. J. GOTTS[4], J. A. AVERY[2], J. L. DOBSON[2], W. SIMMONS[2];
[1]Univ. of Tulsa, Tulsa, OK; [2]Laureate Inst. for Brain Res., Tulsa, OK; [3]Laureate Psychiatric Clin. & Hosp., Tulsa, OK; [4]Section on Cognitive Neuropsychology, Lab. of Brain and Cognition, Natl. Inst. of Mental Health, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Anorexia nervosa (AN) is a psychiatric disorder characterized by body image disturbance and restriction of food intake, leading to a significant loss of body mass. Despite having the highest mortality rate of any mental illness, relatively little is known about the specific neural bases of AN, nor whether it is associated with abnormal neural network organization. We therefore examined resting-state fMRI functional connectivity in 21 unmedicated adolescent and young adult females with a diagnosis of restricting-type AN and 21 healthy control females. At the time of scanning, subjects with AN had been weight-restored to a body mass index (BMI) of at least 18. Data-driven approaches for mapping regions exhibiting group differences in functional connectivity identified 15 brain regions where AN subjects exhibited greater functional connectivity than healthy control subjects. Interestingly, there were no regions where healthy controls exhibited greater functional connectivity than AN subjects. Using these 15 regions, functional connectivity correlation matrices were then constructed for each subject. These matrices were then averaged across all subjects and submitted to k-means clustering in order to identify clusters of brain regions exhibiting similar covariance patterns in the correlation matrices. The k-means clustering algorithm identified two clusters within the data, one representing visual and sensory brain regions and the other consisting of a fronto-parietal network. Multidimensional scaling (MDS) applied to the within-group matrices demonstrated congruence with the k-means clustering results. Statistical tests demonstrated that AN subjects exhibited significantly greater connectivity both within and between the two networks. Importantly, group differences in between-network functional connectivity were larger than group differences in within-network connectivity. This finding is important as it demonstrates that AN is associated with a lack of differentiation between sensory and fronto-parietal networks in the brain. The behavioral significance of this finding was revealed by subsequent statistical analyses demonstrating that in AN subjects the average functional connectivity between networks was positively correlated with psychological maladjustment and negatively correlated with lowest BMI during illness. These findings indicate that illness severity in AN is associated with a lack of differentiation between sensory and fronto-parietal networks in the brain.

Disclosures: K.L. Kerr: None. **S.E. Moseman:** None. **S.J. Gotts:** None. **J.A. Avery:** None. **J.L. Dobson:** None. **W. Simmons:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 841.01/SS23

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CIHR

MEDI

Title: Inter-areal theta-gamma coupling across macaque prefrontal cortices increases with attention and predicts successful stimulus selection

Authors: *B. VOLOH, T. WOMELSDORF;
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Abstract: Selective visual attention is subserved by cortical oscillations in the gamma band (30-100 Hz)[1], while slower theta oscillations (4-10 Hz) have been implicated in long-range networks involved in stimulus expectancy and the preparation of attentional stimulus selection [2]. It remains an open question whether interactions between these oscillations - i.e. cross frequency coupling (CFC) - have a functional role in prefrontal and anterior cingulate cortices (PFC/ACC), structures responsible for implementing attentional selection and updating top-down task representations in order to guide behavior [3]. We tested this question by analyzing local field potentials recorded simultaneously in the macaque PFC/ACC performing a selective attention task. Successful shifting of attention in this task required the integration of cue information with stimulus feature information at unpredictable time periods during task performance. This allowed us to analyze CFC (quantified as the modulation index, MI [4]) before and after attention cue onset. Across all recorded channel pairs, we found a significant increase in MI after cue onset between the phase of a 7 Hz band and the amplitude of a 40-45 Hz band. Channel pairs with a statistically reliable increase in in theta-gamma coupling (TGC) linked different cortical fields in PFC/ACC. Moreover, channels that coupled on correct trials failed to do so on error trials. On these erroneous trials, high frequency activity aligned to distinct and more variable phases compared to correct trials. These results show that coupling between theta and gamma frequencies indexes successful attention shifts and suggests that the phase of low frequency activity at which local high frequency gamma activities couple carries critical information about successful inter-areal coordination during attention and goal directed

behavior. [1] Womelsdorf, T. et al (2006). Nature. 439:733-6. [2] Phillips, J et al (2013). Cereb. Cortex (epub). [3] Lisman & Jensen (2013) Neuron.77(6):1002-16. [4] Tort, A et al. (2010). J Neurophysiol. 104:1195-1210

Disclosures: B. Voloh: None. **T. Womelsdorf:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

Location: Halls A-C

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CIHR

Deutsche Forschungsgemeinschaft (DFG), Collaborative Research Centre 889 "Cellular Mechanisms of Sensory Processing"

Title: Neural interactions in areas 8a and 46 of the primate brain reflect task difficulty and the allocation of attention

Authors: *T. BACKEN[1,2], S. TREUE[2,3], J. C. MARTINEZ-TRUJILLO[1];
[1]Dept. of Physiol., McGill Univ., Montreal, QC, Canada; [2]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; [3]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany

Abstract: Visual attention filters sensory information by selectively enhancing responses to behaviourally relevant stimuli (targets) while suppressing responses to irrelevant distractors. One area in the primate brain that plays an important role in cognitive control and goal-directed behavior is the dorsolateral prefrontal cortex (dlPFC). We have previously shown that single neurons in dlPFC area 8a can discriminate between targets and distractors through their firing rates (Lennert & Martinez-Trujillo, 2011). However, how neurons interact with one another during attentional tasks remains unclear. Here, we investigated such interactions by computing spike count correlations amongst 525 simultaneously recorded pairs of neurons in the left areas 8a/46 of a macaque monkey. Neuronal activity was recorded using a 96-electrode microarray while the animal performed the following task: While fixating at the center of a screen, the animal was presented with two peripheral stimuli and had to indicate a change in the target

stimulus but ignore similar changes in the other (the distractor stimulus). The target stimulus was determined by the stimulus color. We analyzed interactions between neurons during color cue presentation (target selection) and sustained attention. About one third of the neurons showed a spatial preference for one of the two target locations (ipsi- versus contralateral hemifield) and differences in correlated activity were observed based on these spatial preferences. Correlations depended on task difficulty: spatially selective neurons with the same preference were more positively correlated during easier trials (i.e., comprising of weaker distractors) than more difficult ones (i.e., stronger distractors). Likewise, interactions between pairs of neurons with opposite preferences were more negative for trials with weaker distractors. In general, the spatially selective neurons had significantly higher spike count correlation coefficients than the non-selective neurons. Interestingly, the magnitude of correlations varied across task periods (allocation of attention vs. maintenance of it) for neurons prferring contralateral stimuli but not for ipsilateral selective neurons. Our results strongly indicate that neuronal interactions within area 8a/46 of primates play an important role in the allocation of attention to targets in the presence of distractors.

Disclosures: **T. Backen:** None. **S. Treue:** None. **J.C. Martinez-Trujillo:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Program#/Poster#: 841.03/SS25

Topic: F.02. Animal Cognition and Behavior

Title: Reversible visual hemineglect induced by parietal deactivation in the awake behaving cat results in a reduction in theta and gamma oscillations in primary visual cortex

Authors: *W. H. BARNES[1,2], M. C. MUELLER[3,2], R. A. W. GALUSKE[3,2];
[1]Max Planck For Hirnforschung, Frankfurt, Germany; [2]Biol., Technische Univ. Darmstadt, Darmstadt, Germany; [3]Neurophysiol., Max Planck Inst. for Brain Res., Frankfurt, Germany

Abstract: Visual hemineglect in humans not only consists of a neglect of contralateral visual stimuli, but also a neglect of internal representations of visual stimuli. When some neglect patients are asked to describe a visual scene from a particular vantage point, only information on one half of the scene may be recalled. However, if the patient is told to rotate the vantage point by 180 degrees and view the scene from the opposite side, the previously neglected information may be reported, and the previously reported information neglected, pointing to a neglect of

internal representations as well as a neglect of visual stimuli. Using the cat as a neglect model, we induced a reversible visual hemineglect by unilateral deactivation of posterior medial suprasylvian (pMS) sulcus, an area located in parietal cortex. Three cats were trained to perform a visual perimetry task using stationary LED's. The cats were then implanted with cryoloops in pMS sulcus which could reversibly deactivate the tissue on both sides of the sulcus. Neuronal activity was recorded bilaterally in area 18 using chronically implanted 12-channel floating microelectrode arrays. Recording sessions consisted of a baseline period where pMS cortex was active bilaterally and the animals showed no neglect phenomena in a perimetry task. Subsequently, pMS cortex was deactivated unilaterally leading to a profound contralateral visual hemineglect. The analysis of LFP signals recorded under these conditions revealed that unilateral pMS deactivation led to a dramatic loss of oscillatory power in the theta and gamma range for neglected stimuli in the contralateral hemifield, but not for attended stimuli in the ipsilateral hemifield. Loss of high-frequency gamma power for neglected stimuli speaks to the quintessential role gamma oscillations play in attention The loss of theta power might support the attention-to-memory model (Cabeza et al 2008) whereby parietal cortex directs attention to internal goals and representations. Neglect following parietal deactivation could therefore be explained by a failure of internal context-dependent memory search.

Disclosures: **W.H. Barnes:** None. **M.C. Mueller:** None. **R.A.W. Galuske:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: DPZ

CIHR

NSERC

CRC

Title: Feature-based attention modulates spatial summation of responses to two stimuli inside MT neuron's receptive fields

Authors: *J. C. MARTINEZ-TRUJILLO[1], N. MALEK[1], S. TREUE[2,3];
[1]McGill Univ., Montreal, QC, Canada; [2]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; [3]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany

Abstract: Attending to one out of two stimuli presented inside the receptive field (RF) of an extrastriate visual cortical neuron biases the cell's response towards the attended stimulus. Several models have been proposed to predict a neuron's response to pairs of stimuli inside the RF (spatial summation) as well as the response modulation caused by switching attention between the two stimuli. In this study, we further explore this issue by recording, from two awake macaque monkeys, the responses of 77 middle temporal (MT) neurons to two moving random dot patterns (RDPs) inside their RFs. One RDP always moved in the neuron's antipreferred direction, while the other varied from trial to trial and moved in one of 12 directions (tuning pattern), spaced every 30 degrees. 350 milliseconds prior to presentation of the RDP pair, a single moving RDP was presented (the cue) in one of two possible locations, both within the neuron's RF, to indicate which RDP should be attended to. The monkey was required to respond when a direction change occurred in the cued RDP and to ignore changes in the distractor. All direction changes occurred 400 ms after the RDP pair onset. We found that during the first 200 ms of the RDP pair presentation, responses were similar across conditions with the same stimulus configuration, regardless of whether the animal was cued to the antipreferred or tuning pattern. This was considered as the unmodulated response to the pair (Rp). We quantified the attentional modulation as the change in the neuron's responses to antipreferred or tuned stimuli relative to the Rp over the remaining course of the trial. The modulation from Rp was stronger and increased over time when the animals attended to the antipreferred stimulus, while it was much weaker and rather constant over time when the animal attended to the preferred stimulus. The intensity of modulation oscillated between these two observed extremes as the animal attended to the different directions of the tuning pattern in between the antipreferred and preferred directions. Collectively, these results indicate that feature-based attention modulates the rules of spatial summation for two stimuli inside the RF of MT neurons. This may be important for current normalization models of attention.

Disclosures: J.C. Martinez-Trujillo: None. **N. Malek:** None. **S. Treue:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH53667

Title: Unit activity in rat posterior parietal cortex tracks Pearce-Hall attention-for-learning

Authors: *F. SCHIFFINO, V. ZHOU, J. TRAGESER, P. HOLLAND;
Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Within most contemporary learning theories, reinforcement prediction error, the difference between the obtained and expected reinforcer value, critically influences associative learning. In some theories, this prediction error determines the momentary effectiveness of the reinforcer itself, such that the same physical event produces more learning when its presentation is surprising than when it is expected. In other theories, like Pearce & Hall (1980), prediction error enhances attention to potential cues for that reinforcer by adjusting cue-specific attention-for-learning (associability) parameters, biasing the processing of those stimuli so that they more readily enter into new associations. Importantly, in the Pearce-Hall model, associability is determined by the magnitude of these errors, irrespective of direction, which conveys the amount of surprise regarding violated expectancies. Recently, Schiffino, Zhou, & Holland (2014) identified the posterior parietal cortex (PPC) as a site that stores a memory for altered Pearce-Hall associability. Here, we recorded activity of single-units in unilateral rat PPC during a stepwise reversal task designed to identify neurons that tracked associability as opposed to expected reward value. Rats were first trained to hold their noses in an odor port to receive sucrose reward. Then, two light cues, one steady and one flashing, were presented during the hold period to instruct the rat to either enter the left or right liquid cup to receive 2 drops of sucrose. Once rats performed well, the expected reward value was shifted to 3 drops for one cue (Hi) and 1 drop for the other cue (Lo). Unit activity was then recorded across three-block Surprise sessions. In the first block (preShift), the rewards were the same as the end of previous day. In the second block (1st Shift), the reward was shifted for one of the cues (Cue 1) to match the value of the other (Cue 2). In the final block (2nd Shift), the reward of Cue 2 was shifted in the opposite direction and to the preShift value of Cue 1, while the reward for Cue 1 was maintained the same. Thus, Cue 1 signaled a surprising outcome relative to Cue 2 during the 1st Shift block, and Cue 2 signaled a more surprising outcome relative to Cue 1 during the 2nd Shift block. Units tuned to track associability would show no biased activity during the cues in the preShift block, a bias towards Cue 1 in the 1st Shift block, and the same type of bias but towards Cue 2 during the 2nd Shift block. About ¼ of the units that responded to cues by increasing their activity fit those criteria. Evidence for tracking of relative expected reward value was also observed.

Disclosures: **F. Schiffino:** None. **J. Trageser:** None. **V. Zhou:** None. **P. Holland:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust

Title: Glutamatergic control of attentional signals in macaque frontal eye-field

Authors: *C. BRANDT, M. O. DASILVA, A. THIELE;
Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Attention improves perception by affecting different aspects of the neuronal code. It enhances firing rates, it reduces firing rate variability and noise correlations of neurons, and it alters the strength of oscillatory activity. In striate cortex, attention induced rate enhancement requires cholinergic mechanisms (1), while attention induced variance and noise correlation reduction are supported by (glutamatergic) NMDA receptor availability (2). Here we investigate how glutamate affects attentional signals in the frontal eye-field (FEF). Two male macaque monkeys were trained in a covert top-down attention task, where a central color cue indicated on a trail by trial basis where to attend to. The animals had to detect a change of the cued stimulus and ignore changes in un-cued stimuli. They responded by releasing a touch bar to obtain a fluid reward. Attention to the neuron's receptive/movement field significantly increased firing rates ($p<0.05$), and increased response reliability (reduced Fano factors, $p<0.05$). NMDA receptor blockade did not affect attentional rate modulation (assessed by means of ROC and attentional modulation index), but it abolished the attention induced reduction in Fano factors ($p<0.05$). Surprisingly, AMPA/Kainate receptor blockade reduced the attentional rate modulation ($p<0.05$) and it reduced the attention induced Fano-factor reduction ($p<0.05$). Thus, NMDA receptors play a similar role in attentional control in FEF as in primary visual cortex, while AMPA/Kainate receptors (which mostly support bottom up signal processing in striate cortex (2,3)), become involved in the generation of top down signals in FEF. Thus, area, and function specific involvement of neurotransmitters and associated receptors are an important feature of the cortical architecture. 1.Herrero JL, et al. (2008) Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. Nature 454(7208):1110-1114. 2.Herrero JL, Gieselmann MA, Sanayei M, & Thiele A (2013) Attention-induced variance and noise correlation reduction in macaque V1 is mediated by NMDA receptors. Neuron 78(4):729-739. 3.Self MW, Kooijmans RN, Super H, Lamme VA, & Roelfsema PR (2012) Different glutamate receptors convey

feedforward and recurrent processing in macaque V1. Proc Natl Acad Sci U S A 109(27):11031-11036.

Disclosures: **C. Brandt:** None. **M.O. Dasilva:** None. **A. Thiele:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR

NSERC

Title: Instantaneous filtering of visual information by prefrontal cortex neural assemblies

Authors: ***S. TREMBLAY**[1], F. PIEPER[3], A. SACHS[4], J. MARTINEZ-TRUJILLO[2]; [1]Integrated Program in Neurosci., [2]McGill Univ., Montreal, QC, Canada; [3]Inst. for Neuro- & Pathophysiology, Univ. Med. Ctr. Hamburg-Eppendorf (UKE), Hamburg, Germany; [4]Div. of Neurosurgery, Dept. of Surgery,The Ottawa Hosp. Res. Institute, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: In dynamic environments, the brain must rapidly select behaviourally relevant from irrelevant visual information for further cognitive processing. Until now, the existence of this filtering process had only been demonstrated by averaging the activity of individual neurons over series of discontinuous identical experimental trials. In real-life situations, however, the brain must filter sensory information instantaneously based on the simultaneous activity of many neurons. Furthermore, these assemblies of neurons exhibit correlated activities which impact information encoding, an important detail single-unit studies overlook. Here, we chronically implanted multielectrode arrays in area 8A of the prefrontal cortex of non-human primates and showed that the simultaneous activity of assemblies of neurons can be reliably decoded to determine, on a single-trial basis, the location of an attended target among distractors. Additionally, the encoded representation was robust to transient distractors, predictive of behavioural errors, and stable across a timespan of multiple weeks, suggesting potential utility in the implementation of cognitive neural prosthetics.

Disclosures: **S. Tremblay:** None. **F. Pieper:** None. **A. Sachs:** None. **J. Martinez-Trujillo:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR

Title: Comparison of spikes and local field potentials in decoding the focus of visual attention from multi-electrode array recordings in lateral prefrontal cortex

Authors: ***G. DOUCET**[1], S. TREMBLAY[1], R. GULLI[1], F. PIEPER[2], S. ADAM[3], J. MARTINEZ-TRUJILLO[1];
[1]McGill Univ., Montreal, QC, Canada; [2]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: In recent years, much interest has emerged in using the extracellularly recorded fluctuations in voltage potential (local field potential; LFP) to decode information carried between regions of the brain. LFPs are believed to reflect neural input to and local activity within an area of the brain, while local action potentials provide information about the area's output. However, when attempting to decode the information content of an LFP signal, conventional filtering techniques (low-pass at ~300 Hz) are insufficient in segregating spikes components from other LPF sources. Indeed, multiple studies have shown that components of the spike waveforms contaminate LFP signals above 80 Hz (Zanos et al., 2012), biasing the decoded information. In the current study, we aimed at decoding the target of visual attention from simultaneously recorded LFP traces. Neural data was obtained from multi-electrode arrays chronically implanted in the lateral prefrontal cortices (area 8a) of two macaque monkeys involved in a sustained attention task. Decoding performances were first compared between spike-contaminated LFPs and spiking data. Our results demonstrate that LFPs offer comparable performance to spikes in decoding accuracy, although this is only true for the frequency range thought to be corrupted by spike leakage (high gamma). Surprisingly, after removing spike components from the LFPs, using a previously published algorithm (Zanos et al., 2011), we show a negligible decrease in decoding performance, suggesting that the information content of high-frequency LFPs carry similar information to locally recorded action potentials. These

results demonstrate that LFP signals can be used to decode the allocation of attention to targets across the visual field. This may be relevant to the development of cognitive neural prosthetics that often rely on the use of low impedance electrodes.

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Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Title: Burst firing synchronizes prefrontal and anterior cingulate cortex during attentional control

Authors: ***M. OEMISCH**[1], S. ARDID[1,2], S. EVERLING[3], T. VALIANTE[4,5], T. WOMELSDORF[1,2];
[1]Dept. of Biol., [2]Ctr. for Vision Res., York Univ., Toronto, ON, Canada; [3]Dept. of Physiol. and Pharmacology, Ctr. for Functional and Metabolic Mapping, Western Univ., London, ON, Canada; [4]Dept. of Surgery, Div. of Neurosurg., Univ. of Toronto, Toronto, ON, Canada; [5]Div. of Fundamental Neurobio., Toronto Western Res. Inst., Toronto, ON, Canada

Abstract: Attention is realized in the brain by the rapid formation of a coalition of brain cells into a large-scale attention network. Cells in medial and lateral prefrontal cortex control this formation of attention networks, but how the activity of single cells exerts network control is unknown. Here, we show that burst-synchronization could serve as a potent candidate mechanism to achieve network control. We found that cells in macaque prefrontal cortex increased the firing of brief 200 Hz burst events when subjects shifted attention and engaged in selective sensory processing. In contrast to non-burst spikes, burst spikes synchronized at narrow beta (12-20 Hz) and gamma (50-75 Hz) frequencies. Burst synchronization was anatomically specific, functionally connecting the anterior cingulate cortex with the lateral prefrontal cortex, both key players of attentional control. Moreover, distinct cell types contributed differently to burst synchronization, with putative interneuron bursts synchronizing prior to putative pyramidal cell bursts at the beta frequency band. In contrast, gamma bursts were transient impulses with equal timing across cell classes. These findings identify burst synchronization as a possible

mechanism to enable the formation of large-scale attention networks based on 'top-down' information in dorsal anterior cingulate and lateral prefrontal cortex.

Disclosures: M. Oemisch: None. **S. Ardid:** None. **S. Everling:** None. **T. Valiante:** None. **T. Womelsdorf:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: LPDS 2012-08

Title: Predicting successful control of attention-dependent choices from neuronal feature tuning in primate prefrontal and anterior cingulate cortex

Authors: *S. WESTENDORFF[1], D. KAPING[2], S. EVERLING[3], T. WOMELSDORF[1]; [1]Lab. for Attentional Control and Neural Circuits; Dept. for Biol., York Univ., Toronto, Ontario, ON, Canada; [2]Karolinska Institutet, Stockholm, Sweden; [3]Dept. of Physiol. and Pharmacology; Dept. of Psychology, Brain and Mind Institute; Western Univ., London, ON, Canada

Abstract: Neurons in the prefrontal and anterior cingulate cortex (PFC/ACC) are sources of attentional control during goal-directed behavior. Those control functions might be realized by PFC/ACC neurons that convey specific information about task relevant feature dimensions at the time when control processes are required for successful performance. According to this hypothesis, feature selective firing of cells should predict correct over erroneous performance for goal-relevant information while selectivity increases for goal-irrelevant information should predict errors. Here we tested this hypothesis by tracking feature selectivity of neuronal firing across four successive processing stages in an attention task. We recorded single cell activity in 1151 cells across subdivisions of the PFC/ACC in two macaques performing a selective attentional control task. The task included in succession (1) a pre-stimulus baseline period in which no goal-relevant information was available; (2) an instructional cue period that triggered a covert shift of spatial attention to one of two peripheral target stimuli; (3) an epoch requiring a perceptual choice on the attended stimulus and (4) a stimulus-response mapping process which mapped a stimulus rotation to a response target location. We found an increase of selectivity on error trials compared to correct trials for goal-irrelevant information during the pre-stimulus

baseline period. In contrast, selectivity for the spatial location of the correct target stimulus was strongly reduced on error trials at the time of the attentional shift and around the time of perceptual choice. This decrease in selectivity for the spatial location continued until well after the choice. Beginning with the response epoch, direction and expected outcome selectivity were also reduced on error trials. Our results show that performance failures are predicted by changes in the selectivity for different feature dimensions across neurons in PFC/ACC by up to several seconds prior to the erroneous choice. Increased selectivity before any goal-relevant information is available to the animals likely reflects biases from preceding attentional selections and choices, interfering with correct task performance on the current trial. Decreased spatial selectivity could reflect a failed shift of attention and later in the trial a failure to properly maintain the focus of attention on the target stimulus at the time of the choice. These results suggest that for correct performance (1) a lack of biases about trial irrelevant features and (2) proper selectivity for trial relevant features is required across large populations of neurons in the primate PFC/ACC.

Disclosures: **S. Westendorff:** None. **D. Kaping:** None. **S. Everling:** None. **T. Womelsdorf:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: D.04. Vision

Support: CIHR

NSERC

CRC

Title: Neural tuning affects spike-rate correlations during a spatial working memory task

Authors: ***M. LEAVITT**[1], F. PIEPER[2], A. SACHS[3], J. C. MARTINEZ-TRUJILLO[1]; [1]Physiol., McGill Univ., Montreal, QC, Canada; [2]Univ. of Hamburg-Eppendorf, Hamburg, Germany; [3]Div. of Neurosurgery, Ottawa Hosp. Res. Inst., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Neurons in the primate dorsolateral prefrontal cortex (dlPFC) are known to exhibit sustained selective activity during the delay period of spatial working memory (SWM) tasks. It

has been hypothesized that functional interactions between these units may be involved in SWM maintenance, but whether and how these units interact with each other remains poorly understood. In order to investigate this issue, we recorded responses of multiple single units in dlPFC area 8r of two macaca fascicularis using microelectrode arrays. The task consisted of fixation on a central spot for 494-800ms, presentation of a circular sine wave grating at one of 16 randomly selected locations for 506ms, then offset of the grating followed by a delay period that could last between 494-1500ms, and ended with the offset of the central fixation point, cuing the animals to make a saccade to the remembered stimulus location. We recorded the activity of neurons in blocks of 32 channels and sorted spikes using Plexon software (Plexon Inc, TX). We isolated responses of 201 single units for a total of 1319 neuronal pairs. Neurons were classified as being selective for the spatial location of the stimulus during the delay period using a linear decoder (n = 133, or 66%). A neuron's preferred location was determined by fitting a 2-dimensional, 2nd-order polynomial to the firing rate data for each stimulus location and finding the function's maximum. We then computed spike-rate correlations and found that interactions between neurons in the dlPFC vary based on task epoch and neurons' selectivity. Both positive and negative (anti) correlations increase during working memory maintenance. Positive correlations are larger between cells with preferred locations in the same hemifield compared to cells with preferred locations in opposite hemifields (Wilcoxon rank-sum test, $p = 0.017$, Bonferroni-corrected). Negative correlations increase between cells with dissimilar tuning during memory maintenance compared to during stimulus presentation (Wilcoxon rank-sum test, $p = 0.003$, Bonferroni-corrected).

Disclosures: **M. Leavitt:** None. **F. Pieper:** None. **A. Sachs:** None. **J.C. Martinez-Trujillo:** None.

Poster

841. Mechanisms of Attention: Parietal and Prefrontal

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Topic: F.02. Animal Cognition and Behavior

Support: Ottawa Hospital Department of Surgery

Canadian Institutes of Health Research

Title: Single-trial dorsolateral prefrontal cortex neural trajectories predict intended saccade direction

Authors: *C. BOULAY[1,3], F. PIEPER[4,6], M. LEAVITT[4], J. MARTINEZ-TRUJILLO[4], A. J. SACHS[2,3,5];
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Abstract: Prefrontal cortex (PFC) is involved in decision-making processes including attentional modulation, context-dependent visual integration, and planning and initiation of motor responses. If the neuronal activity underlying these processes reflects internal processing rather than stimulus-driven responses then it is expected that the time course of neuronal activity may vary across trials. Investigations into the PFC's role in decision-making and contextual learning might therefore benefit from analysis of single-trial temporal dynamics of multiple simultaneously recorded neurons rather than relying on averaged spike trains. In this study, we recorded single-unit activity and field potentials from microelectrode arrays implanted in the dorsolateral PFC (dlPFC) of two adult macaques (Macaca fascicularis) while they made cued saccades to visual targets. We decoded intended saccade direction from dlPFC activity using three different techniques: (1) a Naïve Bayes classifier with prior spiking probabilities based on mean firing rate; (2) automatic clustering of neural trajectories extracted with factor analysis (FA); (3) automatic clustering of neural trajectories extracted with Gaussian process factor analysis (GPFA; Yu et al., 2009). Only the third technique makes explicit use of single-trial time courses. We found that the third technique classified saccade direction more accurately than the others across all recording sessions for both monkeys. These results suggest that dlPFC neuronal activation time courses differ across trials and are better characterized by analytical techniques like GPFA that make explicit use of single-trial time courses. These findings also have implications for brain-computer interfaces (BCIs) that perform discrete goal selections, rather than continuous process control, because goal-selection is likely encoded in single-trial PFC activity. Subsequent studies will investigate how PFC neuronal activity encodes context and how these encodings change during contextual learning.

Disclosures: C. Boulay: None. **A.J. Sachs:** None. **J. Martinez-Trujillo:** None. **M. Leavitt:** None. **F. Pieper:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 842.01/SS35

Topic: F.02. Animal Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft, NI 618/3-1

Title: Neurons in the crow nidopallium caudolaterale retain visual information across varying working memory periods

Authors: *K. HARTMANN, L. VEIT, A. NIEDER;
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Abstract: Working memory incorporates the capability to retain essential information over short periods of time, to process it and to produce goal-directed behavior. In carrion crows (Corvus corone), we recently reported neuronal correlates of visual working memory in the nidopallium caudolaterale (NCL), a pallial structure in the avian endbrain. Neurons in the NCL were found to be active during constant delay periods in discrimination tasks. So far it remains elusive whether delay-selective neurons can also retain visual sample information across varying time periods We trained two carrion crows (Corvus corone) on a delayed match-to-sample task in a fully-automated touchscreen setup. The task required the crows to remember one of three possible visual items for either 1.5, 3 or 4.5 seconds within a session. While the crows performed this task, we recorded single-unit activity from many electrodes in the NCL. A neuron's delay selectivity was evaluated with a two-factor ANOVA (main factors 'sample item' and 'delay duration') based on discharge rates. Behavioral analyses showed that both crows were able to significantly discriminate the stimuli for all three waiting periods. However, correct choice performance dropped with delay duration in both crows. Reaction times, on the other hand, increased with delay time. Both behavioral parameters indicate increased task demands as a function of memory duration. Preliminary neuronal data show that many neurons were selectively tuned to one of the sample items during the delay period. About 20 % of the recorded neurons exhibited sample selectivity irrespective of delay duration. Our results suggest that single neurons in the NCL of a corvid's brain can bridge several seconds to retain information in visual working memory until this information is needed to perform the necessary choice. Time-independent activity of NCL neurons may thus be an important aspect of active memory maintenance in birds.

Disclosures: K. Hartmann: None. **L. Veit:** None. **A. Nieder:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 842.02/SS36

Topic: F.02. Animal Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft, NI 618/3-1

Title: Audio-visual working memory associations in neurons of the corvid nidopallium caudo-laterale

Authors: *F. MOLL, A. NIEDER;
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Abstract: A growing bulk of evidence shows that the avian nidopallium caudo-laterale (NCL), just like the prefrontal cortex in mammals, is essential for working memory representations of sensory information and for executive control. Single NCL neurons represent visual working memory and rule-guided decisions. However, neuronal representations of cross-modal, cross-temporal association (notably sight and sound) remain unknown in birds. Such representations were shown in the primate prefrontal cortex and constitute important evidence for its highly integrative function. Here, we present similar evidence for crow NCL neurons that associated auditory and visual stimuli across time. We recorded single-unit activity from NCL neurons of two awake carrion crows (Corvus corone) performing in a cross-modal, cross-temporal association task. The crows were trained to associate complex auditory stimuli with visual objects in a computerized procedure. For this, the crow was placed on a perch in front of a touch-screen. To start a trial, the crow had to hold its head within the range of a light barrier. After that, one of two complex auditory stimuli was played back from a speaker for 1.3 sec (sample phase). Followed by a delay of 1 sec during which the screen remained black and no auditory stimulus was present, a visual match or non-match stimulus was shown on the screen (test phase). To receive a reward from a feeder, the crow was required to peck at the test stimulus whenever it matched the previously presented, associated auditory stimulus. Our preliminary data from about 200 recorded single neurons show that more than 30 % of the cells selectively represented the auditory stimulus during the sample phase. Even more cells (about 50 %) associated the auditory stimulus with the upcoming visual stimulus during the delay phase by selectively increased discharge rates. Many delay selective cells showed clear ramping activity, i.e. progressively increasing activity towards the end of the delay, just prior to the appearance of the visual test stimulus. Association coding was significantly weaker in error trials, thus predicting the crows' behavioral choice. These results underscore the highly integrative function of the NCL, binding stimuli from different modalities and bridging such associations across time. In that respect, the avian NCL is on par with primate prefrontal cortex function. The strong correlation of neuronal activation during correct and error trials with the upcoming choice underpins the cardinal role of the NCL in executive function.

Disclosures: F. Moll: None. **A. Nieder:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 842.03/SS37

Topic: F.02. Animal Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft, NI 618/3-1

PhD stipend from German National Academic Foundation

Title: Neuronal activity in the corvid nidopallium caudo-laterale during association learning

Authors: *L. VEIT[1], G. PIDPRUZHNYKOVA[2], A. NIEDER[1];
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Abstract: The ability to form arbitrary associations between sensory stimuli is fundamental for many learned behaviors. We investigated neuronal activity in the nidopallium caudolaterale (NCL) while two crows (Corvus corone) performed a delayed paired association learning task. The NCL is a multimodal integration area in the avian endbrain involved in high-level cognition in corvids. The birds were positioned in front of a touchscreen monitor and presented with a sample image, followed by a brief delay. At the end of the delay, the birds chose one of two simultaneously presented test images. In each session, blocks with well-trained associations were alternated with blocks requiring the learning of new associations. In each learning block, crows learned to associate two arbitrary new sample images to two familiar test images by trial and error. The two test images were kept constant, while the sample images were exchanged for each new pair of associations. In the well-trained blocks, crows responded to two familiar sample images with known associations to the same two test images. A pair of associations was considered learned if the bird reached a performance of at least 80% correct over a group of 40 consecutive trials. The first trial of this successful group was typically reached after a median of 43 (bird 1) and 31 (bird 2) trials (correct and error trials). Both birds reached a performance of 83% after the association was acquired. Learning was also reflected in a change of reaction times, which decreased by approximately 10% for each bird after the learning criterion was met. We recorded the activity of single neurons in NCL. The block structure of the task allowed us to compare neuronal responses for well-known and for new sample items mapping onto the same test items. We found neurons which prospectively encode the chosen test item during the delay for both familiar and newly learned associations. Some of these neurons changed their activity

during the learning process. Thus, selective delay activity in NCL does not simply reflect working memory related to the sample item, but actively processes information for the upcoming behavioral choice. These data provide new insights into the nature of working memory representations in birds, and how these representations are formed during learning. They suggest NCL plays a role in the learning of arbitrary associations between sensory stimuli, a cornerstone of corvids' remarkable behavioral flexibility and adaptability.

Disclosures: L. Veit: None. **A. Nieder:** None. **G. Pidpruzhnykova:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 842.04/SS38

Topic: F.02. Animal Cognition and Behavior

Support: NIDA DA017949

Title: The effects of acute nicotine on contextual safety learning

Authors: *C. F. OLIVER;
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Abstract: Anxiety disorders such as Post-Traumatic Stress Disorder (PTSD) are attributed to deficits in extinction learning with several studies showing that fear extinction is delayed in these patients. In support, PTSD patients fail to learn inhibitory safety signals and children who show safety learning deficits are at a higher risk for developing anxiety disorders in adulthood. Given the high rate of cigarette smoking in anxiety and PTSD patients and the recent finding that an acute dose of nicotine impairs safety learning during extinction, a series of experiments were conducted to investigate the effect of acute nicotine on an animal model of safety learning. Following saline or nicotine administration, mice were trained in a contextual discrimination paradigm in which the subjects received presentations of conditioned stimuli (CS) co-terminated with a foot-shock in one context, context A (CXA), and CS only presentations in another context, context B (CXB). Therefore, CXA was designated as the "dangerous context" whereas CXB was designated as the "safe context". Our results suggested that saline-treated animals showed a strong discrimination between the dangerous and safe contexts while nicotine administration dose-dependently impaired contextual safety learning (Experiment 1). Furthermore, our results demonstrated that nicotine-induced impairment of contextual safety

learning was not a result of increased generalized freezing (Experiment 2) or contingent on the common CS presentations in both contexts (Experiment 3). Finally, our results also showed that increasing the temporal gap between CXA and CXB during training abolished the impairing effects of nicotine (Experiment 4), which suggests that nicotine acts on the memory trace of the context and bridges the temporal gap between two contexts during training. The findings of this study helps link nicotine exposure to the safety learning deficits seen in anxiety disorder and PTSD patients.

Disclosures: C.F. OFliver: None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 842.05/SS39

Topic: F.02. Animal Cognition and Behavior

Support: Supported by the Military Operational Medicine Research Program, US Army Medical Research and Materiel Command.

Title: Ketamine effects on conditioned suppression in rats

Authors: *C. M. GROEBER TRAVIS, D. E. ALTMAN, L. P. SIMMONS, S. DOBRE, R. F. GENOVESE;
Ctr. for Military Psychiatry and Neuroscience, Behavioral Biol. Br., Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: We are evaluating whether ketamine, a dissociative anesthetic, can alter the acquisition, expression or extinction of a conditioned fear, using a conditioned suppression procedure. Rats were trained to lever press on a 30 min VI32 sec schedule of food reinforcement. After response rates stabilized, an inescapable electric shock (IES) was paired with audio/visual conditioned stimuli (CS). To measure the conditioned emotional response (CER), every other day after the IES+CS pairing, rats were presented with the CS only, in the VI32 chamber, for a total of 6 extinction trials. Strength of the CER was determined by comparing the rate of responding before the CS with that after the CS. That is, the CER is evidenced by suppression of behavior. Rats were given differing regimens of ketamine or saline injections after IES only or after each CS. Control rats did not receive IES (CS only), but were given injections after all presentations of the CS. Ketamine (10 mg/ml) or saline was administered in a 4 injection

sequence (100, 50, 50, 50 mg/kg) one hour apart. The first injection was administered IM, in a split concentration: half dose in each leg, immediately after the specified sessions. Three subsequent injections were administered IP. Preliminary results show that, as expected, rats exposed to IES show substantial suppression during initial extinction trials, compared to no shock controls. Although ketamine is relatively short-acting, it appears to have a residual effect on VI32 responding as some rats show a decrease 24 hours or more after the administration of ketamine. After not receiving ketamine for several days, however, response rates return to baseline. Ketamine, in the dose regimen used, does appear to affect responding; however it is too early to make conclusions about the strength or direction of the effects on conditioning. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition. All procedures were reviewed and approved by the WRAIR Institutional Animal Care and Use Committee, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Disclosures: C.M. Groeber Travis: None. **D.E. Altman:** None. **L.P. Simmons:** None. **S. Dobre:** None. **R.F. Genovese:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 842.06/SS40

Topic: F.02. Animal Cognition and Behavior

Support: Supported by the Military Operational Medicine Research Program, US Army Medical Research and Materiel Command.

Title: Effects of ketamine on behavioral changes induced by predator exposure in rats

Authors: *R. F. GENOVESE, S. DOBRE, C. M. GROEBER TRAVIS, C. C. JOHNSON; Ctr. for Military Psychiatry and Neurosciences, Walter Reed Army Inst. Res., Silver Spring, MD

Abstract: We investigated if the dissociative anesthetic, ketamine, could mitigate the adverse effects of predator exposure, potentially by disrupting memory consolidation processes. Rats were exposed to multiple predators (snake, ferret, and cat) in a protected fashion. We have used this procedure previously in our laboratory as a model of traumatic stress. Exposures were followed by ketamine administration. Four injections (1/hr) were given post-exposure and two dose regimens of ketamine were evaluated. Vehicle and sham predator controls were also evaluated. We used exploratory behavior on an elevated plus maze as the dependent measure (difference score; before vs. after exposure). As seen in previous studies, the predator exposure + vehicle produced a significant decrease in EPM activity (difference score significantly non-zero) when evaluated 48h later. Ketamine, however, did not mitigate the decrease in activity and, in fact, the combination (predator exposure + ketamine) produced a greater decrease in exploratory behavior. Additionally, ketamine alone (sham exposure) also produced a significant decrease in exploratory behavior. Our initial analysis suggests that ketamine did not mitigate the adverse effects of predator exposure; however, the effects of ketamine alone have made a meaningful interpretation difficult within this experimental design. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition. All procedures were reviewed and approved by the WRAIR Institutional Animal Care and Use Committee, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Disclosures: R.F. Genovese: None. **S. Dobre:** None. **C.M. Groeber Travis:** None. **C.C. Johnson:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 842.07/SS41

Topic: F.02. Animal Cognition and Behavior

Title: Fructose consumption increased body fat and decreased physical activity but did not impair adult hippocampal neurogenesis in mice

Authors: *A. M. MASNIK[1,2], B. D. PANOZZO[1,2], C. P. KREBS[1,2], A. M. KOBEISSI[1,2], J. G. MUN[1,3], K. DU[1,3], J. S. RHODES[1,2,4];
[2]Dept. of Psychology, [3]Div. of Nutritional Sci., [4]Neurosci. Program, [1]Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: A recent study reported that high-fructose consumption is associated with impaired spatial memory, while another showed reduced markers of neuroplasticity, including adult hippocampal neurogenesis. However, these studies were limited by hypercaloric treatment groups or dietary fructose concentrations that were higher than even the highest levels of intake among humans, and neither combined behavioral and histological measures. Therefore, the purpose of this study was to examine the effect of fructose on behavioral measures of learning and memory and adult hippocampal neurogenesis when administered at a lower dose that better models high levels of fructose consumption among humans in the United States. Over a period of 77 days, male C57BL/6J mice received either a diet with 20% of its total calories from fructose, or glucose as the reference control. On days 30-39, mice received i.p. bromodeoxyuridine (BrdU) injections to label newly-dividing cells. During days 65-77, locomotor activity was assessed using home cage activity tracking, and cognitive performance was assessed in the novel object recognition, rotarod, and contextual fear conditioning tasks. Upon conclusion of behavioral tasks, mice were euthanized to collect brain tissue and peripheral organ measurements. Animals receiving fructose displayed significantly decreased levels of home cage activity, a higher body mass, and increased liver and fat pad mass as compared to the glucose treatment. There were no differences in hippocampal neurogenesis or performance on cognitive tasks. The results of this study suggest that although fructose promotes negative peripheral effects, when consumed at representative levels, its impact on neurological measures may be insignificant.

Disclosures: A.M. Masnik: None. **B.D. Panozzo:** None. **C.P. Krebs:** None. **J.G. Mun:** None. **K. Du:** None. **J.S. Rhodes:** None. **A.M. Kobeissi:** None.

Poster

842. Executive Function: Learning and Memory I

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Program#/Poster#: 842.08/SS42

Topic: F.02. Animal Cognition and Behavior

Title: Water maze swim strategies of mice exposed to proton radiation and a pomegranate-enhanced diet

Authors: *P. V. LORENZO, M. DULCICH, R. HARTMAN;
Loma Linda Univ., Redlands, CA

Abstract: The water maze test involves finding a hidden target in a tank of water situated in a room full of salient spatial cues. Animals can use the spatial cues to extrapolate the target's location, but may also use a variety of non-spatial, strategies, including random search, looping, chaining, scanning, and circling. Neurologically-intact adult rodents will preferentially use spatial strategies, but animals with various types of neuropathology have been shown to compensate by using non-spatial strategies. Recently, we showed that a 2 Gy dose of proton irradiation over 5 minutes did not impair water maze performance when tested 8 weeks later (Dulcich & Hartman, 2013). Additionally, we showed that a pomegranate-enriched diet improved the performance of male, but not female, mice. In the current study, we classified every water maze trial into one of ten search strategies based on the pattern of swim path used to find the platform. A convolution analysis determined the improvement in performance by assessing the shifts in search strategy used across the days of training. The frequency was tallied for each of the ten strategies for each day and group. The predicted performance (PP) was determined by calculating the average swim distance across all training days for each strategy and then multiplying by the frequency of when the strategy was used on each trial on the training day. The animal's actual performance (AP) was calculated by multiplying the frequency from the PP by the average distance traveled on each trial day. Results revealed that the pomegranate diet-animals improved across training days more than the control-diet animals due to the animal changing the search strategy used across trials. The control-diet animals used more looping strategies ($p<.01$), whereas the pomegranate-diet animals used more spatial strategies ($p<.01$). The irradiate females improved more across the training days than the irradiated males due to the changes in search strategies used. Females used more spatial strategies to locate the hidden platform than males ($p<.003$). The results indicate that subtle behavioral changes may not be detected by the standard water maze analyses and that a strategy analysis may provide a more detailed measure of cognitive functioning.

Disclosures: P.V. Lorenzo: None. **M. Dulcich:** None. **R. Hartman:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NNX08AC79G

Title: Analysis and mapping of water maze search strategies in cobalt-60 irradiated rats

Authors: *C. RAY, M. FINLAY, G. NELSON, R. HARTMAN;
Loma Linda Univ., Riverside, CA

Abstract: Radiation exposure can induce significant cognitive changes, which may have implications for issues as diverse as cancer treatment and deep space travel. We examined the effect of γ radiation (5 Gy at 0.5 Gy/min) on the ability of rats to formulate and employ effective spatial learning strategies in the water maze (WM), in which the animals must learn the location of a hidden target in a tank of water situated in a room full of salient spatial cues. Animals can use spatial cues to extrapolate the target's location, but may also use various non-spatial strategies, such as random search, chaining and circling. Neurologically intact rodents preferentially use spatial strategies, but animals with various neuropathologies have been shown to use non-spatial strategies. Performance is generally assessed by measuring the time or swim distance required to find the target. Faster escape latencies and/or shorter distances indicate improvement. Irradiated (n=18) and control (n=18) rats completed ten WM learning trials per day for four days. Performance, as measured by escape latency and swim distance, did not differ between groups. To determine whether the groups used different strategies to achieve similar performance, we classified each WM trial as one of nine search strategies based on the swim path pattern used to find the platform. Strategies are grouped as spatial (spatial direct—swimming directly to the platform; spatial indirect—taking a meandering but expedient path to the target; focal correct—searching in the target's general vicinity), systematic non-spatial (focal incorrect—searching an incorrect but relatively small area; scanning—searching the tank's whole interior; random search), and looping (chaining—swimming in circles in the tank's interior; thigmotaxis—swimming in circles around the perimeter; focal wall searching—intensely searching in one area of the wall then moving to another wall area). We hypothesized that control rats would use mostly spatial strategies to find the platform, but irradiated rats would use non-spatial strategies. Results revealed a correlation between the use of spatial strategies and superior WM performance, making them the most effective strategies. The least effective strategies were random search and thigmotaxis. Controls used the most effective spatial strategies more often (76%) than irradiated rats (54%; $p<.003$). Irradiated rats used the least effective strategies more often (17%) than controls (7%; $p<.05$). These results demonstrate that subtle behavioral effects may not be revealed by standard WM analyses, and that strategy analysis may provide a sensitive measure of cognitive functioning.

Disclosures: C. Ray: None. **M. Finlay:** None. **G. Nelson:** None. **R. Hartman:** None.

Poster

842. Executive Function: Learning and Memory I

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Program#/Poster#: 842.10/SS44

Topic: F.02. Animal Cognition and Behavior

Title: Role of 5-HT5A receptors in the consolidation of memory

Authors: *K. CHÁVEZ, A. MENESES;
CINVESTAV-IPN, México, Distrito Federal, Mexico

Abstract: 5-HT5 receptor occurs in brain areas implicated in learning and memory. Hence, the effects of SB-699551 (a 5-HT5A receptor antagonist) in the associative learning task of autoshaping were studied. The results showed that post-training injection of SB-699551 decreased conditioned responses (CR) during short-term (STM; 1.5h; at 0.1mg/kg) and long-term memory (LTM; 24 h; at 3.0 mg/kg) relative to the vehicle animals. Moreover, considering that there are no selective 5-HT5A receptor agonists, next, diverse doses of the serotonin precursor l-tryptophan were studied during STM and LTM, showing that l-tryptophan (5-100mg/kg) facilitated performance, particularly at 50mg/kg. In interactions experiments, l-tryptophan (50 mg/kg) attenuated the impairment effect induced by SB-699551 (either 0.3 or 3.0 mg/kg). All together this evidence suggests that the blockade of 5-HT5A receptor appear to be able to impair STM and LTM (24 h), while its stimulation might facilitate it. Of course further investigation is necessary, meanly with selective 5-HT5A compounds are necessary.

Disclosures: K. Chávez: None. **A. Meneses:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 842.11/SS45

Topic: F.02. Animal Cognition and Behavior

Title: Transient inactivation of the rodent prefrontal cortex impairs performance on a working memory dependent conditional discrimination task

Authors: D. LAYFIELD, *K. R. URBAN;
Psychology, Univ. of Delaware, Newark, DE

Abstract: The rodent prefrontal cortex, located in the most anterior portion of the brain, has been implicated in a variety of functions, including working memory, decision-making, and integration of various sensory cues into long-term memory (Funahashi and Kubota, 1994, Jo et al., 2007, Horst and Laubach, 2009, Euston et al., 2012). The prefrontal cortex receives fibers from the hippocampus, and lesions of either region impairs function on working memory tasks (Kolb et al., 1974, Eichenbaum et al., 1983, Ainge, 2007, Hallock et al., 2013). These data suggest that the PFC and hippocampus comprise a neural circuit for working memory. Our lab recently developed a new version of the tactile conditional discrimination task designed to test working memory (CDWM); inactivation of the nucleus reuniens (a midline thalamic region reciprocally connected to both PFC and hippocampus) impaired rats' performance on the CDWM task, but not on standard conditional discrimination (CD) (Hallock, 2013). However, the involvement of PFC and hippocampus remained in question. In this study, we have examined the effects of transient PFC inactivation on performance of the CDWM task. Cannulae were implanted bilaterally into PFC and rats were given control infusion of saline, as well as 0.1 μg/mL muscimol. Rats were able to perform the CDWM task after saline infusions, but performance was significantly impaired following infusion of muscimol. This supports the validity of the CDWM task as a working-memory-dependent task, and shows that disconnection of PFC from the working memory circuit is sufficient to prevent proper spatial working memory function.

Disclosures: D. Layfield: None. **K.R. Urban:** None.

Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 842.12/SS46

Topic: F.02. Animal Cognition and Behavior

Title: Effects of co administration of lecithin and caffeine on spatial memory and protein synthesis in Wistar rats

Authors: *B. V. OWOYELE, M. A. TOLUHI;
Physiol., Univ. of Ilorin, Ilorin, Nigeria

Abstract: Caffeine is a psychoactive drug that can be found in many beverages while lecithin is a common name for related compounds called phosphatidylcholines and it is obtained from both animal and plant sources. Consumption of the two substances has been associated with improved memory and amelioration of amnesia in humans but there is paucity of information on the effect of the co administration of the two substances on spatial memory and protein synthesis. Therefore, this study was undertaken to examine the effect of co-administration of Caffeine and Lecithin on spatial memory and creatine kinase in the brain and blood in male rats. Groups of animals comprising of five rats each were orally administered saline(control) caffeine (10mg/Kg), Lecithin (20 mg/Kg), Caffeine + Lecithin (10 mg/Kg +20mg/Kg), Caffeine + Lecithin (5 mg/Kg +30mg/Kg) and Caffeine + Lecithin (15 mg/Kg +10mg/Kg). Animals were pre trained using the Morris water maze prior to the administration of Lecithin and caffeine. Drug administration was for fourteen days once daily. The animals were subjected to the Morris water maze 12 hours after the first day of administration (short term) and after the fourteenth day (long term) of administration of the drugs. They were subsequently sacrificed to obtain serum and brain tissues for the analysis of creatine kinase. The results showed that the concurrent administration of caffeine and lecithin enhanced spatial memory by decreasing the time required to locate the hidden platform. Like wise the brain tissue creatine kinase activity increased while the activity in blood was decreased. In conclusion, the results showed that co-administration of lecithin and caffeine can enhance spatial memory partly by increasing protein synthesis.

Disclosures: B.V. Owoyele: None. **M.A. Toluhi:** None.

Poster

842. Executive Function: Learning and Memory I

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Program#/Poster#: 842.13/SS47

Topic: F.02. Animal Cognition and Behavior

Support: Grants-in-Aid for Scientific Research (B)

Title: Dorsal striatal indirect pathway regulates the response selection accuracy in auditory conditional discrimination

Authors: *K. NISHIZAWA[1], R. FUKABORI[1], K. OKADA[2], M. UCHIGASHIMA[3], M. WATANABE[3], A. SHIOTA[4], M. UEDA[4], Y. TSUTSUI[5], K. KOBAYASHI[1];
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Abstract: The dorsal striatum, which contains the dorsomedial striatum (DMS) and dorsolateral striatum (DLS), integrates the acquisition and performance of discrimination learning. For instance, excitotoxic lesion of striatal neurons disturbs choice accuracy of learned motor response and reduces their responding rate in conditional discrimination. The dorsal striatum receives excitatory inputs from many cortical areas and the thalamic nuclei and projects to the output nuclei through two major pathways composed of the direct and indirect pathways. The balance between opposing inputs from these two pathways is considered to be implicated in motor control though the regulation of basal ganglia output activity. However the mechanism by which the striatopallidal pathway regulates the learning processes of discriminative actions remains unclear. In this study, we induced selective elimination of the striatopallidal pathway by injecting the recombinant immunotoxin into the DLS or DMS of transgenic rats that expressed human interleukin-2 receptor α-subunit under the control of dopamine D2 receptor gene promoter. Then we examined the behavioral consequence of striatopallidal elimination on the performance of auditory discrimination task. Ablation of the DLS- or DMS-derived striatopallidal pathway transiently impaired the performance of conditional discrimination, showing a marked reduction in the selection accuracy of learned motor responses. In addition, the probability of perseverative errors was significantly increased in the animals lacking the striatopallidal pathway from the DMS, but not from the DLS. Although there is a distinct role in modulating perseverative behavior between the DLS- and DMS-derived striatopallidal pathways, these two pathways are necessary for controlling the accuracy of response selection in the conditional discrimination task. This study was supported by Grants-in-Aid for Scientific Research (B).

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Poster

842. Executive Function: Learning and Memory I

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 842.14/SS48

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 24653209

KAKENHI 24530909

Title: Retrieval-induced forgetting in rats' spontaneous object recognition

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Abstract: The act of remembering can cause forgetting. Successful retrieval not only facilitates later recall of the retrieved items, but also impairs later recall of the related or competing items in memory. This detrimental effect of retrieval is referred to as "retrieval-induced forgetting (RIF)" (Anderson et al. 1994). In the present study, we attempted to demonstrate RIF-like phenomenon in rats using a modified spontaneous object recognition test. The object recognition test consisted of a sample phase, retrieval or interference phase, and a test phase with 60 min delay period inserted between the phases. Twenty-five male Long-Evans rats (8 weeks old) were randomly assigned to one of three groups (Control, Retrieval and Interference) and allowed to explore an open field in which two different objects (A, B) were placed in the sample phase. In the retrieval phase for the Retrieval group, two identical objects (B, B), which were the same as one of the objects presented in the sample phase, were placed again. In the interference phase for the Interference group, two identical objects (C, C), which were novel for animals, were placed. In the test phase, two different objects (A, D), one of which was identical to that presented in sample phase (familiar object) and the other was novel, were placed and the time spent exploring each object was analyzed. As a result, animals in the Control and Interference groups explored the novel object longer than the familiar object. In contrast, rats in Retrieval group explored both objects almost equally, suggesting that they could not discriminate between the familiar and the novel objects at the test phase. These results demonstrate the "retrieval-induced forgetting" phenomenon in a spontaneous object recognition test in rats.

Disclosures: **K. Yamada:** A. Employment/Salary (full or part-time):; University of Tsukuba. **M. Ueno:** None. **E. Takano:** None. **Y. Ichitani:** A. Employment/Salary (full or part-time):; University of Tsukuba.

Poster

842. Executive Function: Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI24223004

Title: Functional segregation of prefrontal and premotor cortices in a delayed response task: An rTMS study

Authors: *S. NAKAMURA, T. HOSOKAWA, T. IIJIMA, K.-I. TSUTSUI;
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Abstract: Previous lesion and temporal inactivation studies have shown that the dorsolateral prefrontal cortex (DLPFC) is crucially involved in the retention of information during a short period of time, i.e. short-term memory. The premotor cortex (PMC), which is connected with the DLPFC, is thought to play a key role in planning and preparing a specific motor action on the basis of incoming information from the DLPFC. These brain areas should work together in order to accomplish a complex behavior. Here, we used repetitive transcranial magnetic stimulation (rTMS) as a noninvasive method for disturbing the neural activity and investigated how the DLPFC and PMC contributed to the performance of a delayed response task in which monkeys were required to retain the visuospatial information necessary for the following behavior in their mind. Two monkeys performed a spatial delayed response task, in which one of eight buttons arranged in a circle was illuminated as a cue, and pressing that button after a variable delay period was rewarded. 10-Hz 1-s rTMS was delivered 0.5 s after the beginning of the delay period on every trial. rTMS to DLPFC significantly impaired the task performance in a delay-dependent manner when the target button was located at the hemifield contralateral to the stimulated hemisphere regardless of which hand was used, whereas that to the PMC impaired the task performance when using the contralateral hand of the stimulated hemisphere regardless of the button position and delay length. We further examined whether the impairment we observed here would be rTMS-timing dependent during the delay period. 10-Hz 1-s rTMS was delivered during a fixed delay period at different timing parameters. Although rTMS to DLPFC induced a similar pattern of impairment in the task performance for all the timings we tested, the effect was the largest when rTMS was delivered 0.5 s after the beginning of the delay period. In the case of PMC, we observed a similar pattern of the impairment when rTMS was delivered during the early phase and just before the end of the delay period but not the middle. Thus, the impairment caused by DLPFC stimulation was visual field dependent, whereas that caused by PMC stimulation was effector dependent. Furthermore, the impairment observed here was rTMS-timing dependent during the delay period, and the effect was different between the DLPFC and PMC. These results clearly demonstrate that the DLPFC and PMC function as different functional elements for successful performance of the spatial delayed response task.

Disclosures: **S. Nakamura:** None. **T. Hosokawa:** None. **T. Iijima:** None. **K. Tsutsui:** None.

Poster

842. Executive Function: Learning and Memory I

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Program#/Poster#: 842.16/SS50

Topic: F.02. Animal Cognition and Behavior

Support: NIH T32HD071845

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NIH RO1MH082819

Title: Monkeys use similar discriminative cues across two tests of metamemory

Authors: ***E. K. BROWN**[1], B. M. BASILE[2], V. L. TEMPLER[1], R. R. HAMPTON[1];
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Abstract: Metamemory is the ability to monitor and adaptively control memory. Here, we directly compare performance of rhesus monkeys (*Macaca mulatta*) on two metamemory paradigms: decline-test and information-seeking. We tested the assumption that these two metamemory tests assess the same underlying cognitive capacity. Monkeys performed a four-choice match-to-sample memory task. In Experiment 1, monkeys could decline memory tests on some trials to receive a small, guaranteed reward. Monkeys were significantly more accurate on tests they chose to take than on those they were forced to take, suggesting that they monitored memory to selectively avoid tests when memory was poor. In Experiment 2, monkeys could choose to repeat the sample on some trials. Monkeys were significantly more accurate on tests for which they chose not to repeat the sample than on tests they were forced to take without the option to repeat the sample. This result suggests that they monitored memory to select the repeat-the-sample response when memory was poor. We made the metacognitive response available at different points during trials in both Experiments 1 and 2, to assess which cues control metacognitive judgments. The opportunity to decline tests or repeat the sample was presented prospectively, before test stimuli appeared, or concurrently with test stimuli. In prospective choices, monkeys must base metamemory judgments solely on the current contents of memory, whereas in concurrent choices, the test stimuli provide additional cues that reflect the difficulty

of the memory test. In both metamemory paradigms, monkeys showed a greater benefit of metacognitive responding on concurrent than on prospective tests, suggesting that monkeys monitored both their internal memory states and the immediate familiarity elicited by test items. Similar patterns of metamemory performance across the paradigms used in Experiments 1 and 2 strengthen the hypothesis that similar cognitive monitoring processes are active across the two paradigms, and that metamemory results from the monitoring of multiple cues.

Disclosures: E.K. Brown: None. **V.L. Templer:** None. **R.R. Hampton:** None. **B.M. Basile:** None.

Poster

842. Executive Function: Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant MH058847

Title: Comparing prefrontal and medial temporal lobe in macaque monkeys during a temporal-order-memory task

Authors: *Y. NAYA[1,2], H. CHEN[1], C. YANG[1], W. SUZUKI[2];
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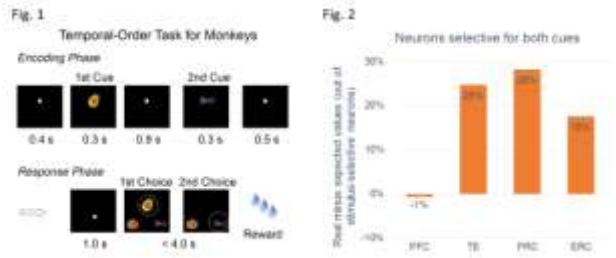
Abstract: A fundamental component of episodic memory is memory for the temporal order of items within an episode. Convergent evidence suggests that both the medial temporal lobe (MTL) and the prefrontal cortex (PFC) contribute to temporal-order memory. Previous physiological studies examined neuronal properties in MTL (Naya & Suzuki, 2011) and PFC (Ninokura et al., 2003 & 2004; Warden & Miller, 2007). However, no studies had yet compared the two brain regions directly. In order to characterize their contributions to temporal-order memory, we recorded single-unit activity from PFC and MTL in two macaque monkeys performed a temporal-order-memory task (Fig. 1) (Naya & Suzuki, 2011). In the task, a sequence of two cue stimuli was shown for 0.3 sec each with a delay interval (0.9 sec) between them. After the encoding phase, the animal was required to touch the two items in the same order as they were presented. We recorded 127 neurons from the dorsolateral PFC. We examined stimulus-selectivity for each recorded neuron using one-way ANOVA and found that 19 PFC neurons exhibited stimulus-selective activities for the first cue ($P < 0.05$) and 15 neurons showed

significant stimulus-selectivity for the second cue. The number of neurons with stimulus-selective responses for both cues (n = 2) did not differ (P = 0.89) from the chance (i.e., 19×15/127). In striking contrast to the PFC, we found significantly larger numbers of neurons showed stimulus selectivity for both cues in the perirhinal cortex (P < 0.001) and the entorhinal cortex (P < 0.001) of MTL as well as area TE (P < 0.005). The hippocampus was excluded from this analysis because it did not have substantial number of stimulus-selective responses for either the first cue or second cue. These results indicate differential contributions of PFC and MTL to the encoding of temporal-order memory with significantly more temporal lobe neurons representing selective information across both to-be-remembered stimulus periods while prefrontal neurons appeared to represent different time periods independently (Figure 2).



Disclosures: **Y. Naya:** None. **H. Chen:** None. **C. Yang:** None. **W. Suzuki:** None.

Poster

842. Executive Function: Learning and Memory I

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Program#/Poster#: 842.18/SS52

Topic: F.02. Animal Cognition and Behavior

Title: Clozapine but not haloperidol prevents and reverses a sub-chronic PCP-induced cognitive deficit in the attentional set-shifting task in the Rat

Authors: *N. IDRIS;
Pharm., Omer Almukhtar Univ., Albayda, Libyan Arab Jamahiriya

Abstract: ***Introduction:*** Cognitive impairment is a pervasive feature of schizophrenia, and is a major determinant of the functional disability that is characteristic of the disorder. Administration of N-methyl-D-aspartate (NMDA) receptor antagonists in rodents has been proposed as an animal model of cognitive dysfunction in this disorder. Evidence from both animal models and human studies implicates a dysfunction of NMDA receptor function may

attribute to pathophysiology of schizophrenia. ***Objectives:*** This study was undertaken to investigate the ability of sub-chronic co-administration of clozapine and haloperidol to both prevent and attenuate the cognitive deficits induced by the NMDA receptor antagonist, phencyclidine (PCP) in the attentional set-shifting task (ASST). ***Methods:*** In the first test, female Sprague-Dawley rats were treated with saline, clozapine 5.0 mg/kg or haloperidol 0.05 mg/kg, 30 min later followed by either saline or PCP 2.0mg/kg twice daily for 7 days, followed by 7 days drug free before tested in ASST task. For the second test, female Sprague-Dawley rats received either vehicle or PCP 2.0 mg/kg for 7 days followed by 7 days drug free. Then rats received clozapine 5.0 mg/kg, haloperidol 0.05 mg/kg or vehicle twice daily for 7 days and were tested 120 min following the last dose of antipsychotic in ASST task. ***Results:*** Sub-chronic PCP significantly ($p<0.01$) increased the number of trials to reach criterion in the EDS phase when compared to vehicle. Atypical antipsychotic, clozapine but not the classical agent, haloperidol, significantly prevented and improved the cognitive impairment induced by PCP in ASST task. Conclusions: These results suggest that antagonism of the consequences of reduced NMDA receptor function could contribute to the superior efficacy of atypical antipsychotic agents in improving cognition in schizophrenia. This cognitive deficit likely reflects clinically relevant and can be used to evaluate the antipsychotic potential of new compounds on cognitive symptoms of schizophrenia.

Disclosures: N. Idris: None.

Poster

842. Executive Function: Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH091174

NSF Graduate Research Fellowship

Title: Modulation of power and synchrony of local field potentials by working memory load in the macaque

Authors: *S. KORNBLITH[1], T. J. BUSCHMAN[2], E. K. MILLER[1];
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Abstract: In humans, working memory maintenance is associated with changes in evoked potentials (Vogel & Machizawa, 2004) and synchrony (Palva et al., 2010) that scale with the number of stimuli to be remembered. To better understand how working memory load modulates local brain oscillations, we recorded simultaneously from the lateral intraparietal area (LIP), frontal eye fields (FEF), and lateral prefrontal cortex (lPFC) of two rhesus macaques while they performed a change localization task (see Buschman et al., 2011). Animals saw a sample array of 2 to 5 colored squares that subsequently disappeared. After an 800-1000 ms delay period, the array reappeared with a change to the color of one of the squares. Animals had to saccade to the changed square. Similar correlations between local field potential power and load were observed in all regions during both the sample and delay periods of the task. In the beta/low gamma band (20-40 Hz), we observed a significant decrease in power with load throughout the sample period and in the beginning of the delay period. This decrease in low gamma power was accompanied by an increase in power in the high gamma band (60-100 Hz). Later in the delay period, effects in the high gamma band dissipated, and we observed a reversal of the direction of correlation in the low gamma band: rather than decreasing with load, power instead increased with load. These effects were visible in all three of the regions from which we recorded. We also observed significant modulatory effects of load upon coherence of oscillations between simultaneously recorded electrodes within and between regions. While the sample was present, high gamma coherence within LIP increased with working memory load, while at sample offset, alpha (10-15 Hz) coherence within prefrontal cortex and between prefrontal sites and LIP decreased with load. Our results show that working memory load exerts effects upon oscillatory power and coherence.

Disclosures: S. Kornblith: None. **T.J. Buschman:** None. **E.K. Miller:** None.

Poster

842. Executive Function: Learning and Memory I

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Program#/Poster#: 842.20/SS54

Topic: F.02. Animal Cognition and Behavior

Title: Involvement of dorsolateral and ventrolateral prefrontal cortex in behavioral adaptation to group reversal

Authors: *T. HOSOKAWA, S. NAKAMURA, Y. MATSUI, M. YAMADA, T. IIJIMA, K.-I. TSUTSUI;
Tohoku Univ., Sendai, Miyagi, Japan

Abstract: To investigate the brain areas related to categorical thinking, we trained two Japanese monkeys (Macaca fuscata) to perform a group reversal task. In this behavioral task, one of eight visual stimuli, half of which were associated with juice and the rest with saline, was chosen to serve as a cue to predict which type of liquid was to be given at the end of the trial. As monkeys learned the stimulus-outcome relation, they showed anticipatory licking after the presentation of a juice-predicting stimulus, and no licking after the presentation of a saline-predicting stimulus. We occasionally reversed the stimulus-outcome relation in all stimuli without any explicit cue. The monkeys showed extremely quick adaptation to the change of this relation after making a single error at the reversal, indicating that they could generalize the relational change experienced for one stimulus for the other stimuli. This result suggests that the monkeys had recognized the stimuli associated with the same outcome as a category. A category of this kind based on experience and learning is termed the "acquired category", in contrast to the "perceptual category" based on perceptual resemblance. We recorded neuronal activity in the dorsolateral prefrontal cortex (dlPFC), ventrolateral prefrontal cortex (vlPFC), and orbitofrontal cortex (OFC) while the monkeys performed the task. We analyzed the activity by multiple regression analysis with the variables stimulus category, reward contingency, and task rule (as the interaction of category and contingency). Prefrontal neurons were found to code one or more than one of these types of information. A large proportion of vlPFC neurons coded category information. A large proportion of dlPFC neurons showed sustained activity related to the task rule. dlPFC neurons showed higher and sustained responses related to the reward contingency, while vlPFC and OFC neurons showed weaker and phasic ones. We further investigated the functions of the prefrontal areas by inhibiting the neural activity by low-frequency repetitive transcranial magnetic stimulation (rTMS). We separately inactivated dlPFC and vlPFC, where we found many task-related neurons, and also the dorsal prefrontal cortex (dPFC) as a control by applying rTMS bilaterally before the monkeys performed the task (twice for each hemisphere at 1 Hz for 5 minutes). Inactivation of dlPFC or vlPFC, but not of dPFC, impaired the behavioral performance after the reversal: the monkeys needed significantly more trials to adapt their behavior to the reversal. These results suggest that dlPFC and vlPFC play critical roles in behavioral adaptation in group reversal based on acquired category information.

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Poster

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Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 842.21/SS55

Topic: F.02. Animal Cognition and Behavior

Title: Sustained activity in mPFC of head-fixed rats performing delayed response task

Authors: *Y. TATEYAMA, K. OYAMA, K. OMORI, T. IIJIMA, K.-I. TSUTSUI;
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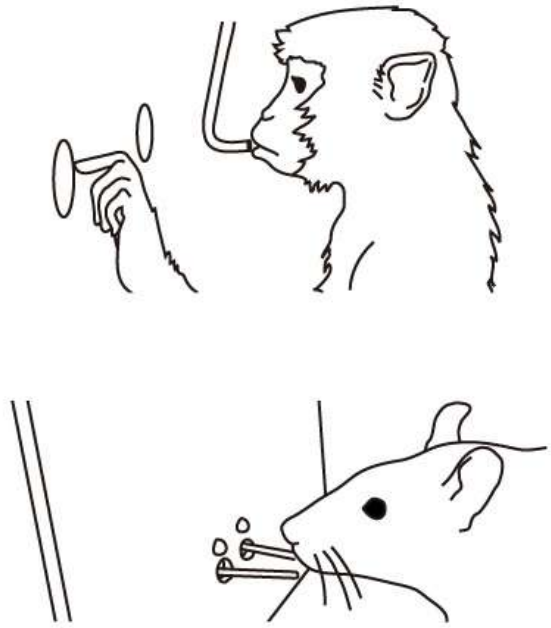
Abstract: In humans and other primates, the dorsolateral prefrontal cortex (DLPFC) is known to be involved in short-term memory. Electrophysiological recordings from behaving monkeys have shown that the DLPFC neurons show sustained activity during the delay period of various delay tasks, such as delayed response and delayed match-to-sample, and therefore, the sustained delay activity is thought to be a neuronal correlate of short-term memory. However, it is still unclear whether the same understanding can be applied to rodents, the most commonly used animal species in neuroscience research. Anatomical studies suggest that there is some homology between the rat medial prefrontal cortex (mPFC) and the monkey DLPFC. Some electrophysiological studies in rats have demonstrated that the rat mPFC neurons show sustained activity. However, these studies were conducted in freely-moving conditions, which is different from monkey studies. The purpose of this study was to investigate whether neurons in the rat mPFC show sustained activity related to short-term memory under head-fixed conditions similar to those used with monkeys' (fig. 1). We recorded single-unit activity with a multi-electrode in the rat mPFC trained in a delayed response task, in which rats were required to memorize the visual cue position and lick a spout in the corresponding position after a delay period. The result was that some neurons in the rat mPFC showed sustained activity during the delay period in the same way as those in the monkey DLPFC.



Disclosures: **Y. Tateyama:** None. **K. Oyama:** None. **K. Omori:** None. **T. Iijima:** None. **K. Tsutsui:** None.

Poster

842. Executive Function: Learning and Memory I

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 842.22/SS56

Topic: F.02. Animal Cognition and Behavior

Title: Effect of acute stress on working memory in male rats sexually motivated

Authors: *E. HERNANDEZ-ARTEAGA[1], M. ALMANZA-SEPÚLVEDA[1], M. HERNÁNDEZ-GONZÁLEZ[1], M. GUEVARA[1], H. BONILLA-JAIME[2], M. OLVERA CORTÉS[3];
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Abstract: Stress is the biological mechanism through which the body attempts to regain homeostasis when affected by internal and external forces (stressors), based on behavioral and

endocrine mechanisms. Stress can affect prefrontal cortex and thus affect executive functions, such as working memory. On the other hand, it is known that sexual behavior is an effective reward that enhances the acquisition and maintenance of working memory. Therefore, this study determines the effect of acute stress on visuospatial working memory (non-matching-to-sample working memory task using a T-maze) in sexually motivated male rats. 32 sexually experts male rats were trained in a T-maze using sexual interaction as a reward during a 4-day training period. According to their performance, the rats were divided into 2 groups: good-learners (n=12) and bad-learners (n=20). Finally, on the fifth day, 6 good-learners and 10 bad-learners were stressed by cold water immersion (CWI, 15°C) for 15 minutes before their execution in the T-maze while the remaining rats formed the control group. The results showed that acute stress by CWI improved working memory in both groups (good- and bad-learners), and decreased their sexual motivation to perform the task.

Disclosures: **E. Hernandez-Arteaga:** None. **M. Almanza-Sepúlveda:** None. **M. Guevara:** None. **M. Hernández-González:** None. **H. Bonilla-Jaime:** None. **M. Olvera Cortés:** None.

Poster

843. Executive Function: Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH/NCI/MSKCC #U54CA137788/CCNY#U54CA132378

NCRR 5G12RR003060-26

NIMHHD 8G12MD7603-28

Title: Exercise improves cognitive function, reduces anxiety and increases BDNF in gonadectomized adult male rats

Authors: *K. Y. SALAS-RAMIREZ[1], M. QADRI[2], K. URIBE[3], C. STREET[2], N. TALUKDER[2], D. WOO[2], G. DEJESUS[2], S. PEREZ[4], C. J. NELSON[5];
[1]Physiology, Pharmacol. and Neurosci., CUNY Med. Sch., New York, NY; [2]The Sophie Davis Sch. of Biomed. Education, CCNY, New York, NY; [3]The City Col. of New York, New York, NY; [4]Lehman College, CUNY, Bronx, NY; [5]Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract: Prostate cancer is the most common cancer in men in the United States with over 190,000 men diagnosed in 2010. Androgen ablation therapy is the standard treatment for advanced prostate cancer and has been found to negatively impact cognitive functioning in men. Physical exercise been shown to improve attention, processing speed, executive function, memory, and working memory in humans and animals. The objective of this study was to determine if exercise can be used as a non-pharmacological intervention for cognitive decline in an animal model for prostate cancer. Thirty six male rats were divided in four groups (1) intact exercise (n=10), (2) intact no exercise (n=8), (3) gonadectomized (gdx) exercise (n=10) and (4) gdx no exercise (n=8). The exercise group was exposed to twenty minutes of forced exercise on a rat treadmill for five days/week. The no exercise group was experimenter handled for an average of 5 mins daily. After 6 weeks of exercise exposure, males were tested for anxiety (elevated plus maze), locomotor activity (open field), working (y-maze), visual (object recognition) and spatial (object placement) memory. Upon the completion of the behavioral studies, brain derived neurotrophic factor (BDNF) in the hippocampus, prefrontal cortex (PFC) and striatum was assessed through ELISA's. Exercise was found to significantly increase generalized locomotion in male rats, when compared to the no exercise group, independent of gdx. There was a significant gdx by exercise interaction for anxiety measures where the gdx exercise males were the least anxious and the gdx no exercise males where to most anxious out of all four groups. Most importantly, exercise significantly improved the cognitive performance in gdx male rats in the working, visual and spatial memory tasks, restoring performance comparable to control rats, independent of gdx. In addition, exercise increased BDNF in striatum in both intact and gdx males, but gdx exercise males had higher BDNF levels in the PFC and hippocampus. These data suggest that exercise is an effective intervention for cognitive decline, especially after androgen ablation by increasing BDNF levels. Further studies will investigate mechanisms by which exercise impacts neural plasticity.

Disclosures: K.Y. Salas-Ramirez: None. **M. Qadri:** None. **K. Uribe:** None. **C. Street:** None. **N. Talukder:** None. **D. Woo:** None. **G. DeJesus:** None. **S. Perez:** None. **C.J. Nelson:** None.

Poster

843. Executive Function: Learning and Memory II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 843.02/SS58

Topic: F.02. Animal Cognition and Behavior

Support: CNPq (Process Number: 153175/2012-5)

Title: Effects of two hypercaloric diets on learning and memory processes in rats (Rattus norvegicus)

Authors: *R. T. PINI[1], L. D. M. F. VALES[1], T. BRAGA COSTA[3], S. S. ALMEIDA[2]; [1]Psychology, Univ. of Sao Paulo, Ribeirão Preto, Brazil; [2]Univ. of Sao Paulo, Ribeirao Preto, Brazil; [3]Univ. of Ribeirao Preto, Ribeirao Preto, Brazil

Abstract: Although widely discussed, the deleterious metabolic effects of obesity on cognitive processes, as learning and memory, remain scarce and poorly known. The aim of this study was to investigate the effects of two hypercaloric diets on learning and memory in rats. Forty male albino Wistar rats were divided into three groups according to nutritional status: Control group (C, n = 13), Cafeteria Diet group (CD, n = 14), and High-fat Diet group (HD, n = 13). Body weight was recorded weekly until 98 days of age, as well as, body weight and accumulation of retroperitoneal and epididymal adipose tissues at the sacrifice day. Learning and memory tasks were evaluated at the Morris Water Maze (MWM). Data of body weight, adipose tissues and MWM test were treated by an analysis of variance (ANOVA) followed by the Newman-Keuls test ($p < 0.05$). HD presented higher body weight when compared to C and CD rats at 77 and 84 days of age. CD and HD showed higher weight at the sacrifice day as compared to C. HD showed higher accumulation of fat on retroperitoneal and epididymal tissue as compared to C and CD, while CD presented higher fat as compared to C. Regarding the MWM test, there was no difference among CD, HD and C animals at the learning and memory test for all the parameters evaluated. On a previous study developed in our lab CD and HD animals receiving the same diets protocols showed better learning performance and better recall of a previously learned information at the memory retention task when tested in two consecutive sessions (12 trials per day), which is an easier task when compared to the present protocol (6 sessions of 4 trials per day). It was concluded that the diets produced obesity and accumulation of fat in the retroperitoneal and epididymal tissues in CD and HD groups. In the MWM test there were no differences among the three groups, indicating that more complex task should reduce differences previously obtained with a task easier to learn. From these data it was concluded that obesity led to metabolic changes, however it was not observed a consistent alteration in the pattern of learning or memory.

Disclosures: R.T. Pini: None. **L.D.M.F. Vales:** None. **T. Braga Costa:** None. **S.S. Almeida:** None.

Poster

843. Executive Function: Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: 5R01DA026297-04-NIH-NIDA

5R01EY017658-05-NIH-NEI

Title: Microelectrode recordings within prefrontal cortex and caudate nucleus during two working memory tasks

Authors: *D. HUIE, K. GHOSE, C. MARTINEZ-RUBIO, T. M. HERRINGTON, E. N. ESKANDAR;
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Abstract: Background: Working memory is the capacity to briefly hold and recall information following removal of the original stimulus and presentation of interim distractions. Though working memory storage has primarily been ascribed to prefrontal cortex (PFC), recent studies have implicated the basal ganglia as a gatekeeper to working memory, permitting certain stimuli to access and change working memory while protecting working memory from distracting input. Though fMRI studies in humans have lent support to this gating hypothesis, it has not been tested with single-unit neurophysiology. Here, we recorded from single neurons in the PFC and anterior caudate nucleus during two working memory tasks. We hypothesize that activity in the caudate nucleus will reflect task-specific working memory demands. Methods: Microelectrode recordings were performed in a rhesus macaque within the anterior caudate nucleus and principal sulcus while the subject engaged in two working memory tasks: a sequential distractor chain (SDC) task and a one-back task. In both tasks, the animal was first shown a cue indicating the task to be performed, followed by a variable-length series of unique images. Following the series of images the subject was presented with two image choices in a two-alternative forced choice task. The correct answer was either the first image in the chain (SDC task) or the last image (one-back task). Results: Preliminary analysis in one monkey revealed that the activity of PFC neurons, but not caudate neurons, contains information about image identity. Neural activity correlating with image identity was present during image presentation and lingered during the delay period following stimulus removal. Both PFC and caudate neurons contain information related to task identity (SDC vs. one-back) during sequential image presentation and immediately prior to choice presentation. Discussion: Corticostriatal networks are thought to play a role in working memory. Our preliminary data support the hypothesis that PFC is involved in the storage of working memory, and that activity in both PFC and caudate reflects variable working memory demands. Ongoing analysis and additional recordings, including simultaneous

recordings in the two areas, will further test the roles of PFC and the striatum in working memory. Loss of working memory is a devastating feature of many neurodegenerative diseases; a detailed understanding of the corticostriatal network's role may illuminate new therapeutic targets for cognitive deficits in these conditions.

Disclosures: D. Huie: None. **K. Ghose:** None. **C. Martinez-Rubio:** None. **T.M. Herrington:** None. **E.N. Eskandar:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Environmental enrichment during development impairs learning in a serial multiple choice task in adult female rats

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Abstract: This study demonstrated that environmental enrichment post-weaning through early adulthood impaired rats' ability to learn a complex cognitive task, namely, the serial multiple-choice task. Environmental enrichment has been shown to have putatively positive effects on neural, behavioral, and cognitive systems in rodents. Enrichment during development increases brain size and cortical thickness, enhances neurogenesis in the hippocampus, and enhances synaptic plasticity (Van Praag, Kempermann, & Gage, 2000). Exposure to environmental enrichment is linked to decreased anxiety-like behavior and improvements in learning and memory (Mesa- Gresa, Ramos-Campos, & Redolat, 2014; Van Praag et al., 2000). Even when exposure to enrichment is brief, it has been found to have long lasting effects (Peña, Prunell, Rotllant, Armario, & Escorihuela, 2009). In this study, female rats were housed in groups of 2-3 per cage from postnatal days 21-60 (P21-60). DuringP21-89, rats experienced either an enriched environment or a standard environment. Environmental enrichment consisted of access to a running wheel, small hiding places, and toys to manipulate and chew. Enrichment was varied weekly so that cages always had 3 different toys and the same toys would not be encountered again for 3 weeks. The standard environment was never changed. Enrichment was discontinued on P90. On P90-95, rats were shaped to nose-poke for water prior. On P96-141 SMC task training was conducted. Rats learned to nose poke receptacles on the 8 walls of an octagonal

chamber in a patterned sequence of responses: 123-234-345-456-567-678-781-818, where digits represent the clockwise position of successive correct receptacles in the circular array and dashes indicate brief pauses that served as phrasing cues. Rats had 90 minutes to complete up to 20 patterns daily for 900 total patterns. The pattern consisted of three element types, namely, within-chunk, chunk-boundary, and violation elements, which are included to assess different cognitive learning mechanisms. Results indicated that environmental enrichment impaired learning for chunkboundary elements but not other element types. Planned comparisons based on a significant enrichment x block of 20 patterns interaction indicated that rats with enrichment made significantly fewer correct responses on chunk-boundary elements than rats without enrichment on days 2-10, 12, and 18. Thus, contrary to prior reports indicating positive effects of early environmental enrichment on adult learning early life, exposure to environmental enrichment impaired performance on serial pattern learning in the SMC task in female rats.

Disclosures: M.E. Miller: None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: R01-EY019882

P30-EY008126

Title: Event-related potentials and oscillatory activity indexing visual working memory capacity limits in nonhuman primates

Authors: *K. FUKUDA, J. D. SCHALL, G. F. WOODMAN;
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Abstract: Visual working memory allows us to actively maintain a severely limited amount of visual information by a network of activities across different brain areas. Studies of human event-related potentials (ERPs) and oscillatory electroencephalogram (EEG) have revealed electrophysiological indices of the capacity-limited working memory storage, but their underlying neural mechanisms still remain to be understood. In contrast, intracranial recordings of nonhuman primates have provided essential insights about working memory activity in the

brain, but it is unclear whether monkey show ERPs and EEG activity that is related to capacity-limited working memory storage as humans do. To address this critical gap in our understanding, our present work establishes the ERP and EEG activity in nonhuman primates that exhibit the hallmark capacity limit during working memory storage. We surgically implanted a set of electrodes on the surface of skull of two macaque monkeys to enable qualitatively similar recording properties as the typical human scalp ERP and EEG recordings. After training, we found that monkeys are capable of maintaining multiple objects (~3 objects) in visual working memory. Moreover, our recordings from the implanted electrodes revealed multiple ERP and oscillatory EEG measures that show the hallmark of a capacity limit across the different epochs of the visual working memory task (i.e., encoding, maintenance and response selection). Our findings establish a set of electrophysiological measures of visual working memory capacity limits in nonhuman primates, building on the success of similar markers in defining the nature of these capacity limits across human subjects.

Disclosures: K. Fukuda: None. **J.D. Schall:** None. **G.F. Woodman:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Navigation trajectories and eye-movement patterns in rhesus monkeys (Macaca mulatta) during learning of object-value associations in a virtual reality environment

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Abstract: Previous studies have shown that visual fixations are instructive in the process of considering, comparing and integrating values of two or more objects in mutually-exclusive forced-choice tasks. Additionally, exogenously manipulating fixations can bias these types of decisions. Whether these dynamics are altered in more immersive, naturalistic situations wherein subjects freely explore their environments has not been determined. How patterns of navigation and visual fixation evolve during learning of object-value associations in this type of task have

also not been explored. To examine this, we designed a virtual reality environment using an open-source video game engine (Unreal Engine 3) that can interact in real-time with Matlab. Two male rhesus monkeys (*Macaca mulatta*) were trained to perform a two-alternative forced choice task in a virtual Y-maze. In each training session, three colored discs were assigned high, intermediate or low reward values. Color-reward value associations were stable within a training session, but colors and their associated reward values were arbitrarily chosen between training sessions. Our analyses of movement trajectories through the virtual Y-maze show that animals are able to rapidly learn object-reward value associations through trial-and-error. When at the branching point of the Y-maze and choosing which colored disc to navigate towards, monkeys rapidly made saccades between both options. Within a trial, the proportion of time spent fixating on each disc predicts which disc will be chosen prior to movement towards that disc. As animals had no indication of object-reward value associations at the beginning of a training session, but learned them through trial-and-error, changes in eye movement patterns over the course of learning were also observed. These results validate virtual reality environments as learning paradigms for use in non-human primate electrophysiological studies. Furthermore, our results support theories that movement trajectories and visual fixations provide useful insights into subjects' learning, decision-making and choice. Since eye movement patterns evolve over the course of learning object-value associations, we suggest that patterns of visual fixation can be used to infer confidence in a subject's choice on a single-trial level.

Disclosures: R.A. Gulli: None. **G. Doucet:** None. **J.C. Martinez-Trujillo:** None. **S. Williams:** None.

Poster

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Title: Inhibitory role of cholinergic interneurons in the dorsomedial striatum via muscarinic M4 receptors on reversal and extinction learning

Authors: *K. OKADA[1], K. NISHIZAWA[2], R. FUKABORI[2], N. KAI[2], A. SHIOTA[3], M. UEDA[3], Y. TSUTSUI[4], S. SAKATA[1], N. MATSUSHITA[5], K. KOBAYASHI[2];
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Abstract: The ability to flexibly adapt current behavior to a changing environment is vital for the survival of animals. The neural mechanisms underlying behavioral flexibility have implicated the contribution of the prefrontal cortex-basal ganglia circuit. In this study, we provide the evidence that the flexibility is suppressed through the cholinergic function in the dorsomedial striatum (DMS). First, we conducted the selective elimination of cholinergic interneurons in transgenic rats by the immunotoxin-mediated cell targeting, to address the role of cholinergic interneurons in the DMS on behavioral flexibility. Elimination of cholinergic interneurons in the DMS, but in dorsolateral striatum (DLS), resulted in the enhancements of reversal/extinction learning on place discrimination task in the cross maze. Cholinergic ablations either in the DMS or DLS did not affect the acquisition of original place learning. The ameliorations were prevented by the infusion of a non-selective muscarinic receptor agonist (oxotremorine methiodide) into the DMS, either during the acquisition or reversal/extinction learning. Furthermore, we conducted the gene silencing of M1 and M4 muscarinic receptor in the DMS, by lentiviral expression of short-hairpin RNA. Selective M4 receptor knockdown imitated the reversal learning enhanced by the cholinergic elimination, whereas M1 receptor knockdown showed the normal performance of reversal learning. Our results indicate that the cholinergic interneurons in the DMS inhibit the behavioral flexibility, mainly through the M4 muscarinic receptor.

Disclosures: K. Okada: None. **K. Nishizawa:** None. **R. Fukabori:** None. **N. Kai:** None. **A. Shiota:** None. **Y. Tsutsui:** None. **M. Ueda:** None. **N. Matsushita:** None. **S. Sakata:** None. **K. Kobayashi:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH MH083809

Title: The role of the medial prefrontal cortex in resolving mnemonic interference: Evidence from an olfactory matching to sample task

Authors: *G. J. PETERS, D. M. SMITH;
Psychology, Cornell Univ., ITHACA, NY

Abstract: A growing body of research suggests that a critical function of the prefrontal cortex (PFC) is to resolve mnemonic interference. Humans with PFC damage are more susceptible to interference and neuroimaging data indicates a PFC role in tasks that require interference resolution. Previously we showed that the medial prefrontal cortex (mPFC) is critically involved in learning and performance of concurrent odor discrimination, but not blocked discrimination learning, suggesting an mPFC role whenever many memory items must be managed effectively for task performance. We also found that the mPFC promotes the retrieval of previously learned odors and is critical for resolving proactive interference (Peters et al. 2013, Learn & Mem: 20(4) 201-9). However, our proactive interference task involved learning two conflicting lists of odor discrimination problems so we could not rule out the possibility that rats with mPFC inactivation performed poorly because they could not respond appropriately to the change from one list to the next. In the present study, we examined the role of the mPFC in resolving interference in a single task that did not involve any changes in task conditions. Rats were trained on an olfactory continuous match-to-sample task in which they were presented with cups containing odorized digging medium (12 odors presented 8 times each, in a predetermined order). They were rewarded for digging when the current odor matched the previously presented odor. Errors were recorded whenever the rat dug in a cup that did not match the previous odor. This task involves substantial interference, particularly when the current odor cue was presented recently but did not match the previous cue. Rats made very few errors when the current odor had not been presented within the last 20 trials. However, performance declined significantly when the current odor was presented 11-20 trials ago and declined further when the odor was presented within the last 10 trials ($F(2,10) = 52.930$, $P < 0.001$), suggesting that memory intrusions from recently presented odors caused interference. In order to determine whether the mPFC is involved in this task, we injected muscimol or saline solution into the mPFC (prelimbic and infralimbic cortices) on alternating sessions using a within subjects design. Rats were significantly impaired on the muscimol sessions, relative to the saline control sessions ($t(5) = 2.740$, $P < .05$). These results indicate that the mPFC plays a role in resolving mnemonic interference, even when there is no change in the strategy or rules needed to perform the task.

Disclosures: G.J. Peters: None. **D.M. Smith:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: 5HT2A receptor blockade in the dorsomedial striatum attenuates a behavioral flexibility deficit in the BTBR mouse

Authors: *D. A. AMODEO[1], A. SYED[2], J. A. SWEENEY[3], M. E. RAGOZZINO[2];
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Abstract: Recent findings indicate that individuals with autism spectrum disorder (ASD) exhibit behavioral inflexibility as shown by a probabilistic reversal learning deficit. The deficit results from a selective inability to maintain a new choice pattern after initially selected (regressive errors). In a similar manner, the BTBR T+ tf/J (BTBR) mouse shows a probabilistic reversal learning deficit due to a specific increase in regressive errors compared to that of B6 mice. Past studies demonstrated that the rodent dorsomedial striatum is one brain area that is critical for reducing regressive errors during reversal learning. Recently, we demonstrated that acute systemic treatment with the 5HT2A receptor antagonist, M100907 attenuated a probabilistic reversal deficit in BTBR mice by specifically decreasing regressive errors. The present experiment examined whether an infusion of M100907 into the dorsomedial striatum attenuates a probabilistic reversal learning deficit in BTBR mice. Mice were tested in a spatial discrimination task using a 80/20 probabilistic reinforcement procedure. In the spatial discrimination, mice were tested on acquisition and reversal learning across two consecutive days. Mice learned to obtain a cereal reinforcement from the "correct" spatial location (reinforced on 80% of trials) compared with the "incorrect" spatial location (reinforced on 20% of trials). The learning criterion in both phases was choosing the 'correct' location on 6 consecutive trials. Five minutes prior to the reversal learning phase, mice received bilateral infusions of vehicle or 0.2 ng M100907 into the dorsomedial striatum. As observed previously, BTBR mice were not impaired on initial learning of the spatial discrimination compared to that of B6 mice. Vehicle-treated BTBR mice were impaired on probabilistic reversal learning compared to that of vehicle-treated B6 mice. M100907 treatment into the dorsomedial striatum attenuated the reversal learning deficit in BTBR mice. M100907 treatment improved probabilistic reversal learning in BTBR mice by selectively reducing regressive errors. M100907

treatment had no effect on reversal learning in B6 mice. The findings suggest that the dorsomedial striatum is one brain area with altered functioning that contributes to behavioral inflexibility in BTBR mice and possibly ASD individuals. Because 5HT2A receptor blockade in the dorsomedial striatum rescued the probabilistic reversal learning deficit in BTBR mice this treatment may correct altered functioning in this region to improve behavioral flexibility.

Disclosures: D.A. Amodeo: None. **A. Syed:** None. **J.A. Sweeney:** None. **M.E. Ragozzino:** None.

Poster

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NSF GRFP

NDSEG

Title: Medial prefrontal cortex inactivation impairs flexible shifting amongst behavioral strategies and associated hippocampal oscillations

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Abstract: Episodic memories include the personal, spatial, and temporal context of events. The environmental cues that distinguish events thereby help evoke memories of prior experience that can guide adaptive behavior. Because many experiences occur in the same spatial context, brain mechanisms are required to retrieve the most relevant memory in a given situation. Here we describe ongoing work examining how interactions between the medial prefrontal cortex (mPFC) and the hippocampus contribute to selective memory retrieval as rats learned spatial discriminations and reversals in a plus maze. We found that the mPFC contributes to spatial reversal learning by reducing proactive interference across successive reversals; inactivating the mPFC bilaterally spares initial spatial discrimination learning but impairs reversal learning. LFPs recorded simultaneously in CA1 and mPFC showed reliable power fluctuations as rats

approached the choice point in the maze. Strong CA1 beta (12-25 Hz) and theta oscillation (5-12 Hz) as well as weaker delta (2-5 Hz) oscillations accompanied initial learning and reversals . Strong mPFC theta (6 Hz) and delta (4 Hz) oscillations occurred simultaneously. Reversal learning was associated with high 4Hz coherence that peaked just before the choice point and returned to baseline levels when the entered the goal arm. In a hippocampus-independent cue-approach task performed in the same maze, 4 Hz coherence remained high until the discriminative cue was presented. The 4 Hz coherence fluctuations were mirrored by changes in 4 Hz power in CA1. Ipsilateral inactivation of the mPFC only reduced slightly 4 Hz power in CA1, but markedly reduced theta and beta power independent of running speed. Ongoing analyses focus on the effect of mPFC inactivation on the coding of information in CA1 during reversal learning. The results thus far demonstrate that interactions between the mPFC and CA1 are crucial for spatial reversal learning and suggest that these are mediated in part by coordinated and dynamic changes in local field potentials in both structures.

Disclosures: K.G. Guise: None. **M.L. Shapiro:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: Dissociations between medial prefrontal cortical regions in conditioned inhibition

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Abstract: Although inhibitory behavior has been the focus of substantial prior research, most studies have focused on premature responding or motor inhibition. Far fewer studies have considered the process by which individuals learn to omit a response. Yet, learning to withhold a behavioral response when signaled by a cue in the environment is an essential aspect of adaptive behavior. Considerable evidence implicates the prefrontal cortex (PFC) in tasks that require the discrimination of salient environmental cues and suppressing dominant response tendencies in favor of more appropriate goal-directed behaviors. Furthermore, several lines of evidence indicate that different parts of the PFC have distinct roles in controlling behavior. In particular,

the prelimbic cortex (PL) is thought facilitate the generation of a strategy to inhibit a prepotent response, while the infralimbic cortex (IL) appears to be more important for maintaining extensively trained inhibitory behaviors. Thus, the present experiments were designed to elucidate the contributions of PL and IL to the acquisition and maintenance of Pavlovian conditioned inhibition. In Experiment 1, rats received lesions before ten days of training in a compound feature negative discrimination task. The results indicate that PL but not IL is required for learning to inhibit food cup responding in the task. In Experiment 2, rats were over-trained in the compound feature negative discrimination task prior to lesioning. A subsequent retention test showed that damage to IL significantly decreased the discrimination between trial types, suggesting that this region is involved in the continued expression of inhibitory learning after thorough training. Interestingly, PL may also be involved in maintaining inhibitory learning, as evidenced by a marginally significant decrease in the discrimination between trial types. These data support the notion that PL and IL have distinguishable roles in modulating inhibition while contributing important information about the specific role for PL in acquisition of a trained Pavlovian response and IL (and potentially PL as well) in performance. Identifying the neural substrates of inhibitory behavior has important implications for understanding the basis for several disorders in which the ability to suppress inappropriate thoughts and actions is impaired, such as Attention Deficit Hyperactivity Disorder (ADHD), schizophrenia and drug addiction.

Disclosures: H.C. Meyer: None. **D.J. Bucci:** None.

Poster

843. Executive Function: Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NRF 2010-0020408

Title: Enriched environment enhances synaptic plasticity and neurohormones underlying improvement of motor and memory functions

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Abstract: Housing animals in enriched environment (EE) with physical exercise enhances behavioral function. However, the mechanism underlying functional improvement and the changes in gene expression patterns have yet to be elucidated. We attempted to investigate the mechanisms associated with exposure to EE by evaluating gene expression. Six week-old CD-1® (ICR) mice were housed in standard cages (SC) or EE comprising a running wheel, novel objects, and social interaction for 2 months (n=16 each). In these mice, motor and cognitive performances were evaluated using rotarod test and passive avoidance test. Gene expression was also investigated in isolated hemispheres of brain using microarray and gene set enrichment analysis (GSEA) (n=3 each). In behavioral assessment, EE significantly enhanced rotarod performance. In addition, EE significantly enhanced short-term working memory when passive avoidance tests were performed. In open field test to investigate general motor function and novelty-induced anxiety, the EE group showed increase of the distance ratio (inside grid crossings to outside grid crossings) and general motor activity (a total of distance) after 8 weeks. Microarray analysis revealed that genes associated with neuronal activity were significantly altered by EE in the brain. Among the genes, EE significantly increase Drd1 (dopamine receptor D1A), Ppp1r1b (protein phosphatase 1r1b), P2ry12 (purinergic receptor P2Y), Pdyn (prodynorphin), and Oxt (oxytocin). On the other hand, the drastic decrease was observed in Slc6a3 (dopamine transporter) as well as Slc6a4 (serotonin transporter), raising the possibility that presynaptic reuptake of these neurotransmitters might be reduced by EE. GSEA showed that genes involved in synaptic transmission and postsynaptic signal transduction were globally upregulated, while those associated with reuptake by presynaptic neurotransmitter transporters were downregulated. Particularly, both microarray and GSEA demonstrated that EE increased opioid signaling, acetylcholine release cycle, postsynaptic neurotransmitter receptors, but decreased Na+/Cl−-dependent neurotransmitter transporters including dopamine transporter Slc6a3 in the brain. Physical exercise and EE enhanced motor and cognitive function through neuroactive gene expression and synaptic plasticity as efficient use of neurotransmitters similar to a neuropharmacologic treatment. This study was supported by a grant from National Research Foundation (2010-0020408).

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Poster

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Topic: F.02. Animal Cognition and Behavior

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C06RR015455

Title: Greater cognitive flexibility in female rats relative to males in an automated set-shift task

Authors: N. F. BRYANT[1], B. M. COX[1], S. B. FLORESCO[2], *C. M. REICHEL[1];
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Abstract: Deficits in cognitive flexibility are associated with a number of neuropsychiatric disorders, including schizophrenia and substance abuse, which rely on prefrontal cortical function. Furthermore, sex differences in cognitive flexibility exist in neuropsychiatric patients, although the neural mechanisms are unknown. Here, we identify baseline sex differences in cognitive flexibility of rats by assessing the ability to shift behavior in response to new information using an Automated Set-Shift Task (ASST). Male and female Sprague Dawley rats were placed in operant chambers (equipped with levers and lights) for daily sessions, and received a sucrose reward for correct trials. The rats completed ASST in phases: pre-training, visual cue discrimination, extra-dimensional shift (EDS), and a reversal. Previous studies have established that different sub-regions of the prefrontal cortex (PFC) mediate different components of behavioral flexibility. Lesions or inactivation of the medial PFC disrupts EDS, whereas orbitofrontal lesions disrupt reversals. Therefore this task allows us to further investigate the neural mechanisms that underlie different components of cognitive flexibility. Additionally, we analyzed specific error types made during the EDS and reversal, perseverative errors, which measure the rats ability to shift strategies from the previously learned rule, and regressive errors, which measure the rats ability to maintain the new rule. On test days, estrous cycle phase was examined in females to determine if cognitive performance varied across cycle. Females out performed males during the early phases of the task, specifically during pre-training and visual cue discrimination phases. The females also exhibited greater flexibility than the males in the EDS and the reversal learning tasks. Specifically, in the EDS, they made fewer regressive errors than the males, and in the reversal, they required fewer trials to achieve criterion performance. Preliminary findings suggest that estrous cycle phase effects the performance of the females, but specific cycle effects are still being investigated. To date, underlying sexual dimorphisms that are responsible for the behavioral manifestations are unknown. As such, c-Fos expression (as an index of neuronal activity), will be presented from the prefrontal cortices. As sex-specific rodent models of neuropsychiatric disorders are almost non-existent, these experiments may allow for the development of such models and subsequently facilitate a sex-specific approach to pharmacotherapies.

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Poster

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Support: MRC intramural program MC-A060-5PQ14

JSPS Postdoctoral Fellowships for Research Abroad (KW)

Title: Neuronal activity of monkey prefrontal cortex during foraging for multiple targets

Authors: ***M. KUSUNOKI**[1,2], K. WATANABE[1,3], M. KADOHISA[1,2], J. DUNCAN[1,2]; [1]Dept. Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom; [2]Cognition and Brain Sci. Unit, MRC, Cambridge, United Kingdom; [3]Japan Society for the Promotion of Sci., Tokyo, Japan

Abstract: Prefrontal cortex plays an important role in the acquisition and retention of working memory. To address working memory for multiple concurrent items, monkeys were trained with a foraging task in which animals explored a choice array, searching for a number of rewarded target locations, and remembered these locations once they were found. A circular array of four to eight choice locations, marked by white squares, was presented on a touch panel display with an initial fixation point (FP). Each trial started when the animal held a home key and fixated the FP for 1-2.5 sec, then the animal released the home key and touched one of the choice locations when FP changed in color. After 350-450 ms of touch-hold period, the color of the selected location changed to green or red, revealing that the location was a target or a non-target, respectively. When a target was selected, a drop of soft food was given as a reward. The number of the targets was one to three and the trials were repeated until the animal had touched all the target locations, then a cycle of foraging trials ended. The end of each cycle was indicated by blinking the target array. Then cycles with the same targets were repeated three to five times to form a set of cycles. When a new set started, the animals searched targets efficiently with few repeated touches on the same locations in the first cycle (exploration phase). After the initial exploration, their performance improved quickly, with almost no error touches in the later cycles (memory phase). While animals performed the foraging task, we recorded single unit activities in the dorsolateral prefrontal cortex (DLPFC). We found many neurons showing selectivity for the

chosen target before and around the touch period. Many of these neurons showed different activities in the first cycle from those in the repeated cycles, some firing vigorously in the first cycle and others not. There were also neurons specifically firing after the touched location was revealed as target or non-target in the first cycle, but little sustained activity carrying target information from one touch to the next. The results suggest interplay of active and passive memory mechanisms in complex, multi-step behavior.

Disclosures: M. Kusunoki: None. **K. Watanabe:** None. **M. Kadohisa:** None. **J. Duncan:** None.

Poster

843. Executive Function: Learning and Memory II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

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Title: Neural changes between puberty and adulthood related to working memory and response inhibition

Authors: *X. ZHOU[1], D. ZHU[1], K. SAMSON[1], C. J. LEES[1], A. J. BENNETT[2], E. SALINAS[1], T. R. STANFORD[1], C. CONSTANTINIDIS[1];
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Abstract: The prefrontal cortex undergoes a protracted period of maturation that extends beyond puberty and into early adulthood. Concomitant changes have been well documented in executive function, working memory, and impulse control, but little is known about the changes that prefrontal neurons undergo over this time period. To address this question, we tracked behavioral performance and neurophysiological activity in areas 8a and 46 of the prefrontal cortex in monkeys as they transitioned from the time of puberty into adulthood, evaluated by morphological, radiographic, and hormonal indexes of development. Four male macaque monkeys (*Macaca mulatta*) were thus tested with an oculomotor delayed response task, designed

to assess working memory, and an anti-saccade task, designed to assess the ability to withhold impulsive responses. The monkeys performed the working memory task robustly around the time of puberty, though modest further improvements in performance were still observed during adulthood (percentage of correct trials, excluding breaks in fixation, for young: 86.4%±2.4%; adult: 97.7%±0.8%). Activity of prefrontal neurons increased in the adult stage, with the biggest increase in firing rate observed during the delay period of the task, even after accounting for effects of performance. Mean delay-period activity of 124 neurons recorded in the peri-pubertal stage was 11.5 spikes/s; mean delay-period activity of 156 neurons recorded in the adult stage was 15.1 spikes/s, in sessions matched for behavioral performance. A much greater behavioral improvement was observed for the anti-saccade task during adulthood (percentage of correct trials for young: 57.8%±5.6%; adult: 76.5±9.3%). Curves of performance plotted as a function of cue viewing time (tachometric curves) shifted to the left in adulthood, indicating that adult monkeys required less time to detect and interpret the visual stimulus in order to produce a correct saccade away from the target. This improvement in performance was associated with changes in activity that were evident already in the baseline fixation period of the anti-saccade task, prior to the presentation of the cue. The mean baseline rate was 11.3 spikes/s among 266 neurons recorded during the peri-pubertal stage vs. 18.6 spikes/s among 314 neurons recorded during the adult stage. Our results indicate that the immature prefrontal cortex is characterized by lower firing rate during the delay period of a working memory task and the baseline period of the anti-saccade task, suggesting that the development of cognitive capacity is directly related to changes in firing of prefrontal neurons.

Disclosures: X. Zhou: None. **D. Zhu:** None. **K. Samson:** None. **C.J. Lees:** None. **A.J. Bennett:** None. **E. Salinas:** None. **T.R. Stanford:** None. **C. Constantinidis:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH091492

Title: Sex differences in response latencies during an object reversal task in male and female marmosets

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Abstract: Aging in both women and men is associated with a decline in sex hormones and cognitive function; however, research examining the efficacy of hormone replacement therapy on cognitive decline has been mixed. To clarify this relationship, it is necessary to use a more controlled animal model. The common marmoset (Callithrix jacchus) has been proposed as a new primate model of human aging, and may be particularly useful in studying the effects of sex steroids on cognition. In this study, we examined the effect of sex hormones on object reversal in 10 gonadectomized (GDX) male marmosets (mean age 5.5 years) and 11 GDX female marmosets (mean age 3.7 years). Females were implanted with Silastic capsules containing 17-β estradiol (E2, n = 6) or empty capsules (control, n = 5) and males received weekly injections of either testosterone cypionate (T, n = 5) or oil vehicle (control, n = 5). After controlling for differences in age, we found that the females and males did not differ in the number of trials to reach a 90% learning criterion. However, we found that males (M = 7.2 s + 0.68 SEM) had significantly longer response latencies than females (M = 4.47 s + 0.65 SEM), regardless of hormone replacement ($F(1, 16) = 6.97$, $p < .02$). Additionally, we observed that sex affected response latencies depending on trial outcome ($F(1,16) = 6.98$, $p < .02$), with males (M = 7.2 s + 0.68 SEM), but not females (M = 4.47 s + 0.65 SEM), taking significantly longer to respond on incorrect trials. This suggested that males were making errors due to distraction, were more affected by the uncertainty of their choices, or were less motivated by the reinforcement than females. T levels in males were not correlated with response latencies, but were negatively correlated with performance on reversal 3 ($r(8) = .67$, $p = .04$). In contrast, E2 levels in females were associated with shorter response latencies ($r(9) = -.61$, $p = .04$) and poorer performance ($r(9) = .68$, $p = .02$) on reversal 2. We conclude that GDX female and male marmosets performed similarly on the object reversal task, in spite of a slower rate of responding in males. Sex hormones had subtle effects on performance: elevated hormone levels impaired learning performance on this task in both sexes, but the mechanisms involved speed-accuracy trade offs in females and speed-independent mechanisms in males. Future studies using gonadally intact animals are necessary to more fully understand the relationship between sex hormones and cognitive performance in both sexes. Supported in part by NIH grant # MH091492

Disclosures: **M.G. Laclair:** None. **J. Chang:** None. **A. Lacreuse:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant F31 MH099782

McGovern Institute Mark Gorenberg Graduate Student Fellowship

NIH Grant RO1 MH60379

Title: Representation of habitual action sequences in the sensorimotor corticostriatal circuit

Authors: *N. LEMAIRE[1], A. GRAYBIEL[2];
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Abstract: Well-ingrained habits usually consist of a series of actions which are performed in an automated manner to obtain a reward. Lesion studies have revealed that the sensorimotor/dorsolateral striatal circuit is required for the performance of such habits, but how neural activity in this area subserves this functional role is not understood. I designed a task in which each rat learned to perform a unique sequence of three lever presses to obtain a reward. I found that when rats performed the correct sequence in late training sessions, regardless of the specific sequence learned, the response types of the projection neurons and fast-firing interneurons in the dorsolateral striatum fell into three groups: phasic responses around the time of the first lever press, phasic responses around the last lever press/reward delivery, and an extended response during reward consumption. Units exhibited one or a combination of these response types, but the exact timing of each response was precise within units and varied across units. The same units responded differently during incorrect sequence performance. Furthermore, the occurrence of units consistently responsive to specific motor events such as lever presses or lever-to-lever transitions was rare. This data suggests that dorsolateral striatal neurons exhibit a beginning and end activation pattern in a wide variety of habitual action sequences and do not directly encode the specific motor or sensory elements of the behavior. Simultaneously recorded units from associative dorsomedial striatum were much less modulated by this task, consistent with the selective importance of dorsolateral striatum in the performance of habitual behaviors. Finally, I used halorhodopsin to inhibit the terminals from motor cortex in sensorimotor striatum in a subset of trials to examine how this cortical input may be driving the task-related responses of the striatal neurons.

Disclosures: **N. Lemaire:** None. **A. Graybiel:** None.

Poster

843. Executive Function: Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: DC 04845

CVS, CNCS

Title: Supramodal processing in medial prefrontal cortex during audiovisual working memory

Authors: *B. PLAKKE, M. D. DILTZ, L. M. ROMANSKI;
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Abstract: The medial prefrontal cortex (mPFC) is well known for its role in decision making and conflict monitoring, but it is also involved in reward and memory processing. Previous work has demonstrated it receives connections from a wide array of brain regions including the thalamus, limbic structures, striatum, and sensory cortices with robust projections from the superior temporal gyrus, which provides auditory and multisensory input. The mPFC and anterior cingulate (ACC) have been implicated in the processing of vocalizations and may be involved in decisions during audiovisual processing, though few experiments have been done using auditory or audiovisual stimuli. Our previous work has shown that ventrolateral prefrontal cortex (VLPFC) responds to and integrates faces and vocalizations. Neurophysiological recordings during audiovisual working memory tasks demonstrate a role for VLPFC in remembering faces and vocalizations. In the current study, we asked whether mPFC might play a role in audiovisual working memory, particularly related to decision making. We recorded from mPFC (areas 24, 32) in rhesus macaques while they performed an audiovisual non-match-to-sample (AV-NMTS) task. During the task, subjects attended the Sample (an audiovisual movie clip of a species-specific vocalization paired with a corresponding facial gesture) and were required to detect a non-matching stimulus, i.e. one whose auditory or visual track differed from the Sample movie. Subjects received a juice reward for correctly detecting the non-match with a button press. Preliminary data from 218 single-units showed that mPFC neurons were active during several task epochs including the sample presentation (55%), the delay period (57%) and the non-match (63%) period. Examination of the non-match period revealed few neurons with preference for the face or vocal component. Only a small number of cells had responses to passively presented faces and/or vocalizations. This differs from VLPFC where neuronal responses during the non-match period or during passive presentation were shown to be specifically related to face or vocalization components (Hwang et al., 2008). Overall, this supports the notion that mPFC neuronal activity may be supramodal and mainly reflects the decision or mnemonic processing during the task.

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Poster

843. Executive Function: Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01MH095894

DOD W81XWH-11-1-0584

FRSQ 25559

Title: Contributions of anterior cingulate gyrus and anterior insula to social learning of food preferences

Authors: ***E. DU**[1,2], J.-F. GARIÉPY[3,2], D. L. XIE[1], J. ERB[1], M. L. PLATT[4,3,2,1];
[2]Duke Inst. for Brain Sci., [3]Ctr. for Cognitive Neurosci., [4]Dept. of Neurobio., [1]Duke Univ., Durham, NC

Abstract: Social learning is the process by which behavior is shaped by the actions of other individuals. Although individuals of many species engage in social learning, the neural mechanisms mediating this process remain poorly understood. To address this question, we developed an experimental paradigm in which monkeys learn the value of novel foods either from direct experience or by watching a demonstrator monkey taste the food. To assess learning, we quantified the time monkeys spent looking at good and bad foods as an implicit measure of value. Gaze was monitored at 1000 Hz with an infrared video eye-tracker (EyeLink) while foods were presented. We found that monkeys spent more time looking at good foods, whether learned through direct experience or through observation of a demonstrator monkey. Viewing preferences reversed within one session following a reversal in color-flavor associations. To determine the neural mechanisms mediating social and nonsocial learning, we injected muscimol, a GABA-A receptor agonist, into areas of prefrontal cortex implicated in learning, prior to reversing color-flavor associations. We hypothesized that inactivation of anterior cingulate gyrus (ACCg) would impair social learning, but that inactivation of insula would impair learning of flavors from direct taste experience. As predicted, we found that inactivating ACCg temporarily impaired social learning, while leaving learning from direct experience intact.

Saline injections into the same area had no effect on learning. By contrast, muscimol injected into anterior insula temporarily impaired learning from direct taste experience, but left social learning intact. Saline injections into anterior insula had no impact on learning. These findings suggest parallel processing streams involving the ACCg and anterior insula mediate learning from others and learning from direct experience of taste.

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Poster

843. Executive Function: Learning and Memory II

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Program#/Poster#: 843.20/TT8

Topic: F.02. Animal Cognition and Behavior

Title: Behind the Mask: Neurobiological indicants of emotional resilience and cognitive function in wild raccoons (Procyon lotor)

Authors: *K. G. LAMBERT[1], M. BARDI[1], T. LANDIS[1], M. M. HYER[1], A. RZUCIDLO[1], S. GEHRT[2], C. ANCHOR[3], D. JARDIM MESSEDER[4], S. HERCULANO-HOUZEL[4]; [1]Psychology, Randolph Macon Col., Ashland, VA; [2]Envrn. and Natural Resources, Ohio State Univ., Columbus, OH; [3]Forest Preserve District of Cook County, Elgin, IL; [4]Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: At the turn of the 20th century, before the rat became the prototypical mammalian model of behavioral and neuroscience laboratory explorations, scientists attempted to systematically evaluate the cognitive abilities of raccoons (Pettit, 2010). Due to the raccoon's apparent cognitive prowess, scientists encountered insurmountable challenges to maintaining these animals in the laboratory. Since that time, very little research has been conducted on the neurobiology of these animals. Accordingly, the purpose of the current study was to extend systematic explorations of raccoons to appropriate field settings. As these animals are observed in natural field settings, and representative brains examined as they become available, interesting findings are emerging. Data in the current series of observations of animals residing in natural habitats in Illinois and Florida reveal the following findings: (1) the raccoon cortex and cerebellum have high neuronal densities (assessed via isotropic fractionation) at levels that are intermediate between primates and non-primate mammals (2) the raccoon cortex contains von Economo neurons thought to be involved in advanced social/cognitive functions, (3) raccoons in

urban habitats that forage for food in human refuse sources reveal significantly less resilient stress hormone ratios (higher CORT, lower DHEA) than counterparts foraging in natural areas such as streams, (5) adult raccoons exhibit extended bouts of social behavior such as reciprocal grooming and rough-and-tumble play and, finally (6) raccoons exhibit advanced problem solving abilities and persistent novelty exploration. More specifically, the density profile of neurons in the cerebral cortex suggests an apparently unique evolutionary positioning of the raccoon. For example, the raccoon cortex (22 g) contains 453 million neurons whereas the pig cortex, a larger mammal with a larger cortex weighing 36 g, contains 293 million neurons. The convergence of evidence emerging from these preliminary studies in relevant field settings suggests that continued investigations of this species will likely reveal new insights related to the neurobiology of opportunistic mammals.

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Poster

843. Executive Function: Learning and Memory II

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Program#/Poster#: 843.21/TT9

Topic: F.02. Animal Cognition and Behavior

Support: NI 618/5-1

Title: Dopamine D1 and D2 receptors differentially modulate neuronal rule coding in primate prefrontal cortex

Authors: ***T. OTT**[1], S. N. JACOB[1,2], A. NIEDER[1];
[1]Animal Physiol., Inst. of Neurobiology, Univ. of Tübingen, Tübingen, Germany; [2]Charité Berlin, Berlin, Germany

Abstract: The capacity to flexibly change behavior in a goal-directed way according to abstract rules depends on the integrity of the prefrontal cortex (PFC). We have shown previously that single neurons in the PFC encode abstract numerical rules. The cellular mechanisms giving rise to the rule-related neuronal activity are, however, poorly understood. Since the PFC receives strong projections from the dopaminergic midbrain modulating executive functions such as working memory, we hypothesized that dopamine receptors in the PFC are involved in

regulating abstract rule coding. Two rhesus monkeys (Macaca mulatta) were trained to compare numerosities and to switch flexibly between two abstract numerical rules based on a rule cue. The “greater than” rule required the monkeys to release a lever if the first test display showed more dots than the sample display, whereas the “less than” rule required a lever release if the number of items in the test display was smaller compared to the first test display. We recorded single neurons in the lateral PFC while simultaneously applying the dopamine D1 receptor (D1R) agonist SKF81297, the D1R antagonist SCH23390, or the D2 receptor (D2R) agonist quinpirole to the vicinity of the cells using iontophoresis. We report that both dopamine D1Rs and D2Rs facilitated rule coding of PFC neurons, albeit by distinct physiological mechanisms: D1R stimulation suppressed neuronal firing while enhancing responses to the preferred rule, an effect mainly mediated by narrow-spiking (putative inhibitory) neurons. D2 receptor stimulation, instead, excited neuronal firing while suppressing responses to the nonpreferred rule primarily via broad-spiking (putative pyramidal) neurons, thus also enhancing neuronal rule coding. Thus, prefrontal dopamine is essential to maintain rule coding in the PFC. These findings highlight complementary modulatory contributions of dopamine receptors to the neuronal circuitry mediating executive functioning and goal-directed behavior.

Disclosures: **T. Ott:** None. **S.N. Jacob:** None. **A. Nieder:** None.

Poster

843. Executive Function: Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant 081162

Title: Dynamical patterns of clustered gamma activity in the frontoparietal system

Authors: ***T. A. ROMANO**[1], S. L. BRESSLER[1,2], R. F. SALAZAR[2], N. M. DOTSON[2], C. M. GRAY[2];
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Abstract: The prefrontal cortex (PFC) and posterior parietal cortex (PPC) function as a FrontoParietal Network (FPN) and play a role in higher cognitive functions, such as working memory (WM). Despite the network's well-established involvement in WM, little is known

about the spatio-temporal properties of gamma activity in the FPN during WM. Unit activity and local field potentials (LFPs) were recorded from PFC (n= 113) and PPC (n= 160) in two macaque monkeys performing a rule-based, oculomotor delayed match-to-sample (DMS) task. Time-frequency (t-f) plots of spectral power were computed from MultiVariate AutoRegressive (MVAR) LFP time-series models separately for correct and incorrect trials. Cluster analysis was applied to t-f power plots of FPN LFP activity. Sustained, ramping, and sensory-related dynamical t-f patterns of gamma activity were characterized from electrode clusters. Patterns related to WM were distinguished from other co-occurring processes, such as anticipation or visual response to sample. We observed sparsely distributed electrode clusters that included both PFC and PPC sites, and t-f varying dynamical patterns of FPN gamma activity. During correct trials, as compared to incorrect trials, a larger number of electrodes displayed sustained gamma activity that was either elevated or depressed relative to the presample period, and sensory-late gamma activity that was elevated from presample period. The observed dynamical patterns of gamma activity display heterogeneity of time-dependent dynamics and these dynamics are important for successful WM in the FPN.

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Poster

844. Executive Function: Network Activity

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Topic: F.02. Animal Cognition and Behavior

Support: R01MH077779

T32GM008244

Title: Modulation of neural synchrony in prefrontal and parietal cortex of monkeys as a function of executive processing demand in a context processing task

Authors: J. A. WESTERBERG[1], R. K. BLACKMAN[2], S. SAKELLARIDI[3], *M. V. CHAFEE[4];
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Abstract: We investigated prefrontal network dynamics in monkeys during the performance of a context processing task that engages both working memory and executive control, and is known to measure cognitive deficits in schizophrenia. In this task, monkeys viewed a cue followed by a probe stimulus (both consisting of a pattern of dots). The motor response to the probe depended on the preceding cue. One cue pattern was designated 'A' and one probe pattern was designated 'X'. 'AX' trials required a target response (leftward joystick movement). All other cue and probe patterns were collectively designated 'B' (cues) or 'Y' (probes), and any cue-probe sequence that was not 'AX' required a nontarget response (rightward movement). We used multi-electrode arrays to record local field potentials (LFPs) in prefrontal and posterior parietal cortex during task performance. We utilized two trial sets to vary executive processing demand. In 'Prepotent' sets, most trials were of the 'AX' sequence, setting up the habit to respond 'target' whenever the 'X' probe appeared. In 'Balanced' trial sets, the four possible trial sequences were presented in equal proportion, so that 'X' probes were associated with target and nontarget responses with equal frequency. Neural synchrony, as measured by oscillatory power in time-frequency analyses of the LFPs, was strongest during the probe period and at the time of the motor response. This was the time in the trial when the identity of the cue and probe had to be compared to determine response direction. Synchrony during the probe period was markedly stronger on 'A' cue trials, when the identity of both the cue and probe had to be taken into account to determine response direction, in comparison to 'B' cue trials, during which no cue-probe comparison was required (all 'B' cues required a fixed 'nontarget' response irrespective of the probe). This difference was particularly pronounced on balanced trial sets when the 'A' cue was maximally informative about the direction of the required response to the probe. We in addition observed several differences between cortical areas. Synchrony was stronger in the gamma band in parietal cortex, and in the beta band in prefrontal cortex. In prepotent trial sets, we found that power in the alpha band in parietal cortex was enhanced during the delay period following 'B' cues. This may reflect the greater need to use 'B' cue information stored in working memory as a 'no go' signal to override the prepotent target response in the prepotent sets. These findings provide evidence that patterns of neural synchrony in prefrontal networks reflect the use of working memory to mediate conditional responding to subsequent stimuli.

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Poster

844. Executive Function: Network Activity

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Topic: F.02. Animal Cognition and Behavior

Support: UNH Research Leveraging Initiative Grant

Title: Encoding of information about actions and context in rat prefrontal cortex: Effects of thalamic inactivation

Authors: *B. A. WORMWOOD[1], M. J. FRANCOEUR[2], K. D. ONOS[1], R. L. A. MILLER[1], C. R. LEHET[1], E. K. BRASLEY[1], L. E. MANZO[1], B. M. GIBSON[1], R. G. MAIR[1];
[2]Psychology, [1]Univ. of New Hampshire, Durham, NH

Abstract: Medial thalamus is organized to regulate the activity of prefrontal cortex (PFC) and PFC-related circuits that play a critical role in flexible goal-directed responding. The mediodorsal nucleus is the primary source of specific thalamo-cortical projections to middle layers of PFC and receives direct cortico-thalamic and indirect cortico- striato-pallidal input from PFC. Midline and intralaminar nuclei are important sources of nonspecific thalamocortical projections to deep and superficial layers of PFC as well as thalamic input to anatomically-related areas of the basal ganglia and hippocampus. Medial thalamic lesions have well-known effects on prefrontal function that include impairment of delayed non-matching to position tasks (DNMTP). To elucidate the influence of medial thalamus on prefrontal function, we examined the effects of temporary thalamic inactivation on the response properties of prefrontal neurons in rats performing a 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). 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. Consistent with previous results, unilateral inactivation had no significant effect on behavior. We compared waveforms, inter-spike-interval histograms, and cluster analyses to identify single neurons that were active across all three days. For most neurons, comparable PETH results were observed on days 1 and 3, confirming the stability of our recordings. On day 2, inactivation was consistently associated with decreased neural activity and degraded PETH responses in the ipsi-inactivation hemisphere. These results are consistent with the hypothesis that the medial thalamus plays an active role in shaping response properties of PFC neurons.

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Poster

844. Executive Function: Network Activity

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Support: UNH Research Leveraging Initiative Grant

Title: Encoding of information about action and context by medial thalamus in the rat: Comparisons to prefrontal cortex

Authors: *R. L. MILLER[1], K. D. ONOS[2], M. J. FRANCOEUR[2], B. A. WORMWOOD[2], E. B. SMEDLEY[2], C. J. THERIAULT[2], E. E. RYDER[2], B. M. GIBSON[2], R. G. MAIR[2];
[1]Univ. of New Hampshire, Newmarket, NH; [2]Univ. of New Hampshire, Durham, NH

Abstract: Medial thalamus plays a critical role in prefrontal function. Previous work in our lab has shown that lesions of the mediodorsal nucleus (MD) produce delay-dependent impairment of delayed matching and non-matching (DNMTP) to position tasks while larger lesions involving adjacent midline (M) and rostral intralaminar (IL) nuclei produce delay-independent impairment of these tasks, comparable to the effects of prefrontal lesions. To elucidate the influence of MD, M, and IL on prefrontal function we recorded the activity of neurons in these nuclei in rats performing a dynamic delayed non-match- to- position (DNMTP) task. Activity was recorded with tetrode arrays that were advanced incrementally through thalamus across 40 to 60 recording sessions. 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) and with spatial location as place fields. Isolated activity was recorded from 2,030 neurons in 3 rats completed to date. Significant behavioral correlations defined by PETH responses beyond the 99% confidence interval were observed for 267 (13%). PETH analyses revealed a wider range of response types than we observed in parallel studies of prefrontal cortex. Of the 267 neurons with behavioral correlates, 140 (52%) were consistent with the 10 response types that accounted for 95% of behaviorally correlated cells in prefrontal cortex. In general, thalamic neurons exhibited higher levels of activity and PETH patterns that were not as sharp as those found in prefrontal cortex. Video tracking analyses revealed place fields consistent with PETH results. Our results are consistent with the hypothesis that the medial thalamus plays an active role in shaping response properties of prefrontal cortex.

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Poster

844. Executive Function: Network Activity

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Topic: F.02. Animal Cognition and Behavior

Support: UNH Research Leveraging Initiative Grant

Title: Complex encoding of information about actions and context in rat prefrontal cortex

Authors: ***M. J. FRANCOEUR**, K. D. ONOS, B. A. WORMWOOD, R. L. A. MILLER, C. R. ELLIS, D. C. CHASE, H. A. COLE, B. M. GIBSON, R. G. MAIR;
Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Medial prefrontal cortex (mPFC) plays a critical role in executive functions that allow us to respond flexibly while performing goal-directed actions. To understand how adaptive behavior emerges from neurons that comprise mPFC we recorded neural activity in rats as they performed a dynamic delayed non-match- to- position (DNMTP) task. Activity was recorded with tetrode arrays that were advanced incrementally through mPFC across 40 to 60 recording sessions. 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) and with spatial location as place fields. Isolated activity was recorded from 900 neurons in 6 rats. Significant behavioral correlations defined by PETH responses beyond the 99% confidence interval were observed for 288 (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 preparation to respond (before trials began), execution of specific actions, action-outcomes, and memory delay. Video tracking analyses revealed place fields consistent with PETH results. For instance, cells with movement-related PETHs showed maximal activity on paths connecting levers while cells with reinforcement-related PETHs had maximal activity at locations where reinforcement was delivered. A subset of neurons demonstrated conjunctive coding responding to both behavioral events and specific locations within the arena, for instance reinforcement-related activity at only one of four locations where reinforcement was delivered. The distribution of responses was

indicative of both functional specialization and broad distribution of shared information about actions and reinforcement in subareas of prefrontal cortex. Our results suggest that executive function mediated by mPFC emerges from discrete populations of neurons that encode basic information related to the planning, execution, and outcome of learned actions and the context in which they occur.

Disclosures: **M.J. Francoeur:** None. **K.D. Onos:** None. **B.A. Wormwood:** None. **R.L.A. Miller:** None. **C.R. Ellis:** None. **D.C. Chase:** None. **H.A. Cole:** None. **B.M. Gibson:** None. **R.G. Mair:** None.

Poster

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Support: NIMH Grant MH081163

NINDS Grant NS059312

McKnight Memory and Cognitive Disorders Award

Title: Large-scale correlation dynamics reveal interplay between integration and segregation during visual working memory

Authors: ***N. M. DOTSON**, C. M. GRAY;
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Abstract: Cognitive processes, such as working memory, require large-scale cooperation among widespread cortical and sub-cortical brain regions. Importantly, these processes must achieve an appropriate balance between functional integration and segregation, which are thought to be mediated by task dependent spatiotemporal patterns of correlated activity. In a previous study, we used cross-correlation analysis to estimate the incidence, magnitude, and relative phase angle of temporally correlated activity from simultaneous local field potential recordings (8 - 25 Hz) in prefrontal and posterior parietal cortex in monkeys performing an oculomotor, delayed match-to-sample task. We found long-range intra-parietal and fronto-parietal correlations that displayed a bimodal distribution of relative phase values, centered near 0° and 180°, suggesting a possible basis for functional segregation among distributed networks.

Both short- and long-range correlations displayed striking task-dependent transitions in strength and relative phase, including 180° shifts in the relative phase. We have subsequently found a similar set of phase relationships from simultaneous recordings in the frontal, temporal, parietal and visual cortices using a 256-channel microelectrode recording device, which spans an entire cerebral hemisphere. Commonly, nearby recordings maintain a near 0° relative phase relationship, while long-range correlations are concentrated near 0° and 180°. For example, the relative phase of V1 signals are largely all near 0°; while, simultaneously, these sites are closer to 180° with recordings in parietal and prefrontal areas. These findings illustrate that cognitive events are accompanied by wide spread changes in the pattern of temporal coordination across the brain.

Disclosures: **N.M. Dotson:** None. **C.M. Gray:** None.

Poster

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Support: NIMH Grant MH081163

NINDS Grant NS059312

McKnight Memory and Cognitive Disorders Award

Title: Analysis of the spatio-spectral patterns underlying visual working memory and their architectonic underpinnings

Authors: ***S. J. HOFFMAN**, N. M. DOTSON, T. LYNN, C. M. GRAY;
Cell Biology/Neuroscience, Montana State Univ., Bozeman, MT

Abstract: Electrophysiological techniques with the requisite time and spatial resolution to accurately describe cortical dynamics during cognitive processing are in their infancy. To advance the field, we developed a large-scale recording device, for use in non-human primates, with 256 independently moveable microelectrodes, spaced at 2.4 mm intervals, spanning an entire cerebral hemisphere. Recordings have been completed in one animal performing an object-based, delayed match-to-sample task. Data were collected over 6 months, yielding 817 recordings of neuronal activity from 46 cortical areas. An analysis of the spectra from signals

across the cortex indicated that many cortical areas exhibit distinct spectral properties. Furthermore, this analysis revealed a distinct pattern of spectral content (power multiplied by frequency normalized by the sum of power across all frequencies) across the cortex. Prefrontal areas showed the highest percentage of spectral content between 25 and 35Hz. Posterior parietal and temporal areas were dominated by 12-25Hz power, while visual areas showed the highest concentration of low, 1-12Hz power. In foveal V1, a small set of recordings, exhibited strong activity in the gamma band (35-90Hz) during the presentation of a centrally presented sample stimulus. Prefrontal areas also exhibited a high percentage of spectral content in this range during working memory. An important question evident from this spatio-spectral patterning is if the underlying architectonics are correlated with spectral content. To address this, we are determining the relationship between spectral content and architectonic parameters including pyramidal cell density and laminar distribution, myelin density, and the laminar distribution and characterization of the bands of Baillarger.

Disclosures: **S.J. Hoffman:** A. Employment/Salary (full or part-time):; Gray Matter Research. **N.M. Dotson:** None. **T. Lynn:** None. **C.M. Gray:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH RO1-MH082017

Gatsby Foundation

Swartz Foundation

Title: Neurophysiological mechanisms supporting flexible, context-specific, operant and pavlovian behavior

Authors: *J. MUNUERA[1], M. RIGOTTI[1,3], S. FUSI[1], D. C. SALZMAN[1,2,4];
[1]Neurosci., [2]Psychiatry, Columbia Univ., New York, NY; [3]Physical Sci., IBM T. J. Watson Res. Ctr., Yorktown Heights, NY; [4]Psychiatry, NYPSI, New York, NY

Abstract: In everyday life, the relationship between actions and outcomes can vary depending upon context. The presentation of a stimulus thereby can lead to the performance of distinct

actions in different situations. This flexible behavior relies on an ability to represent information about stimuli, contexts, and potential actions and outcomes. Further, agents must integrate this information appropriately to support flexible behavior. A neurophysiological signature of the integration of information about multiple parameters can be found in single neurons when cells respond selectively to combinations of parameters, exhibiting non-linear "mixed selectivity" [Rigotti et al., Nature 2013]. We sought to understand how the encoding and integration of information about multiple parameters relates to performance of a task that demands flexible actions to acquire rewards. We targeted the amygdala and 2 parts of the prefrontal cortex interconnected with the amygdala, the anterior cingulate and orbitofrontal cortices (ACC and OFC). Rhesus monkeys learned to associate each of 4 images (conditioned stimuli, CSs) to an operant action (hold or release a button) in two different contexts. Each context was defined by the set of operant and reinforcement contingencies for the CSs. For each CS, either, both, one, or neither of the operant and reward contingencies differed in the two contexts. In both contexts, correct action execution resulted in reward delivery (unconditioned stimulus, US) for 2 of the CSs, and in successful trial completion without a reward for the other 2 CSs. Failure to complete trials correctly led to a time out and repetition of the trial type. Both pavlovian (anticipatory licking) and operant behaviors indicated that the monkeys understood the rules that linked stimuli and contexts to actions and rewards. All task-relevant variables, including stimulus identity, context, operant action, and expected reinforcement were encoded in in each area, with many single neurons exhibiting mixed selectivity. The encoding of operant actions in amygdala and OFC was particularly surprising given prior studies. These data suggest that the complexity of the task resulted in the sculpting of neural responses properties such that populations of neurons simultaneously integrated information about the many variables needed to support good performance. Further analyses aim to establish whether and how the encoding and formatting of the different types of information account for flexible behavior.

Disclosures: **J. Munuera:** None. **M. Rigotti:** None. **S. Fusi:** None. **D.C. Salzman:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: K08 NS078100

Title: Medial frontal control of striatal neuronal ensembles during interval timing

Authors: E. B. EMMONS, K. L. PARKER, *N. S. NARAYANAN;
Neurol., Carver Col. of Med. / Univ. of Iowa, Iowa City, IA

Abstract: Frontostriatal circuits are central to the temporal organization of behavior and are dysfunctional in human disorders such as Parkinson's disease. Here we investigated the nature of the interactions between the medial frontal cortex and striatum in an interval timing task, an elementary cognitive task that involves working memory and attention. We trained rats to perform an interval timing task with 3 and 12 second delays. Animals were then implanted with a cannula for focal drug infusions in the medial frontal cortex and a recording electrode in the dorsal striatum. Concordant with prior work, we found that medial frontal inactivation with muscimol and D1 dopamine blockade with SCH23390 impaired interval timing performance. In addition, striatal neurons were prominently modulated during the interval timing task. Medial frontal D1 blockade did not consistently change the activity of striatal neurons encoding movements such as lever press or release. However, medial frontal D1 blockade altered neuronal activity encoding time as well as field potentials within the striatum. These data suggest that medial frontal networks provide cognitive signals to striatal ensembles. In addition, these results provide evidence that the medial frontal cortex exerts top-down control of other brain areas during performance of an interval timing task. These data are useful in understanding the neural basis of a highly-conserved elementary cognitive behavior and provide insight into how corticostriatal systems interact to guide cognition.

Disclosures: E.B. Emmons: None. **K.L. Parker:** None. **N.S. Narayanan:** None.

Poster

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Support: NIH Grant 7R01MH062349-12

Title: Gating neural activity by a disinhibitory mechanism in cortical circuit

Authors: *G.-Y. R. YANG, J. D. MURRAY, X.-J. WANG;
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Abstract: A disinhibitory circuit has recently been studied across multiple areas in the mouse cortex. The circuit consists of three major types of cortical interneurons that are specialized in their synaptic targets. Specifically, vasoactive intestinal peptide positive (VIP) neurons inhibit somatostatin-positive (SOM) neurons, which in turn inhibit dendrites of pyramidal cells. In this way, VIP neurons disinhibit pyramidal dendrites. *In vivo* experiments have demonstrated that this disinhibitory circuit is recruited by specific behavioral contexts, such as active sensing or receipt of reinforcement signals. However, the function of this disinhibitory circuit remains unclear. To explore the role of dendritic disinhibition in regulating pyramidal cell activity, we studied simple compartmental neuron models endowed with active dendritic processes such as NMDA spikes. We found that disinhibition can effectively mediate a control pathway that opens the gate for signal propagation. Importantly, this control pathway can operate without interfering with the content coded in incoming signals. We found that neuronal dynamics depend critically on the temporal sparseness of dendritic excitation in the incoming stimulus and of dendritic inhibition from SOM cells. If the control pathway is mediated by disinhibition, it can open the gate to signal propagation without directly exciting the pyramidal cell and therefore avoid being confused with the stimulus input. We studied a cortical circuit model comprised of pyramidal cells and three interneuron types: SOM, VIP, and parvalbumin positive (PV). The circuit is constrained by experimentally-measured connectivity and cellular/synaptic dynamics. We analyzed the dynamical regimes wherein disinhibition can be recruited by top-down control signals, regulating signal propagation and network computations. These extended circuit computations can subserve cognitive operations in tasks that require gating.

Disclosures: G.R. Yang: None. **J.D. Murray:** None. **X. Wang:** None.

Poster

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Support: UBACYT 20020100100043

R01-MH086507

Title: GABAergic control of Working Memory: A computational model of the prefrontal cortex

Authors: *S. E. LEW[1,2], K. Y. TSENG[2];
[1]Univ. de Buenos Aires, Capital Federal, Argentina; [2]2Department of Cell. & Mol. Pharmacol., The Chicago Med. Sch. at RFUMS, Chicago, IL

Abstract: A fine tuning between GABAergic transmission and pyramidal cell firing by dopamine in the prefrontal cortex (PFC) has been proposed to play a critical role in the regulation of working memory processes as disruptions of such interactions can result in reduced cognitive impairments similar to those seen following PFC lesion in animal models. Here we employed a modified version of the well-established computational model of working memory developed by Brunel and Wang (2001) to determine how dopamine modulation of GABAergic transmission in the PFC enables input selectivity in pyramidal cells to sustain working memory and its reset. Our PFC model is comprised of a network of 2,000 neurons with an inhibitory/excitatory ratio of 0.25, and includes the following physiological features of dopamine action: (i) dopamine facilitation of prefrontal GABAergic transmission via activation of local fast-spiking interneurons (FSI); (ii) D1 facilitation of NMDAR-mediated response in both pyramidal neurons and FSI. Overall, our data indicate that behavioral outcomes associated with an initial dopamine elevation will increase FSI activity. As a result, the signal detection ratio in the PFC network increases by virtue of reduced recurrent activity in pyramidal neurons. Further simulations revealed that a fine homeostatic interplay between dopamine and FSI is needed to enable PFC output selectivity and stability. A similar dopamine-FSI interaction is required for the formation and retention of working memory, especially in the presence of distractor stimuli. Finally, our model also predicts that phasic activation of FSI by dopamine is an effective mechanism to reset the PFC working memory state back to baseline. Thus, a critical gain of prefrontal FSI function by dopamine is necessary for maintaining PFC network stability, which enables working memory retention and reset.

Disclosures: S.E. Lew: None. **K.Y. Tseng:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Foundation for Anesthesia Education and Research Mentored Research Training Grant-Basic Science

Title: Recovery of consciousness from isoflurane anesthesia is mediated by a structured network of discrete intermediate brain states

Authors: *A. HUDSON[1], D. P. CALDERON[2], D. W. PFAFF[2], A. PROEKT[3];
[1]Anesthesiol., David Geffen Sch. of Medicine, UCLA, Los Angeles, CA; [2]Lab. of Neurobio. and Behavior, The Rockefeller Univ., New York, NY; [3]Anesthesiol., Weill Med. Col. of Cornell Univ., New York, NY

Abstract: How does the brain traverse the vast space of potential neuronal activity states to recover those compatible with consciousness after a gross perturbation? There are several distinct possibilities. The simplest possibility is that the trajectory is a random walk through the parameter space. Another possible trajectory is a smooth directed path towards the region in the parameter space compatible with consciousness. Finally it is possible that en route to recovery, brain activity abruptly jumps between several distinct individually stabilized intermediate states. To distinguish among these possibilities, we analyzed cortico-thalamic local field potentials (LFPs) recorded from rats during recovery of consciousness from isoflurane anesthesia. The titratability of isoflurane allowed us to parametrically perturb ongoing brain dynamics. Our results suggest that, en route to recovery, brain activity is confined to a low dimensional subspace defined by characteristic spectral signatures of the LFPs. Furthermore, differences between states are associated with changes in the global coherence among multiple cortical and thalamic electrodes. Within this subspace, spectral characteristics of the LFPs cluster into discrete metastable states that persist for minutes. Transitions between these metastable states are structured, such that some states form “hubs” that connect groups of otherwise disconnected “spur” states. Thus, this network architecture suggests that the brain must pass through one or more of the hub states to reach wakefulness. These observations begin to characterize the intrinsic dynamics that allow recovery of consciousness from a large perturbation resulting in loss of consciousness and characterized by burst suppression.

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Poster

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Title: Task-dependent modulation of regular-firing and fast-spiking neurons in medial prefrontal cortex during sensory discrimination

Authors: *Z. ZHOU, K. K. SELLERS, C. YU, F. FROHLICH;
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Abstract: The ability to discriminate sensory stimuli defines the limits of sensory processing. Little is known about how the brain performs discrimination of complex sensory input. Here, we delineated the network dynamics of single unit activity in medial prefrontal cortex (mPFC) during discrimination of two-dimensional shapes. We hypothesized that excitatory neurons and inhibitory interneurons in mPFC play differential roles in this visual discrimination task. To test this hypothesize, we trained female ferrets (Mustela putoris furo) to discriminate between two abstract shapes presented on a screen. In this task, ferrets indicated the conditioned stimulus by nose poke (touch) of the touchscreen, and received a water reward from a lick spout. Trials with high and low contrast versions of shapes were interleaved to assess behavior and network dynamics for “easy” and “difficult” demands to discriminate the stimuli. We recorded extracellular neural activity in mPFC with 16-channel electrode arrays. Putative excitatory or inhibitory units were classified by peak-to-peak duration of spike waveforms, and organized by response during trials. Behavioral performance in the easy vs. difficult condition differed significantly with respect to percent correct ($p=0.0001$, ANOVA) and nose-poke reaction time ($p<0.005$, ANOVA). We found three main groups of neurons (by response dynamics) with exclusive modulation of activity for one of the three phases of individual trials: neurons with activity modulated during stimulus viewing (8.0%, n=53), nose-poke (17.1%, n=113), and reward acquisition (14.2%, n=94). Interestingly, we found a larger proportion of putative fast-spiking inhibitory interneurons for the stimulus viewing phase (16.4%, n=24). When units were sorted by task difficulty, the majority of units were again active in specific trial phases: stimulus viewing (easy: n=55; difficult: n=50), stimulus touch (easy: n=91; difficult: n=90), and reward acquisition (easy: n=109; difficult: n=97). Our data demonstrates that freely-moving ferrets are well-suited for combined behavioral and electrophysiological studies. We found that a substantial set of both putative pyramidal cells and inhibitory interneurons in mPFC are exclusively activated by specific aspects of the sensory discrimination task with invariance to task difficulty. In addition, the two cell types differed in their relative contribution to network dynamics, as a function of task phase. Together these data support a model of active engagement of different networks within mPFC as a function of specific cognitive demands.

Disclosures: **Z. Zhou:** None. **K.K. Sellers:** None. **C. Yu:** None. **F. Frohlich:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01MH085666

Title: DREADDing the mediodorsal thalamus and prefrontal inhibition: Potential partners in executive function

Authors: ***B. R. FERGUSON**, W.-J. GAO;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The mediodorsal thalamus (MD) is suggested to play a critical role in executive function through its extensive innervation of the Prefrontal Cortex (PFC). These glutamatergic MD projections synapse not only onto pyramidal neurons, but also gamma-aminobutyric (GABA)-ergic inhibitory interneurons. However, it remains unknown how PFC neurons, particularly inhibitory interneurons, are modulated by MD thalamocortical input. We hypothesized that MD efferents drive PFC inhibition, and further these specific connections are recruited and enhanced in times of acute stress. To explore this possibility, we utilized Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that allow us to selectively target glutamatergic MD neurons, and transiently inhibit their activity in adult SD rats. Following MD inhibition both *in vivo* and *in vitro*, we observed a reduction in PFC inhibition as measured by recording of IPSCs using whole-cell patch clamp electrophysiology. Additionally, we demonstrated that following acute restraint stress, there is an elevation of PFC inhibition, which is coupled with increased MD excitability. These results suggest that the MD directly drives GABAergic neurotransmission in the PFC, supporting its role as functioning critically both in cognition and potentially the cognitive augmentation seen in rodents performing cognitive tasks following acute exposure to stress. In contrast, when the MD is compromised this could have functional consequences leading to cognitive deficits, such as those which manifest in SZ. Future directions involve exploring how PFC inhibition is effected when MD activity is downregulated during exposure to an acute stressor.

Disclosures: **B.R. Ferguson:** None. **W. Gao:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Itch relief is a rewarding behavior

Authors: *L. TA-JEN;
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Abstract: To address whether itch relief is a rewarding behavior, there are several questions need to be investigated. First, whether rewarding circuitry is activated when mice scratch. VTA(ventral tegmental area)- N.acc(nucleus. Accumben) is the most well known rewarding circuitry. When mice get reward like a piece of cheese , the axon terminal projected to N.acc of dopamingeric neuron in the VTA will release dopamine. Therefore, we detected dopamine release in N.acc with microPET. Second, whether scratching behavior will be affacted after lesion of the rewarding circuitry. 2,4,5-trihydroxyphenethylamine (6-OHDA) is used to kill dopamingeric neurons and can retrograde from axonal terminal to cell body to make effect. Thus, I did Intra-accumben injection of 6-OHDA and behavioral experiments of itch. Finally, I did conditioned place performance(CPP) to see whether mice tend to stay in the space where they had ever got itch and scratching or not.

Disclosures: L. Ta-Jen: None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH100820

Title: Unique oscillatory patterns in the rat prefrontal cortex after NMDA receptor blockade

Authors: *B. KOCSIS;
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Abstract: N-methyl-D-aspartate receptor (NMDA-R) hypofunction has been strongly implicated in the pathomechanism of schizophrenia. NMDA-Rs are involved in various aspects of cortical information processing, and their dysfunction leads to cognitive deficits. Prefrontal cortex pathology has been strongly implicated in schizophrenia-relevant cognitive deficits. Acute administration of NMDA-R antagonists elicits psychotic symptoms in human and schizophrenia-relevant signs in rodents, including a strong increase in gamma activity in different cortical areas. This study compared the changes in gamma and low-frequency oscillations in PFC after injection of NMDAR antagonist MK-801 (0.2mg/kg) in seven freely moving rats with those in several surface EEG recordings over the frontal, parietal and occipital cortices. Low-frequency oscillations: In control conditions, on-going hippocampal theta rhythm is usually associated with short (1-10 s) intermittent theta segments in the PFC during active waking whereas theta never appears in PFC during REM sleep. On the other hand, PFC is frequently generating characteristic low-frequency oscillations of its own at ~2 Hz and 4 Hz during waking which may co-occur or alternate with hippocampal theta rhythm. These 2 Hz and 4 Hz rhythms are represented by sharp peaks in the autospectra during active waking behaviors and thus are different from the wide-band delta activity characteristic for quiet waking and slow wave sleep in both PFC, other cortical regions, and hippocampus. After NMDA-R blockade the power of low-frequency oscillations drastically increased in the PFC, including both the intrinsic 4 Hz rhythm and theta which onder this condition persisted for extended periods (1-10 min and longer). Gamma activity: As in other cortical areas, enhancement of gamma activity was also present in the PFC but the frequency characteristics and its temporal dynamic was different. The rise of gamma activity in the PFC started slower and later than in other regions and only reached peak level ~2 hrs after MK-801 injection. The spectrum of MK-801 induced gamma activity was dominated by a large peak in the 50-70Hz range, significantly larger than in any other leads, and showed relatively weak increase in the near-40 Hz range, compared with other cortical areas. PFC gamma activity was strongly modulated by both 4 Hz and theta oscillations. The specific changes in PFC oscillations may affect not only local processing but also long-range functional coupling between PFC and other cortical and hippocampal areas.

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Poster

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Title: Integration of executive control signals across prefrontal cortical-striatal and hippocampal neural ensembles

Authors: *I. OPRIS[1], D. FETTERHOFF[1], C. A. SEXTON[1], L. M. SANTOS[1], J. L. LONG[1], J. V. NOTO[1], B. C. PARISH[1], O. D. JURCHESCU[2], M. ENACHESCU[3], G. GERHARDT[4], D. SONG[5], V. Z. MARMARELIS[5], R. E. HAMPSON[1], S. A. DEADWYLER[1], T. W. BERGER[5]; [1]Wake Forest Sch. of Med., Winston-Salem, NC; [2]Physics, Wake Forest Univ., Winston Salem, NC; [3]Ctr. for Surface Sci. & Nanotechnology, Univ. Politehnica, Bucharest, Romania; [4]Anat. & Neurobio., Univ. of Kentucky, Lexington, KY; [5]Biomed. Engin., USC, Los Angeles, CA

Abstract: Executive control of behavior, according to Karl Lashley, is a crucial ability emerging from the hierarchical architecture of the brain circuitry. Recent results (Opris et al., 2013; Bahlmann et al., 2014) indicate that prefrontal cortical microcircuits on rostro-caudal axis may reflect such hierarchical organization of executive control. This suggests that prefrontal cortical microcircuits integrate information from parietal and temporal (hippocampal) lobe areas and process the executive variables to be expressed in behavior via cortical-striatal-thalamic-cortical loops. We hypothesize that prefrontal microcircuits communicate with parietal/temporal cortices via supra-granular layers, and top-down to subcortical structures (caudate-putamen) and hippocampal subfields CA3-CA1. To test this hypothesis, four non-human primates (NHPs, rhesus macaques) were trained in a rule based spatial vs. object delayed match-to-sample (DMS; Hampson et al., 2013; Opris et al., 2011, 2012a,b, 2013) task with 2-7 images placed on eight symmetric locations on the screen with variable delay epochs of 1-60s. We recorded simultaneously from the prefrontal cortical layers L2/3-L5 and hippocampal CA1-CA3 granular layer and from the prefrontal cortex and striatum (caudate and putamen) with a combination of multi-electrode array (MEA) and microdrive with multiple tetrodes. The conformal MEA consisted of two quartets of platinum recording sites (separated by 1350 um) placed respectively in L2/3 and L5 in PFC, or CA3 and CA1 in hippocampus. To facilitate integration of cognitive information we stimulated in CA1 and recorded in prefrontal cortical layer L2/3, and similarly stimulated in L5 and recorded in hippocampal CA3 subfield. Our results show that subgroups of prefrontal cortical, striatal and hippocampal neurons exhibit enhanced firing on sample/match-

phase when the spatial rule was in effect and less firing when object-rule was cued. Also, when MIMO stimulation was applied the animal's correct performance increased for a particular location under the same spatial rule. These unique neural recordings demonstrate that prefrontal, striatal and hippocampal CA1-CA3 neurons integrate and process information that is relevant for spatial location of objects. These results have implications for the design of wireless cognitive prosthetics for patients suffering with various cognitive dysfunctions such as schizophrenia, autism or dementia.

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Poster

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Title: A cognitive function of the default mode network in monkeys: Shifting of selective attention?

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Abstract: The default mode network (DMN) is a set of brain regions activated during rest in human (Shulman et al. 1997) and monkey (Mantini et al., 2011). It is engaged during internally

focused tasks including autobiographical memory retrieval, envisioning the future, and conceiving the perspectives of others. In addition the DMN is hypothesized to support a broad low-level focus of attention when monitoring the external world for unexpected events (Buckner et al. 2008). Defining an overarching function common to such widely different conditions, however, is implicitly difficult. An intriguing possibility is, that 'shifting operations' between series of internal thoughts, memories, and during passive, seemingly indifferent observation of the environment might be the glue across these conditions. If so, shifts in spatial attention should also engage the DMN. We tested this hypothesis using monkey fMRI during a covert selective attention task, previously used in humans (Molenberghs et al. 2007). Two pairs of shapes were peripherally presented and each pair contained a relevant and irrelevant shape. Monkeys fixated in the center and had to respond manually when the relevant stimulus dimmed. An event consisted of the replacement of the current stimulus pair by the other pair. In 1/3 of the trials this change between pairs elicited a spatial shift in attention as the relevant stimulus was replaced by an irrelevant one. An event-related analysis (N=3) revealed a high degree of overlap (>70%) in cortex posterior to the CS, between shift-related activations and the monkey DMN as defined in the consensus map of Mantini et al. (2011) (comparing rest vs. active task conditions in 15 expts). In contrast, shift-related activations anterior to the central sulcus, overlapped only to 8.15% with the monkey DMN, indicating possible functional subdivisions of the DMN. In the precentral sulcus and the ACC, shift- and DMN specific activations clustered adjacently. Sustained contralateral attention overlapped with the stimulus representations and activated an entirely different set of areas, except for portions of the ACC, the IPS, and area 12. Our data show that the posterior core of the DMN is clearly activated during shifting attention from one location to another, potentially mediated by frontal areas during top-down attentional control. It is therefore tempting to hypothesize that shifting operations in general, be it across memories, thoughts, and internally generated representations, are one important defining feature of the DMN. Cognitive shifting operations also break down in DMN-associated pathologies such as Alzheimer's disease, autism and schizophrenia (Buckner et al. 2008).

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Acute and chronic effects of the traditional herb Cistanche Tubulosa alone or in combination with Gingko Biloba or nerve growth factor on hippocampal networks grown on microelectrode array neurochips

Authors: *D. SALTER[1], M. A. MURRAY[1], D. FAST[2], A. RAJGOPAL[2], K. JUEGELT[3], O. H. U. SCHRÖDER[3], B. M. BADER[3], A. VOSS[3];
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Abstract: *Cistanche Tubulosa* (*Schrenk.*)*Wight* (CT), a perennial parasitic plant that grows in China and Pakistan deserts, is frequently used as a tonic herb by the Traditional Chinese doctor. Gingko leaf extract (*Gingko Biloba*-GB) derived from the Gingko tree is a common traditional treatment used for neurocognitive health. Effects of these extracts on neural activity, however, is not clearly elucidated. Here, we use microelectrode array (MEA) neurochip recordings to evaluate activity changes in hippocampal networks elicited by various concentrations and combinations of two cultivated, standardized extracts of CT and GB. After acute application to 4 week old primary hippocampus cultures, multiparametric analysis revealed both CT and GB induced mild but measurable activity changes within four functional activity categories: general activity, burst structure, oscillation and synchronicity. An isobolographic approach revealed interaction between GB and CT at specific concentrations becoming most apparent at 100 ug/ml when the GB/CT combination is composed of 10-25% GB; the effects of 10% being most pronounced. This concentration and combination increased the spike organization into bursts, induced a stronger burst structure and increased bursting regularity and synchronicity. Increasing amounts of GB (>50%) elicited an inhibition of general activity. Further, when GB/CT is 75/25%, the potency of GB increased 5-fold over GB alone. Chronic CT treatment (30 ul/mg) of hippocampal cultures on MEAs from 4-28 days *in vitro* resulted in an enhancement of spontaneous activity through strengthened bursting activity. Nerve growth factor (NGF) applied acutely to CT treated cultures further increased the response over that of NGF applied to vehicle-treated cultures; notably increasing general activity, lengthening burst duration, increasing pattern regularity, and improving synchronicity within the networks. Recorded cultures were further analyzed by immunocytochemistry, fluorescent microscopy and quantitative image analysis for morphological findings. Chronically treating hippocampal networks with CT did not increase neuronal cell number, but did increase the global number of synapses as well as the number of synapses per neurite. These findings support the notion that these traditionally used herbs are neuro-active and can interact with endogenous growth factors within neural systems. We also show that chronic repeated-dose treatment with CT induced morphological alterations and increased hippocampal network activity *in vitro.*

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NeuroProof GmbH. **O.H.U. Schröder:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **B.M. Bader:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **A. Voss:** A. Employment/Salary (full or part-time):; NeuroProof GmbH.

Poster

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Title: Neural correlates of strategy shifting in the mouse prefrontal cortex, striatum and hippocampus

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Abstract: In the face of ever-changing reward contingencies animals must continually identify and execute new behavioral strategies. In rodents, such strategy shifting is often studied using a plus maze task, in which animals must switch between an egocentric response strategy (f.ex. 'turn left') and an allocentric spatial strategy (f.ex. 'go east'). Lesion studies have shown that the medial prefrontal cortex (mPFC) is required for switching between these two strategies, whereas the striatum and the hippocampus are involved in executing response and spatial strategies, respectively. Yet exactly how the mPFC enables strategy switching remains unclear. One possibility is that the mPFC does so through its interactions with the hippocampus and striatum, with which it is anatomically connected. In the current study we addressed this by recording simultaneously from all three structures in mice as they switched between spatial and response strategies in a plus maze. On each trial of the task, animals were placed in either the south or north arm of the plus maze and had to enter either the east or west arms in order to obtain a food reward. Which arm was rewarded was determined by one of two rules: for the 'response rule', animals had to make a specific body turn at the intersection (f.ex. always enter the arm on the left). For the 'spatial rule', animals always had to go to an arm in a specific spatial location (f.ex. the east arm). Once animals learned one of these rules, reward contingencies were changed so

that reward could only be obtained by following the other rule. Mice were able to learn both the spatial and the response rule and switch between them multiple times. As a first approach to investigating the neural correlates of strategy shifting we examined activity in the mPFC of mice during the switching phase of the plus-maze task. Specifically, we focused on trials in which the rewarded response was inconsistent with the previously rewarded rule. On these trials, we observed greater gamma power in the mPFC when animals made a correct vs. an incorrect response ($p<.01$, sign-rank test, n=16 strategy switches from 6 mice). In contrast, mPFC gamma power was not higher when animals made a correct response on trials where the rewarded response was consistent with the previous strategy. These results suggest that the mPFC is selectively involved in strategy shifting, in agreement with lesion studies. In order to gain further insights into the role of the mPFC we are currently examining how its interactions with the striatum and hippocampus change during the strategy shifting task.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Dissociable salience networks in the macaque brain anchored in right anterior insula

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Abstract: Salience network (Seeley et al., 2007) is a resting state network heavily involved in affective processing and includes major nodes such as amygdala, anterior insula (AI) and anterior cingulate cortex (ACC). Research on human salience network has demonstrated two separable networks anchored by ventral and dorsal AI (vAI and dAI), respectively implicated in attention and affective experience (Touroutoglou et al., 2012). In monkeys, despite a burgeoning number of resting state studies (Mantini et al., 2013; Belcher et al., 2013; Hutchison et al., 2011), it is still not known whether there is a salience network. We used a hypothesis-driven seed-based

approach to investigate whether salience network exists in the monkey brain and has similar dorsal and ventral extents, which may give us insights into how affect is experienced by monkeys. Subjects were 4 rhesus monkeys (1 female, 4-6kg, 4-7y.o.) (Mantini et al., 2013). Each subject underwent twenty 10-min resting state scans in a 3T Siemens scanner, during which they sat in a sphinx position while continuously fixating a point on a blank screen. fMRI data were slice time corrected, motion corrected, linearly detrended, coregistered to the anatomical image, spatially normalized to F99 atlas space, and smoothed with a 2mm fwhm Gaussian kernel. Regions of interest (ROIs) were generated as 2mm-radius spheres centered on right frontal insula (FI), vAI and dAI coordinates homologous to the respective human seeds (Seeley et al., 2007; Touroutoglou et al., 2012). At subject level, whole-brain connectivity was calculated for the average time course from each ROI and then converted to z-scores; at group level, correlation maps were produced by fixed-effect analysis (Mantini et al., 2011). Right FI seed reveals a salience network consisting of dorsal ACC, bilateral AI and amygdalae. Right vAI and dAI have distinct connectivity patterns. The vAI seed is preferentially connected to dorsal ACC, pregenual ACC, ventral AI and bilateral amygdalae, closely resembling human vAI network. The dAI seed is preferentially connected to posterior cingulate, dorsal and ventral AI, putamen, and dorsolateral prefrontal cortex, lacking some parietal targets in human dAI network. To our knowledge, this is the first study that clearly demonstrates the existence of salience network in the macaque brain at 3T. Importantly, we identified two distinct networks anchored by vAI and dAI seeds. Furthermore, while the vAI-seeded network closely resembles the human vAI network, the dAI-seeded network exhibits some different characteristics than the human dAI network. Our findings have implications for understanding affective experience in monkeys.

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Poster

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Title: Cingulocerebellar interactions during interval timing

Authors: *K. L. PARKER, N. S. NARAYANAN;
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Abstract: Anatomical, structural and functional magnetic resonance imaging, and lesion studies advocate for cerebellar involvement in cognition. A network involving functional connectivity between the cerebellum and non-motor areas in the anterior cingulate has been implicated in cognitive tasks. However, the precise role of this circuit and its interconnections remain unknown. To explore this issue, we infused retrograde tracer (red beads) in the anterior cingulate and anterograde tracer (green beads) in the cerebellar dentate deep nuclei. We found these structures to be disynaptically connected via two distinct routes as previously reported. Single synapse red prefrontal beads and green cerebellar beads colocalized on neurons within the ventrolateral thalamus. Convergence of these tracers confirms a pathway through which the cerebellum may influence neurons in the anterior cingulate. We have previously shown that D1 dopamine receptors in the anterior cingulate are required for temporal control during an interval timing task. This task involves attention, working memory, and timing. We probed cerebellar involvement during interval timing using cerebellar infusions of GABA agonist, muscimol, in animals trained to estimate 3 second and 12 second delays. This manipulation profoundly impaired interval timing, particularly at the 3 second delay, while retaining motor function. Next, we recorded neuronal activity in animals implanted with electrophysiological recording electrodes in the anterior cingulate and cerebellar dentate nuclei. Our results reveal strongly modulated neuronal activity in both areas during both delays. Specifically, cerebellar neurons slowed neuronal activity prior to reward availability and then burst in correlation with the animals' peak time of response; prefrontal neurons exhibited ramping following stimulus onset. These data indicate that the cerebellum may modulate the anterior cingulate during temporal control and support an essential role in cognition.

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Department of Veterans Affairs and the American Legion Brain Sciences Chair

Title: Differential effects of NMDA receptor blockade on precise spike timing in local prefrontal and parietal cortical circuits of monkeys performing an executive control task

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Abstract: We used multielectrode arrays in the prefrontal and parietal cortex of monkeys to investigate the effects of injecting on alternating days either saline or the NMDA antagonist phencyclidine (PCP; 0.25 - 0.30 mg/kg i.m.) on the fine-scale timing of action potentials in local cortical circuits. Monkeys performed a dot-pattern expectancy (DPX) task that required both working memory and executive control. In each trial monkeys viewed a cue stimulus followed by a probe stimulus, each consisting of a pattern of dots. The animals responded to the probe stimulus with a leftward or rightward joystick movement, depending on the identity of both cue and probe stimuli. On trials in which the cue stimulus was the dot pattern we labeled 'A' and the probe stimulus was pattern 'X' (the 'target' condition), the monkeys moved to the left. Cue stimuli that were not 'A' were collectively labeled 'B', and probe stimuli that were not 'X' were collectively labeled 'Y'. All 'AY', 'BX', and 'BY' trials ('non-target' conditions) required a rightward response. The monkeys performed trials within two types of set: prepotent and balanced. In the prepotent sets, most trials were of the 'AX' sequence, setting up a tendency to make the 'target' response. In the balanced sets, all four possible stimulus sequences were presented in equal frequency. We identified pairs of neurons as being putatively monosynaptically connected if the cross-correlogram derived from their spike trains exhibited a significant peak or trough at a lag between 2 and 5 ms, and identified cells with common input as those with significant peaks centered near 0 ms. Spike timing differed between cortical areas, with more neurons in parietal cortex exhibiting monosynaptic interactions and more neurons in prefrontal cortex exhibiting common input. In addition, PCP had differential effects on spike timing in the two cortical areas. In parietal cortex, PCP injection reduced the proportion of neuronal pairs exhibiting monosynaptic interactions to about 50% of baseline levels. In contrast, PCP seemed to exert the opposite effect in prefrontal cortex, increasing the proportion of neuron pairs exhibiting monosynaptic interactions by about 50%. PCP also affected the proportion of neuron pairs exhibiting common input in prefrontal cortex, reducing the frequency of these pairs by about 30% relative to baseline conditions (saline). In contrast, the proportion of cell pairs exhibiting common input in parietal cortex was relatively unaffected by PCP. These data suggest that NMDA receptors play differential roles in spike timing within local prefrontal and parietal circuits.

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Title: Prefrontal D1 stimulation rescues mesocortical dopamine deficits during interval timing

Authors: ***Y.-C. KIM**[1], S. L. ALBERICO[2], N. S. NARAYANAN[1];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder causing not only motor impairment but also cognitive dysfunction. Cognitive symptoms can affect up to 75% of PD patients. Available treatment options are limited as the mechanism is unknown. Our overall goal is to develop better targeted therapies for cognitive symptoms. To study this in detail, we investigated mesocortical dopamine projections in mice performing an interval timing task. This task is well suited to study cognitive dysfunction in PD because it depends on dopamine, requires corticostriatal circuits, involves executive functions such as working memory and attention to time, and is consistently impaired in PD patients. We found that depleting dopamine in mesocortical circuits by injecting 6-OHDA in the VTA impaired interval timing. We then attempted to rescue deficits in interval timing using electrical and optogenetic stimulation strategies. We stimulated targets of mesocortical projections in the medial frontal cortex using conventional electrical stimulation both in animals with and without VTA dopamine depletion. We found weak effects of electrical stimulation. To specifically stimulate prefrontal D1 dopamine receptor neurons, we used BAC transgenic mice in which cre-recombinase is expressed only in neurons expressing the D1 dopamine receptor. We first trained D1Cre+ mice to perform a fixed 12s interval timing task. We injected with AAV-LoxP-ChR2 in the medial frontal cortex, 6-OHDA in the VTA, and implanted optical fibers in the medial frontal cortex. Animals were photostimulated randomly on 50% of trials at 2, 20, and 40 Hz during performance of timing tasks with 473nm blue laser. Photostimulation occurred either early (0-6 s) or late in the interval (6-12 s). We found that 20 Hz photostimulation was most effective in rescuing interval timing deficits. We also explored prefrontal D1 neuronal activity with

electrophysiological recording by using optogenetics to identify these neurons. Our data indicate that neurons expressing D1-type dopamine receptors could be targeted by novel therapies for cognitive symptoms of PD.

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Support: NSERC RGPIN 227493-09

Title: Neural correlate of visual working memory in the macaque monkey

Authors: C. LI, J. BARBER, *M. PARE;
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Abstract: Working memory refers to a cognitive process that allows temporary retention of information. Persistent activity has been regarded as a potential neural correlate of working memory, as it has been observed during the retention interval of working memory tasks in both humans and monkeys. In particular, human studies have identified such activity in event-related potentials (ERP) over the posterior cortex. Identifying similar signals in monkeys is essential to bridging the gap between these species and fully understanding the neural basis of human working memory. To that end, we recorded ERP in two female rhesus monkeys with electrodes implanted in their skull over occipital and parietal cortices. The animals performed three tasks, including a visual sequential comparison task in which memory load was manipulated. As in human studies, we found that the amplitude and polarity of the neural activity during the retention interval reflected the spatial location of the target stimulus, was predictive of trial outcome, and scaled with the number of items that had to be remembered. We also examined ERP following partial blockade of NMDA receptors, whose activation has been hypothesized to support persistent activity. Intramuscular injections of sub-anesthetic doses of the NMDA antagonist ketamine deteriorated both the task performance and the neural activity during the retention interval. These findings provide a link to the single-neuron mechanisms of working

memory in monkeys and further validate the monkey as a model of human visual working memory.

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Title: The neurophysiology of stress-related impairment of prefrontal cognitive function

Authors: D. M. DEVILBISS, *C. W. BERRIDGE;
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Abstract: The prefrontal cortex (PFC) plays a critical role in the regulation of goal-directed behavior. Delayed response tasks of working memory are regularly used to examine the neurobiology of PFC-dependent function. PFC neurons display persistent discharge during delay intervals of these delayed-response tasks when information must be retained across time. The persistent nature of delay-related activity is posited to involve recurrent/self-excitatory neuronal activation. However, PFC neurons also code information and events outside the delay interval of these tasks, including outcome (reward and error). Recurrent activation also contributes to task related discharge outside the delay interval. Stress impairs PFC function, as measured in these delayed-response tasks, and may involve the degradation of persistent discharge and recurrent activation during delay intervals. However, the neurophysiological mechanisms responsible for stress-related impairment in PFC-dependent function remain poorly understood. To address this issue, the current study examined the effects of stress on the discharge activity of medial PFC (mPFC) neurons in rats tested in a T-maze delayed-response task of spatial working memory. Three main effects of stress were observed. First, stress potently suppressed the discharge rates of neurons strongly tuned to specific task events, including, delay reward and error, while simultaneously increasing the activity of weakly tuned neurons. Collectively, this effectively collapses the normally robust patterns of task-related activity across functional groups of PFC

neurons. Second, stress increased recursive activation of PFC neurons with strong, delay-related tunings, while suppressing recursive activation of neurons weakly tuned to delay-related information. Finally, stress suppressed the ability of individual PFC neurons to multiplex multiple task events (e.g. reward neurons that also respond to the delay; delay neurons that also respond to error or reward). Stress-related suppression of discharge outside a neuron's preferred tuning predicted task performance, especially neurons tuned to the delay interval. These latter findings provide further support for the hypothesis that successful goal attainment involves multiplexing of task-related events across multiple populations of PFC neurons, including outcome evaluation signals coded by delay-tuned neurons. Collectively, these findings indicate that stress elicits a diversity of actions on PFC neuron recurrent activation and task representations, including the suppression of outcome evaluation signaling.

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Poster

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Title: A self-optimising network

Authors: ***A. M. BRUNO**[1], M. D. HUMPHRIES[3], W. N. FROST[2];
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Abstract: The activity of neural populations has been studied in many contexts, such as sensory processing, working memory, decision-making, and motor programs. Such networks are

typically viewed as relatively fixed structures, and thus are often studied by averaging population activity over many trials. However, studies in both vertebrate and invertebrate systems are uncovering evidence that many neural networks are surprisingly labile, suggesting this static perspective may be too narrow. Here we present evidence from the marine mollusk *Aplysia* of a network that evolves not just trial-to-trial, but moment-to-moment over the time course of a single motor program. Strikingly, the network simplifies its functional structure as it responds to sensory input, undergoing a dramatic coalescence during the first tens of seconds of operation. In light of our findings, we propose sensory stimuli can act to reorganize the very networks they activate, possibly to optimize their own processing. We investigated the neural mechanisms of network self-optimization in *Aplysia* during fictive locomotion. We obtained multi-neuron datasets of the rhythmic motor program using large scale optical recording with a fast voltage sensitive dye. We then interrogated the population of recorded neurons in functional and network space. We first sought to identify sets of coactive neurons as ensembles using a community detection based consensus clustering algorithm. We then tracked the functional relationship between ensemble members over time. We found that the functional relationship between members of the different ensembles was significantly augmented over the first tens of seconds of the motor program. In addition to tracking the changes in the functional relationships between neurons, we treated the population as an entity to identify the set of discrete activity states the network visits, then tracked the sequence of states through time to expose the point where the network converged to a stable repeating temporal structure. We found this point also occurred tens of seconds into the motor program. Intriguingly, these same convergent dynamics were evident topographically, as an activity pattern on the ganglion surface. Of particular interest, we found repeated elicitation of the fictive behavior resulted in enhanced coalescence by all measures. Previous work from this lab discovered an intrinsic modulatory system in a related species that causes the network to rapidly alter itself every time it is activated by sensory stimuli. As components of *Aplysia*'s system closely mimic the other, we pursue the idea that coalescence represents the impact of intrinsic neuromodulation on the network.

Disclosures: **A.M. Bruno:** None. **M.D. Humphries:** None. **W.N. Frost:** None.

Poster

844. Executive Function: Network Activity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 844.28/TT38

Topic: F.02. Animal Cognition and Behavior

Support: NIH intramural support

Title: A curvature-processing network in macaque visual cortex and its functional implication

Authors: *X. YUE[1], M. VERGAMINI[1], I. POURLADIAN[1], R. TOOTELL[2], L. UNGERLEIDER[1];
[1]NIH, Bethesda, MD; [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: Psychophysical studies have demonstrated a special role for curved (as opposed to straight) features in face recognition. Therefore, delineating the functional architecture of curvature processing is essential for our understanding of shape recognition, face recognition in particular. The current study was designed to test for a cortical specialization of curvature processing, and the functional role curvature plays in face recognition. Functional MRI was acquired in three fixating macaques (4.7T), in response to round vs. rectilinear shapes of different types. Stimuli included: 1) images of rounded vs. rectilinear real world objects; and 2) computer-generated arrays of 3D shapes spheres vs. pyramids. We also presented images of monkey faces and objects to localize the well-studied face network. By contrasting fMRI response to images of round vs. rectilinear shapes, our results demonstrated a network of cortical areas selective for the processing of curved features along the ventral visual pathway. The network includes three patches: a posterior curvature-biased patch located in the near-foveal representation of dorsal V4 (PCP), a middle curvature-biased patch located in the posterior superior temporal sulcus (STS) within TEO (MCP), and an anterior curvature-biased patch located in anterior TE just ventral to the STS (ACP). These three patches are organized hierarchically. MCP is posterior to the posterior face patch, 2.2 cm apart from the peak-to-peak activation; ACP largely overlaps the anterior face patch, with the activation peaks only 0.37 cm apart. This close topographical relationship between the curvature and face networks implies a functional link between these two processing streams, which we investigated in a psychophysical experiment using human subjects. Stimuli used in the psychophysical experiment included computer-generated images of faces and high-pass filtered chairs. On each trial, a sample image (a face or chair) was presented briefly, followed immediately by a mask to interrupt visual processing. The mask was then followed by a 2-choice recognition test. Two kinds of masks were used: curved and straight. The presentation time of the sample images varied from trial to trail using a staircase method to make performance comparable across conditions. Our results showed that subjects (n=16) required significantly longer viewing time of the sample images to perform the face recognition task with curved masks compared to straight masks ($t(15)=3.25$, $p < 0.01$). This pattern was reversed for the chair recognition task ($t(15) = -2.34$, $p < 0.05$). These results support the idea that curvature and face processing are functionally related.

Disclosures: X. Yue: None. **M. Vergamini:** None. **I. Pourladian:** None. **R. Tootell:** None. **L. Ungerleider:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.01/TT39

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1ZIAMH002784

Title: Adult neurogenesis is essential for attention-shift from current behavior toward novel cues

Authors: *C. S. WEEDEN, H. A. CAMERON;
Unit on Neural Plasticity, NIH, Bethesda, MD

Abstract: New neurons continue to be produced in the adult hippocampus throughout life, but their function is not yet clear. Most studies aimed at addressing the function of adult neurogenesis have focused on learning and memory, the most widely known functions of the hippocampus. However, early studies of rats with hippocampal lesions found that these animals are impaired in some very simple attention shifting tasks. We investigated the role of adult neurogenesis in these behaviors. Using GFAP-TK transgenic rats recently developed by our group, we can selectively inhibit adult neurogenesis by oral administration of the drug valganciclovir. Rats were placed in an open field chamber that contained a water bottle and were repeatedly exposed to an auditory cue while behavior was recorded. Raters blind to treatment conditions scored orienting responses, or turning of the head toward the sound source. Under these conditions, rats lacking adult neurogenesis and controls exhibited indistinguishable orienting responses. When water deprived, all rats explored the chamber similarly and upon discovery of the water bottle, both groups spent the remainder of the session drinking water. However, when the auditory cue was presented control rats stopped drinking in order to orient toward the cue source for several seconds, but rats lacking adult neurogenesis continued to drink and displayed fewer and weaker orienting responses. This study demonstrates that new neurons are required for normal shifting of attention from ongoing behavior to novel cues with unknown salience. This shortcoming in sensory processing could potentially underlie some of the previously observed learning and memory deficits in rodents lacking adult neurogenesis and could also explain some paradoxical behavioral improvements observed in the presence of superfluous cues. Finally, these findings provide confirmation of widely forgotten hippocampal lesion studies, and suggest that a complete understanding of hippocampal function must include its role in attentional processes.

Disclosures: C.S. Weeden: None. **H.A. Cameron:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.02/TT40

Topic: F.02. Animal Cognition and Behavior

Title: Adult hippocampal neurogenesis affects motivation to obtain sucrose reward in an operant task

Authors: R.-M. KARLSSON, *H. A. CAMERON;
NIMH, NIH, Bethesda, MD

Abstract: Decreased hippocampal neurogenesis may play a role in the pathogenesis of anxiety and depression. Diminished interest or pleasure, anhedonia, is one of the hallmark symptoms of major depression but is poorly understood. Previous findings in our laboratory have shown that mice lacking neurogenesis show decreased sucrose preference, which is a standard measure for studying anhedonia in rodents. The aim of the present study was to further investigate the role of adult hippocampal neurogenesis in motivation to obtain rewards using an effort based task. We inhibited adult neurogenesis using valganciclovir in transgenic mice and rats that express herpes simplex virus thymidine kinase (TK) under the control of the GFAP promoter. TK and wild-type (WT) littermate controls were mildly food restricted and trained to lever press for chocolate flavored sucrose tablets on fixed ratio (FR) and exponentially progressive ratio (PR) tasks. Treated TK (v-TK) mice showed normal acquisition of lever press on a FR schedule and minimal pressing on the inactive lever, suggesting normal learning of lever-reward association. However, when switched to a PR schedule, mice lacking adult neurogenesis showed significantly reduced responding compared to WT controls. Similar to our mouse study, v-TK rats showed normal FR responding to sucrose reward but significantly reduced responding when switched to PR schedule. This is the first study to demonstrate that rodents lacking adult hippocampal neurogenesis have decreased motivation to work for a sucrose reward in an effort based task, consistent with the anhedonic phenotype seen in the sucrose preference test. Future studies will investigate the basis and extent of these motivational changes.

Disclosures: R. Karlsson: None. **H.A. Cameron:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.03/TT41

Topic: F.02. Animal Cognition and Behavior

Support: NIH 1ZIAMH002784

Title: A role for adult neurogenesis in the adaptation to an unpredictable, threatening environment

Authors: *L. R. GLOVER[1,2], T. J. SCHOENFELD[1], R.-M. KARLSSON[1], D. M. BANNERMAN[2], H. A. CAMERON[1];
[1]Section on Neuroplasticity, NIH, NIMH, Bethesda, MD; [2]Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom

Abstract: New neurons are born in the dentate gyrus throughout life. Previous work in our lab showed that new neurons diminish behavioral responses to stress. It is unclear, however, precisely how they do this and what contribution they make towards emotionality. One possibility is that these neurons affect the appraisal process or alter the perception towards an uncertain threat. We used mice that express the HSV-tk transgene (TK) under a GFAP promoter to selectively ablate adult-born neurons and to investigate responses to unpredictable, aversive experiences. In a fear conditioning task, a tone or light that always predicted an upcoming shock ('perfect conditioning') produced similar freezing and startle behaviors in TK and wild type (WT) littermate controls. However, when additional cues were added so that only 50% of cues predicted shocks ('imperfect conditioning'), TKs froze and startled less than WT mice. This same pattern of results was reflected in neural activation of the mature granule cells and CA3 pyramidal cells as measured by c-fos activation; TKs trained in the imperfect condition showed decreased activation relative to WTs and all mice in the perfect condition. Interestingly, cued fear conditioning has traditionally been seen as a hippocampus-independent task, but these findings show that the hippocampus is engaged when ambiguity about the cue is introduced. Because we see increased freezing during the cue in TKs even with imperfect conditioning, it appears that the cue-shock association is present, suggesting that these behavioral changes do not reflect a lack of learning. To look for lasting consequences of an unpredictable, aversive experience, mice were tested in the novelty-suppressed feeding task following perfect or imperfect fear conditioning. Following imperfect conditioning, WTs showed greater latency to eat food in a novel environment, while perfect conditioning had no effect on these mice. TKs, however, showed intermediate increases in latency regardless of the type of conditioning. Clamping stress hormones at low levels prevented the increased latency in WTs after imperfect

conditioning but had no effect on TKs. These findings suggest that new neurons enhance protective stress-related behaviors in response to unpredictable threat and also regulate responses to future novel situations in a glucocorticoid-dependent manner. These changes could bias behavior to optimally adapt to adverse environments.

Disclosures: L.R. Glover: None. **T.J. Schoenfeld:** None. **R. Karlsson:** None. **D.M. Bannerman:** None. **H.A. Cameron:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.04/TT42

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: R01 NS047357

R56 NS047357

Title: V1 interneurons subpopulations expressing different transcription factor combinations are sequentially generated from the p1 domain

Authors: *J. T. ANDERSON[1], R. F. ANDRE[1], J. B. BIKOFF[2], T. M. JESSELL[2], F. J. ALVAREZ[1];
[1]Physiol., Emory Univ., ATLANTA, GA; [2]Howard Hughes Med. Institute, Kavli Inst. for Brain Science, Columbia Univ., New York, NY

Abstract: The spinal cord contains a diversity of interneurons (INs) that lends to its vast computational power; however, all adult INs differentiate from just a few progenitor groups and little is known about their mechanisms of diversification. Here, we focus on V1 INs, which originate from p1 progenitors, express the transcription factor (Tf) engrailed-1 (En1) and generate ipsilaterally projecting inhibitory INs. Previously, we showed that V1 neurogenesis can be divided into early (E.9.5 to E10.5) and late phases (E11 to E12), each producing distinct V1s (Benito-Gonzalez and Alvarez, 2012. J Neurosci 32:1156-70). These include early phase Renshaw cells (RCs) and late phase Ia inhibitory interneurons (Ia-INs), with each expressing a different Tf; all RCs express MafB while at least some IaINs express Foxp2. A more recent genetic survey of V1s uncovered a larger repertoire of Tfs subdividing V1s into further subclasses (Bikoff, Machado, Drobac, Mentis and Jessell, SfN 2012). Thus, we used 5-ethynyl-

2'-deoxyuridine (EdU) birthdating and Tf expression to test whether temporal control of neurogenesis can also explain this larger diversity. EdU was injected in timed pregnant females of three different transgenic lines. In one line (V1-En1) all V1 INs are labeled with tdtomato, in a different line (V1-MafB) MafB-expressing V1s additionally express EGFP and finally in a dual reporter line (V1-Foxp2), Foxp2(+) and FoxP2(-) V1s respectively express EGFP or tdtomato. EdU was injected at E10 for early phase INs and E11.5 and E12 to distinguish different late generated V1s. Spinal cords from postnatal day (P) 5 pups were processed to reveal the lineage labeling with fluorescent proteins, EdU incorporation and immunocytochemical detection of the following Tfs: MafB, Foxp2, Pou6f2, OTP, Foxp4 and Sp8. The results indicate that early and late generated V1s correspond with V1s that express specific Tfs. Early V1s included MafB and Pou6f2 V1s. MafB V1s could be divided in a dorsal population, that is a subgroup of Pou6f2 V1s, and a ventral population that corresponds to RCs. Ventral-MafB RCs represented a larger proportion of neurons generated at E10 than dorsal-MafB or Pou6f2. Thus, RCs are generated earlier than other V1s within the early group. In contrast, the majority of lineage labeled Foxp2 V1s and all V1s retaining Foxp2 expression at P5 correspond to late generated V1s. Within Foxp2 V1s a dorsal OTP-expressing group is generated later than a ventral group that is Foxp4 positive. Finally, Sp8 V1s seem generated in the late wave and independent of the Foxp2 lineage. The data therefore suggests that a large number of V1 phenotypes express differences in their time of neurogenesis.

Disclosures: J.T. Anderson: None. **R.F. Andre:** None. **J.B. Bikoff:** None. **T.M. Jessell:** None. **F.J. Alvarez:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.05/TT43

Topic: F.04. Neuroethology

Title: Social dominance in the anemonefish a. ocellaris

Authors: *R. DEANGELIS, J. RHODES;
Beckman Inst., Urbana, IL

Abstract: Social Dominance in the Anemonefish *A. ocellaris* Social interactions between members of the same species can have long lasting influences on behavior and morphology. One dramatic example is socially influenced post-maturational sex change, a trait that is common in

coral reef fishes. However, the neuroendocrine mechanisms are not well understood. Most previous work has been conducted in species with a harem mating system and where the males are behaviorally dominant (e.g., Bluehead wrasse and Bluebanded goby). In this study, the anemonefish, *Amphiprion ocellaris*, was used a complementary novel model organism. Anemonefish are monogamous and the female is the larger, dominant sex in the pair. Any other fish in the territory besides the pair are non-reproductive. Anemonefish are easy to breed and keep in captivity, and socially influenced sex change can be experimentally induced in the laboratory. If the female is removed from the territory, then the male changes sex into the female and the most dominant member of the non-reproductive fish becomes the male. Although dominance establishment in *A. ocellaris* precedes female gonadal development, and is thought to occur relatively rapidly, the specific behavioral chronology and neuroendocrine correlates are unknown. Therefore, juvenile clownfish were placed in 20 gallon aquaria in groups of 4, and aggressive and affiliative behaviors, as well as time spent in the shelter were recorded for 2 months. After three weeks, fish received a single injection of bromodeoxyuridine (BrdU) at 50 mg/kg to label dividing cells. Along with behavioral measures, blood was also taken every two weeks to measure 11-ketotestosterone, estradiol, and cortisol. The experiment is currently in progress. Preliminary data suggest that behavioral dominance is established within a few days, and that the dominant individual spends a majority of time in the shelter. Brains will be sectioned and quantified to measure number and size of arginine vasotocin neurons in the preoptic area (POA) and to measure numbers of BrdU positive cells in multiple brain regions of the social decision circuit. Gonads will be sectioned and stained to measure proportion of testicular and ovarian tissue. We hypothesize that individuals at relatively higher levels in the dominance hierarchy will display increased numbers of new neurons in social decision regions, larger AVT positive cells in the POA, more ovarian tissue in the gonads, higher levels of E2, and lower levels of 11-KT and cortisol. Preliminary findings suggest our *A. ocellaris* model will be useful for uncovering neuroendocrine correlates of socially induced sex change.

Disclosures: R. Deangelis: None. **J. Rhodes:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.06/TT44

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

Brain Canada

Title: Circuit tagging by CREB facilitates the formation of new fear memories

Authors: *A. J. RASHID, C. YAN, A. DECRISTOFARO, L. HSIANG, A. P. YIU, P. W. FRANKLAND, S. A. JOSSELYN;
Brain and Behaviour, The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Past experience can influence the subsequent formation of memories. We investigated whether formation or recall of an amygdala-dependent fear memory can influence the subsequent formation of a second distinct fear memory. In mice trained in two different auditory fear conditioning tasks with distinct tones (CS1:7.5 kHZ, CS2: 2.8 kHz), the second memory was enhanced if the first training session occurred within 6 h, but not 24 h, before the second session. The second memory was also enhanced if animals recalled the first memory 6 h before training for the second memory. In addition to enhanced memory, mice trained with the 6 h (but not 24 h) delay between training sessions showed 1) increased freezing to tones with frequencies between CS1 and CS2, suggesting greater fear generalization, 2) higher overlap between the proportion of lateral amygdala (LA) neurons activated following the recall of each memory, as determined by the co-expression of activity-dependent transcripts during recall of each memory, and , the ability to extinguish both memories by repeated exposure to only CS2. These results suggest that temporally limited cellular and network changes induced by formation of a fear memory primed those neurons to be preferentially involved in encoding of a second memory. We hypothesized that these circuit changes may involve the activity of the transcription factor CREB. In support of this, we observed that CREB activity peaked 6 h after fear conditioning and returned to baseline after 24 h, and the effects of previous training on memory formation could be mimicked by virally increasing CREB levels in a subset of LA neurons. Preferential activation of neurons with increased CREB (i.e. memory allocation) involved increased neuronal excitability, as optogenetic reduction of excitability of virally transduced neurons prevented allocation. Furthermore, communication with surrounding neurons also regulated allocation as co-expression of CREB with tetanus toxin light chain, which prevents neurotransmitter release, no longer confined the fear memory trace to those neurons with increased CREB. Collectively, these results indicate that CREB activity associated with one memory can transiently “tag” a circuit in the LA, thereby facilitating subsequent new learning.

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Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.07/TT45

Topic: F.04. Neuroethology

Support: NSERC

Title: Hippocampal neurogenesis varies seasonally in male Eastern Chipmunks

Authors: *G. A. SCOTT[1], R. DASENDRAN[1], H. LEHMANN[2], A. N. IWANIUK[3], D. M. SAUCIER[1];
[1]Fac. of Sci., Univ. of Ontario Inst. of Technol., Oshawa, ON, Canada; [2]Dept. of Psychology, Trent Univ., Peterborough, ON, Canada; [3]Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Adult hippocampal neurogenesis is observed across a wide array of species. Although its exact functional role is debatable, a popular view is that hippocampal neurogenesis contributes to the formation and maintenance of hippocampal-dependent long-term memory. Some recent findings suggest that in a number of wild species, including various birds and rodents, rates of hippocampal neurogenesis undergo seasonal change in response to seasonally-specific behaviours such as mating and food-caching, which ostensibly exert differential loads on spatial memory capacity. However, research into this phenomenon in wild mammals is sparse, and the current findings are in conflict with respect to whether seasonal changes in neurogenesis are a general phenomenon in wild rodent species or not. In the present study, we examined hippocampal neurogenesis in male Eastern Chipmunks (Tamias striatus), a hibernating rodent in which both sexes engage in food-caching and which has two distinct breeding seasons (March/April and June/July, respectively). Chipmunks were collected from the beginning of April to the end of October. The collection season was divided into four time blocks that included the spring breeding season (March/April), the non-breeding period in May, the summer breeding period (June/July), and the fall non-breeding period (August-October). Chipmunks were captured in live traps and perfused in the field rapidly within 3 hrs of capture. The brains were sectioned and labelled for doublecortin (DCX), a marker for immature neurons. DCX-positive cells were counted throughout the dentate gyrus using unbiased/assumption-free stereology. It was found that Eastern Chipmunks exhibit high rates of neurogenesis, confirming the presence of this phenomenon in this species. Additionally, the numbers of immature neurons is highly heterogeneous among individuals (~2,000 cells to ~60,000 cells). The number of DCX+ cells was significantly lower in the spring breeding season than the fall non-breeding season, but no other significant differences were observed. Future work will correlate these changes with age and hippocampal volume, as well as examining the same variables in female chipmunks. Overall, the current observed differences in the number of DCX+ cells could reflect seasonal variations in

steroid hormones (high in spring, low in fall) and/or an increased demand in spatial memory during the fall when chipmunks are caching food.

Disclosures: **G.A. Scott:** None. **R. Dasendran:** None. **D.M. Saucier:** None. **A.N. Iwaniuk:** None. **H. Lehmann:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.08/TT46

Topic: F.02. Animal Cognition and Behavior

Title: Voluntary exercise rescues behavioral deficits induced by neonatal alcohol exposure and increases adult hippocampal neurogenesis in mice

Authors: *G. F. HAMILTON, P. J. BUCKO, C. P. KREBS, D. S. MILLER, J. S. RHODES; Univ. of Ilinois Urbana-Champaign, Champaign, IL

Abstract: Developmental alcohol exposure in humans can result in a wide range of deficits collectively referred to as Fetal Alcohol Spectrum Disorders (FASD). FASD-related impairments in cognition and learning persist into adulthood and are accompanied by structural changes in the hippocampus. In rodent models of FASD, neonatal alcohol exposure reduces the survival of newly generated hippocampal neurons when measured in adulthood and also impairs hippocampal-dependent behavior. Aerobic exercise has been shown to both increase levels of adult neurogenesis and to also enhance behavioral performance. For this study, we utilized two different models of a third trimester alcohol exposure. In Experiment 1, animals received a treatment of either saline or a 20% ethanol solution at 5g/kg split into two doses, two hours apart, on postnatal day (PD) 7. For Experiment 2, animals received either saline or 20% ethanol treatments at 5g/kg (split into two doses, two hours apart), on PD 5, 7 and 9. In both experiments, animals were weaned on PD21 and, beginning on PD 35, all animals received either a running or sedentary intervention for 45 days. From PD 36-PD 45, all animals received i.p. injections of 50 mg/kg bromodeoxyuridine (BrdU) to label dividing cells. Behavioral testing on the Passive Avoidance task and the Rotarod occurred between PD 72-PD 79. The number of surviving BrdU+ dentate granule cells was measured at PD 80 for Experiment 1 and PD 81 for Experiment 2. No long-lasting influence of either alcohol paradigm on the number of surviving BrdU+ cells was evident. In contrast, running significantly increased the number of BrdU+ cells across postnatal treatments in both experiments. Behavioral results for the Passive Avoidance

indicate the detrimental impact of neonatal alcohol exposure. Specifically, on Day 1, a PD 5, 7, and 9 alcohol exposure produced long-term impairments while PD 7 did not produce deficits. Exposure to voluntary exercise rescued alcohol-induced deficits on Day 1. On Day 2, no effect of postnatal treatment was evident but runners displayed improved performance over sedentary mice in both the alcohol-exposed and saline-exposed groups. Results of Rotarod performance showed a beneficial impact of voluntary exercise in both Experiments. Alcohol-induced deficits were only evident in the PD 5, 7 and 9 paradigm. Overall, these data illustrate the long-term, possibly detrimental, influence of neonatal alcohol exposure and the potentially beneficial impact of voluntary exercise on the alcohol-exposed brain.

Disclosures: G.F. Hamilton: None. **P.J. Bucko:** None. **C.P. Krebs:** None. **D.S. Miller:** None. **J.S. Rhodes:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.09/TT47

Topic: F.02. Animal Cognition and Behavior

Support: the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no211696

Title: Involvement of LCPUFA based diets on brain structure and function from genesis to senescence

Authors: *A. J. KILIAAN, C. I. F. JANSSEN, I. A. C. ARNOLDUSSEN, V. ZERBI; Anat., Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Many clinical and animal studies demonstrate the importance of long-chain polyunsaturated fatty acids (LCPUFA) in neural development and neurodegeneration. Also several other dietary components have been recognized for their effects on cognitive abilities. Dietary factors can affect multiple brain processes by regulating neurotransmitter pathways, synaptic transmission, membrane fluidity and signal-transduction pathways. This talk will focus on involvement of LCPUFA based diets on inflammation, cerebral blood flow and neural plasticity and connectivity (as visualized with rs fMRI and DTI) from genesis to senescence based on our recently published and new preliminary data, in mice models for Alzheimer and on the most recent literature.

Disclosures: **A.J. Kiliaan:** None. **C.I.F. Janssen:** None. **I.A.C. Arnoldussen:** None. **V. Zerbi:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR

EJLB Foundation

Alzheimer's Society of Canada

Restracomp

NSERC CGS

Title: Searching for a fear memory engram in lateral amygdala

Authors: ***C. YAN**[1,2], A. P. YIU[1], V. MERCALDO[1], B. RICHARDS[1], A. J. RASHID[1], J. PRESSEY[3], M. M. TRAN[1], S. A. KUSHNER[5], M. A. WOODIN[3], P. W. FRANKLAND[1,2,4], S. A. JOSSELYN[1,2,4];
[1]The Hosp. For Sick Children, Toronto, ON, Canada; [2]Inst. of Med. Sci., [3]Cell and Systems Biol., [4]Physiol., Univ. of Toronto, Toronto, ON, Canada; [5]Dept. of Psychiatry, Erasmus Med. Ctr., Rotterdam, Netherlands

Abstract: Memories are thought to be sparsely encoded in the brain. For instance, during formation of an auditory fear memory, while the majority of principle neurons in lateral amygdala (LA) receive both tone and shock information, only a small proportion of these neurons are activated during fear recall. This suggests that although many neurons are eligible, only a few are chosen to encode any one fear memory. The mechanism by which these "winning" neurons are selected to encode a memory is still not fully understood. In previous studies, our lab and others found that neurons with increased cAMP responsive element binding protein (CREB) level are more likely to be selected to encode a memory. Moreover, subsequent permanent ablation or reversible silencing of this small population of neurons abolishes memory expression, suggesting that they represent a crucial part of the memory trace. How does CREB

bias this selection/allocation process? In Hebbian plasticity, an active post-synaptic cell is more likely to fire in response to pre-synaptic input and strengthen the intervening synapse and, therefore is more likely to be involved in memory formation. As previous research shows that CREB can modulate neuronal intrinsic excitability, we hypothesized that one key factor that determines which neurons are recruited to a memory trace is relative excitability. Here we tested this hypothesis using multiple methods to manipulate excitability in small population of LA principal neurons. First, we virally expressed a dominant negative KCNQ2 channel (hQ2-G279S, dnKCNQ2) and an inwardly rectifying potassium channel, Kir2.1, to increase and decrease intrinsic excitability, respectively. Second, we took advantage of chemico- and optogenetics tools and increased excitability shortly before training. We found increasing excitability in the hours or minutes before memory formation is sufficient to enhance the memory strength by biasing the memory trace into neurons with increased excitability. In conclusion, our evidence suggest that during formation of a memory trace, neurons are allocated to a memory trace based on relative excitability.

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Poster

845. Neurogenesis and Plasticity

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.11/TT49

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DA0270847 to J.S. Rhodes

NIH NRSA F30 DA034480-01A1 to M. L. Mustroph

Erik Haferkamp Memorial Scholarship to A.L. Holloway

Title: New neurons are not necessary for exercise to abolish conditioned place preference for cocaine

Authors: *M. L. MUSTROPH[1], J. R. MERRITT[2], A. L. HOLLOWAY[2], D. S. MILLER[2], H. PINARDO[3], C. N. KILBY[3], J. S. RHODES[3];

[1]Neurosci., [2]Beckman Inst., Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Beckman Inst., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Recent evidence suggests wheel running can abolish conditioned place preference (CPP) for cocaine in mice. Running significantly increases the number of new neurons in the hippocampus, and new neurons have been hypothesized to enhance plasticity and behavioral flexibility. Therefore, we tested the hypothesis that increased neurogenesis was necessary for exercise to abolish cocaine CPP. Male nestin thymidine kinase transgenic mice were conditioned with cocaine, then housed with or without running wheels for 28 days. Half the animals were fed valganciclovir in their chow to induce apoptosis in newly divided neurons, and the other half were fed standard chow. The first 10 days, mice received daily injections of bromodeoxyuridine (BrdU) to label dividing cells. Levels of running were similar in animals fed valganciclovir or normal chow. Valganciclovir significantly reduced number of new neurons(BrdU+ NeuN+ cells) in the dentate gyrus of both sedentary and runner animals. However, even though valganciclovir-fed runners displayed similar levels of neurogenesis as sedentary normal-fed controls, they displayed the same abolishment of CPP as runners with intact neurogenesis. Results demonstrate that new neurons are not necessary for running to abolish CPP in mice.

Disclosures: M.L. Mustroph: None. **J.R. Merritt:** None. **A.L. Holloway:** None. **D.S. Miller:** None. **H. Pinardo:** None. **C.N. Kilby:** None. **J.S. Rhodes:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.12/TT50

Topic: F.02. Animal Cognition and Behavior

Support: CREST, Japan Science and Technology Agency (JST)

KAKENHI 22115013

Title: Competitive neurogenesis reduces the dimensionality of the pattern separation problem

Authors: *A. J. DECOSTANZO, T. FUKAI;
Brain Sci. Inst. Lab. for Neural Circuit Theory, RIKEN, Wakoshi, Japan

Abstract: Neurogenesis in the adult hippocampal dentate gyrus (DG) is demonstrated to facilitate behavioral discrimination between similar contexts or objects, referred to as pattern

separation. The vast majority of adult-born cells die, and the adult DG retains approximately the same total number of granule cells throughout the lifetime of the animal. Meanwhile immature cells compete with each other and with mature cells for survival. Previously we found that this competition can enhance pattern separation in a way that strongly depends upon sparse coding. In the present study we further develop our computational model to demonstrate that the competitive neuronal selection process counterintuitively reduces the dimensionality of the representation in the DG. We show how this synaptic competition alters the population code to select the most useful separating dimensions. Furthermore, the optimal neurogenesis rate is proportional to the novelty of the environment, yet always remains surprisingly low at less than 0.5% of the total DG population per day. This corresponds well with values that have been experimentally demonstrated. Thus competitive neurogenesis represents an efficient biological strategy for pattern separation.

Disclosures: A.J. DeCostanzo: None. **T. Fukai:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.13/TT51

Topic: F.02. Animal Cognition and Behavior

Support: ISAO (Internationale stichting Alzheimer onderzoek) Grantno 11528

EU 7th framework Grantno 211696

Title: Effect of a specific multi-nutrient diet on systolic blood pressure and cerebral hemodynamics in aging apoE4 and apoE-ko mice

Authors: *M. WIESMANN[1,2], V. ZERBI[1,2], D. JANSEN[1,2], L. MELLENDIJK[1,2], L. M. BROERSEN[3], A. HEERSCHAP[1], J. A. H. R. CLAASSEN[1,2], A. J. KILIAAN[1,2];
[1]Radboudumc, Nijmegen, Netherlands; [2]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands; [3]Nutricia Research, Nutricia Advanced Med. Nutr., Utrecht, Netherlands

Abstract: ***Introduction*** It is well-established that the cholesterol-transporter apolipoprotein ε (apoE) genotype is strongly associated with the development of both cardiovascular and neurodegenerative diseases like Alzheimer's disease (AD). In addition, hypertension and the presence of the apoE4 allele have shown to lead to a significantly greater amyloid burden [1]. In

the present study, we tested the effects of long-term consumption of a specific multi-nutrient diet in two models for vascular risk factors in AD: the apolipoprotein ε4 (apoE4) and the apoE knockout (apoE-ko) mice. Dietary intervention is a non-pharmacological way to prevent the genotype-induced occurrence of these pathologies. This specific multi-nutrient Experimental diet was developed to support neuronal membrane synthesis and maintenance of vascular health. ***Approach and Results*** We investigated the relationship between systolic blood pressure (SBP), cerebral blood flow (CBF) and a dietary intervention in wild-type (WT) C57bl/6j controls and two models for atherosclerosis and hypercholesterolemia: respectively the apoE-ko and apoE4 -mice. At 2 months of age, the mice were put on the diets (Control + Experimental) for the remainder of the experiment. SBP was monitored twice each month via tail cuff plethysmography starting from 16 month until 18 month of age. At the end of 18 month, CBF was measured with MR by flow-sensitive alternating inversion recovery. Directly following the MR measurements, brains were collected and biochemical and immunohistochemical analyses were performed. From 16-18 month of age, apoE-ko mice had an increased SBP compared to their WT littermates, which was lowered by the Experimental diet. In this transgenic mouse model, the Experimental diet increased also the cortical CBF. At 18 month of age, only Control fed apoE4 mice had a raised SBP compared to their WT littermates. This effect was not visible in apoE4 mice fed the Experimental diet. The cortical and thalamic CBF was decreased at 18 month of age. All animals fed the Experimental diet had a higher CBF than Control fed animals. In addition, biochemical and immunohistochemical data will be shown. ***Conclusions*** We provide new evidence for a relationship between apoE and risk factors for AD. Our data suggest that this specific multi-nutrient diet has beneficial effects on early pathological consequences of hypercholesterolemia and vascular risk factors for AD. ***References*** 1. Rodrigue, K.M., et al., *Risk factors for β-amyloid deposition in healthy aging: Vascular and genetic effects.* JAMA Neurology, 2013. **70**(5): p. 600-606.

Disclosures: **M. Wiesmann:** None. **V. Zerbi:** None. **D. Jansen:** None. **L. Mellendijk:** None. **L.M. Broersen:** None. **A. Heerschap:** None. **J.A.H.R. Claassen:** None. **A.J. Kiliaan:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.14/TT52

Topic: F.02. Animal Cognition and Behavior

Support: The Brain and Behavior Research Foundation (NARSAD Young Investigator Award)

Canadian Foundation for Innovation

Ministry of Research & Inovation

Title: Cognitive flexibility, chronic stress, and suppressed hippocampal neurogenesis

Authors: E. K. LUI[1], E. MARANDI[1], N. PURI[1], M. SALIM[1], M. CHAHAL[1], J. QUADRILATERO[2], *E. SATVAT[1];
[1]Sch. of Publ. Hlth. & Hlth. Systems, [2]Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Functional adult hippocampal neurogenesis appears to be critical for learning novel changes or new strategies in a familiar environment. Chronic stress and corticosterone (CORT) treatment have been shown to inhibit adult hippocampal neurogenesis. The effects of chronic stress on learning and memory are nonetheless complex, task specific, and reversible. We investigated the effects of chronic CORT treatment with and without a recovery period on cognitive flexibility using a water maze protocol that incorporated reversal training. We hypothesized that chronic CORT treatment, which has been shown to suppress adult hippocampal neurogenesis, would attenuate cognitive flexibility. We further investigated the effects of a short recovery period following chronic CORT treatment on learning and memory as well as on cognitive flexibility. New neurons were labeled by injecting rats with bromodeoxyuridine (BrdU 4x50 mg/kg) on the first day after drug treatment (40 mg/kg CORT or vehicle). Drug treatment continued for either 22 days in Experiment 1 (without a recovery period) or 14 days in Experiment 2 (with a 3-day recovery period). Spatial learning was assessed on days 18 to 20 in the hidden platform version of the water maze. The two groups of rats without a recovery period showed equal performance in the water maze; however, CORT-treated rats with a recovery period showed superior spatial learning and memory compared to vehicle-treated rats. Cognitive flexibility was assessed on day 21 by reversal task, in which the hidden platform was placed in a new location. As expected the CORT-treated rats without a recovery period showed deficit in cognitive flexibility, measured by longer latency to find the new location of the hidden platform. This deficit was reversed in the CORT-treated rats that had a 3-day recovery period. Rats were trained for the reversal task for an additional day and underwent a probe test 24 hours later. Regardless of drug treatment and recovery period, rats spent significantly more time in the target quadrant than in the opposite quadrant. Brain tissues were collected half an hour after the probe test and are currently being analyzed for functional neurogenesis.

Disclosures: E.K. Lui: None. **E. Satvat:** None. **E. Marandi:** None. **N. Puri:** None. **M. Salim:** None. **M. Chahal:** None. **J. Quadrilatero:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.15/TT53

Topic: F.02. Animal Cognition and Behavior

Support: AstaReal Co., Ltd (Japan)

The MEXT supported Body and Mind Integrated Sports Sciences (BAMIS) Research Project

The MEXT supported Human High Performance (HHP) Research Project

Title: Marine-plant-derived astaxanthin enhances adult hippocampal neurogenesis and spatial learning in mice

Authors: *J. YOOK[1], M. OKAMOTO[1], T. MATSUI[2], M. LEE[1], H. SOYA[1];
[1]Exercise Biochem., Univ. of Tsukuba, Tsukuba, Japan; [2]Fellow of the Japan Society for promotion of science, Tokyo, Japan

Abstract: BACKGROUND: Diet as well as exercise can play an important role in enhancing hippocampus-related learning and memory. For example, nutritional components such as DHA have beneficial effects on brain health by increasing adult hippocampal neurogenesis (AHN). Recently, astaxanthin (ASX), a red carotenoid pigment found in the marine plant Haematococcus pluvialis has been shown to have potential neuroprotection effects. ASX is currently being researched as a super-antioxidant and as a potential means of protection against and treatment for negative phenomena such as cancer and inflammation by reducing oxidative stress from periphery organs to the brain. However, the effects of a diet that includes ASX are not known, nor is the mechanism behind neural plasticity and cognition under ordinary conditions. Here we investigated whether dietary ASX enhances AHN and spatial learning and memory. METHOD: We used male C57BL/6J mice (12 weeks old) kept individually, and performed two experimental procedures. The first experiment was conducted to define the most effective concentration of ASX for AHN. The mice were randomly divided into 4 groups according to ASX concentrations. Mice received standard chow supplemented with ASX (AstaREAL 20 F; Fuji Chemical Industry Co., Ltd., Japan) at concentrations of 0.02%, 0.1% and 0.5% or a placebo (ASX= 0%). All mice received injections of 5-bromo-2'-deoxyuridine (BrdU, 50 mg/kg, i.p.) before the treatment to evaluate survival of newly generated cells. Immunohistochemistry for AHN was performed on coronal sections of the brain. Based on the results of the first experiment, in the second experiment, we checked the effects of ASX diet (0.5%) on spatial

learning and memory. The mice were randomly assigned to 0% and 0.5% ASX diets for 4 weeks, and, during the final week of the diet, were trained with the Morris water-maze (MWM) for 4 days and then given a probe test 24 hours after the last training session. RESULTS & DISCUSSION: Results of AHN analyses revealed that proliferation cells (Ki67+) significantly increased with 0.1% and 0.5% ASX diets compared to the 0% ASX diet. New-survival cells (BrdU+/NeuN+) were significantly higher for the 0.5% ASX diet. These results suggest that dietary ASX causes a dose-dependent increase in AHN, significant with a 0.5% ASX diet. Results of the MWM showed that the 0.5% ASX diet led to significantly increased performance for learning, but not for spatial memory compared with the 0% ASX diet, demonstrating enhanced spatial learning. CONCLUSION: Together, our results suggest that dietary ASX enhances AHN and spatial learning in adult mice, and these results will better enable us to assess diets for brain fitness.

Disclosures: J. Yook: None. **M. Okamoto:** None. **T. Matsui:** None. **M. Lee:** None. **H. Soya:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.16/TT54

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: R01 NS047357

R56 NS047357

Title: MafB and Foxp2 expression divide mammalian V1 interneurons into subpopulations with non-overlapping neurogenesis, transcription factor expression, development, firing properties and circuit functions

Authors: A. F. RIVARD[1], J. T. ANDERSON[1], J. B. BIKOFF[2], T. M. JESSELL[2], *F. J. ALVAREZ[1];
[1]Physiol., Emory Univ., Atlanta, GA; [2]Howard Hughes Med. Institute, Kavli Inst. for Brain Science, Columbia Univ., New Yorl, NY

Abstract: Spinal interneurons (INs) originate from a few progenitor domains conserved from fish to mammals. Each generates a canonical IN class that diversifies according to increasing

spinal circuit complexity in different species. Many inhibitory INs controlling the ventral motor circuits of the ipsilateral cord derive from p1 progenitors and belong to the V1 class. In swimming vertebrates V1s are a homogenous IN group that provides inhibitory feedback modulation of motor output and gates sensory inputs driving reciprocal contralateral inhibition during alternate swimming axial contractions. Mammals contain a larger V1 diversity correlating with the development of limbs and terrestrial locomotion. Previously, we described two functionally different mammalian V1s, Renshaw cells (RC, recurrent inhibition to motoneurons) and Ia inhibitory interneurons (IaIN, reciprocal inhibition of flexors and extensors), that respectively express the transcription factors (Tfs) MafB and Foxp2 and are generated sequentially during neurogenesis. However, these TFs are also expressed in other V1s and in non-V1s and undergo diverse developmental regulation in different INs. Using novel genetic intersectional approaches to specifically label the MafB and Foxp2 V1 lineages we found that MafB V1s represent around 12% of all V1s in the lumbar cord and are divided in two populations located in the dorsal and ventral most regions of the V1 distribution. Postnatally they express different Tfs (Pou6f2 dorsal, MafA ventral). All ventral MafB-V1s correspond to RCs. In contrast, around 60% of V1s expressed Foxp2 during development. These are divided in dorso-ventral groups according to postnatal expression of OTP (dorsal) and Foxp4 (ventral) or latero-medial groups according to timing of Foxp2 expression. The lateral group expresses Foxp2 during neurogenesis and migrates laterally towards limb motoneurons, many display synaptic connectivity typical of IaINs. A medial group migrates towards axial motoneurons and upregulates Foxp2 later in embryogenesis. Many of these neurons maintain Foxp2 expression until P5. A final group regulates Foxp2 expression for a short time. MafB and Foxp2 V1s exhibit distinct firing phenotypes. More significantly, many MafB V1s express a T-current mediated low-threshold spike (LTS) that provides these cells with burst firing capacity. Foxp2 V1s lack LTSs and express “fast spiking” phenotypes typical of lateral inhibitory INs throughout the brain. Late-generated Foxp2 V1s are therefore a phylogenetically novel V1 class that evolved properties well-adapted to the necessities of phasic lateral (reciprocal) inhibitory control of limb musculature.

Disclosures: **A.F. Rivard:** None. **J.T. Anderson:** None. **J.B. Bikoff:** None. **T.M. Jessell:** None. **F.J. Alvarez:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: American Federation of Aging Research (AFAR

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NINDS Grant 1R01NS087950

NINDS Grant R21NS078660

NINDS Grant 1R01NS081716

NIMH Grant 5R00MH085944

Pew Foundation

Title: Neural network contribution of adult neurogenesis on pattern separation behavior

Authors: *J.-M. ZHUO[1], M. DESAI[2], M. E. BUCKLIN[1], K. GURREA[1], N. T. M. ROBINSON[1], B. D. ALLEN[2], J. G. BERNSTEIN[2], M. P. ELAM[1], K.-L. T. LE[1], H. ZENG[3], E. S. BOYDEN[2], A. JASANOFF[2], X. HAN[1];
[1]Biomed. Engin., Boston Univ., Boston, MA; [2]MIT, Cambridge, MA; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The continued addition of new neurons into adult hippocampus via neurogenesis provides a unique neural network modulation mechanism beyond reorganizing synaptic connections between existing neurons. Adult-born dentate granule cells (abDGCs) can integrate into existing hippocampal neural circuits, and influence behaviors. However, it remains elusive how hippocampal adult neurogenesis modulate neural network *in vivo*, and how learning affects this modulation in relevant cognitive functions such as pattern separation. Here, we analyzed the functional roles of age-defined abDGCs using optogenetics, fMRI and behavioral methods. We discovered that optogenetic silencing of a few hundred 5-10 week old abDGCs impaired spatial pattern separation behavior. Using fMRI in conjunction with optogenetics (opto-fMRI), we further demonstrated that silencing abDGCs during this critical maturation window led to robust blood-oxygen-level dependent (BOLD) signal changes in a large scale neural network, including the contralateral hemisphere. Once passing this maturation window, silencing abDGCs failed to

modulate spatial pattern separation behavior, and produced much smaller BOLD signals that were limited to the local hippocampal areas. In addition, naïve abDGCs without appropriate training would produce little behavioral impact, even though they are capable of modulating a large scale neural network. Together, these observations provide direct evidence that a small population of age-defined abDGCs could produce a transient but robust neural network influence, and learning is required to shape abDGCs' neural network modulation for relevant behavioral functions.

Disclosures: J. Zhuo: None. **M. Desai:** None. **M.E. Bucklin:** None. **K. Gurrea:** None. **N.T.M. Robinson:** None. **B.D. Allen:** None. **J.G. Bernstein:** None. **M.P. Elam:** None. **K.T. Le:** None. **H. Zeng:** None. **E.S. Boyden:** None. **A. Jasanoff:** None. **X. Han:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.18/TT56

Topic: F.02. Animal Cognition and Behavior

Support: SFB 497/A9

DFG BR-2215

Title: Role of Bcl11b in transcriptional control of adult hippocampal neurogenesis and function

Authors: *R. SIMON[1], L. BAUMANN[1], J. FISCHER[1], F. SEIGFRIED[1], J. ANDRATSCHKE[1], H. SCHWEGLER[2], S. BRITSCH[1];
[1]Inst. of Mol. and Cell. Anat., Univ. of Ulm, Ulm, Germany; [2]Inst. of Anat., Otto-von-Guerike-University, Magdeburg, Germany

Abstract: Neurodegenerative diseases are often linked to adult neurogenesis impairing synaptic plasticity, learning and memory. The hippocampus, in particular the dentate gyrus, is one of only two brain regions where adult neurogenesis occurs. With advancing age, the proliferative activity of hippocampal stem cells and the neuronal differentiation capacity continuously decline, resulting in a dramatic reduction of neurogenesis between the age of 2 months and 2 years in a rodent's life. The molecular mechanisms underlying the balancing of neurogenesis, neuronal differentiation, and organ homeostasis within the brain are incompletely understood. Bcl11b/Ctip2, a Krueppel-like zinc finger transcription factor, is expressed in the developing as

well as adult nervous system. We have shown that Bcl11b is specifically expressed in postmitotic granule cell neurons of the dentate gyrus throughout development to adulthood, however expression is excluded from the progenitor compartment. Using conditional gene targeting in mice we demonstrated that Bcl11b is essential for postnatal development of the dentate gyrus. Loss of Bcl11b expression results in a hypoplastic dentate gyrus caused by reduced progenitor proliferation and arrested differentiation of postmitotic granule cell neurons. Furthermore, Bcl11b/Ctip2-mutant neurons fail to integrate into the hippocampal neuron circuitry. As a consequence, Bcl11b mutants exhibit severe deficits in learning behavior (1). Bcl11b expression is sustained throughout adulthood raising the possibility of Bcl11b involvement in adult neurogenesis. Preliminary data employing a tetracyclin-dependent inducible mouse model demonstrates an important function of Bcl11b in adult neurogenesis already two months after ablation of Bcl11b in adult animals. Most strikingly ablation of Bcl11b in the adult mouse leads to impaired learning and memory behavior. Together our data indicate that Bcl11b is required for the maintenance of adult hippocampal biology and function. (1) Simon R, Brylka H, Schwegler H, Venkataramanappa S, Andratschke J, Wiegreffe C, Liu P, Fuchs E, Jenkins NA, Copeland NG, Birchmeier C, Britsch S (2012) A dual function of Bcl11b/Ctip2 in hippocampal neurogenesis. ***EMBO J*** **31:** 2922-2936

Disclosures: **R. Simon:** None. **L. Baumann:** None. **J. Fischer:** None. **F. Seigfried:** None. **J. Andratschke:** None. **H. Schwegler:** None. **S. Britsch:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.19/TT57

Topic: F.02. Animal Cognition and Behavior

Support: MED/2009/214-423

Title: Rice bran extract improves brain mitochondrial function in aging NMRI mice

Authors: ***S. HAGL**[1], D. BERRESSEM[1], N. GREBENSTEIN[2], J. FRANK[2], G. P. ECKERT[1]; [1]Goethe-Universität Frankfurt, Frankfurt, Germany; [2]Univ. of Hohenheim, Stuttgart, Germany

Abstract: In the last few decades, life expectancy was rising constantly due to improvements in health care and technology which lead to increased incidence of age-related diseases including Alzheimer's disease. Research revealed that mitochondria are significantly involved in aging

processes that ultimately lead to neurodegeneration. A healthy lifestyle including a diet rich in antioxidants and polyphenols represents one strategy to protect the brain and to prevent neurodegeneration. Key components of Rice Bran Extract (RBE) are tocopherols, tocotrienols and γ-oryzanol. RBE has been reported to have anti-inflammatory, antioxidant, cholesterol-lowering and anti-diabetic activities. Since we could recently show that RBE feeding increased brain mitochondrial function in young guinea pigs (Pharmacol. Res. 2013, 76:17-27) we now tested the effect of RBE administration on brain mitochondrial function in aged mice. Aged (18 months old) NMRI mice were fed with RBE (340mg RBE/kg body weight) via oral gavage once a day for 3 weeks. 3 and 18 months old NMRI mice fed with vehicle served as control groups. We assessed mitochondrial function by measuring mitochondrial respiration in isolated brain mitochondria and mitochondrial membrane potential (MMP) and ATP levels in dissociated brain cells (DBC). Additionally, we determined levels of mitochondrial marker proteins using Western Blot analysis and examined blood plasma and brain tissue concentrations of key components of RBE. ATP level, complex I respiration, the respiratory control ratio and protein levels of PGC1α were significantly decreased in aged NMRI mice. RBE administration was able to entirely compensate this age-related mitochondrial dysfunction by elevating PGC1α protein levels and citrate synthase activity and by improving the resistance of DBC against sodium nitroprusside induced nitrosative stress. According to these results, RBE is a very promising candidate neutraceutical in the prevention of age-related neurodegenerative diseases like Alzheimer's disease.

Disclosures: S. Hagl: None. **D. Berressem:** None. **N. Grebenstein:** None. **J. Frank:** None. **G.P. Eckert:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.20/TT58

Topic: F.02. Animal Cognition and Behavior

Title: Altered bone morphogenetic protein signaling mediates age-related changes in neurogenesis and cognition

Authors: *E. A. MEYERS, A. M. BOND, J. A. KESSLER;
Dept. of Neurol., Northwestern Univ., Chicago, IL

Abstract: Many aspects in mental function are preserved into old age, however the ability to acquire and rapidly adjust to novel information diminishes later in life. This decline in cognition is associated with a decrease in neural progenitor cell (NPC) proliferation in the hippocampus, the center for learning and memory acquisition. Several signaling pathways have been identified in modulating neurogenesis in the adult subgranular zone (SGZ) of the hippocampus, however the driving force behind age-related cognitive impairment is still unknown. Bone morphogenetic proteins (BMPs) are regulators of cell cycle in stem cell niches throughout the lifespan. Co-modulation of BMP4 and its antagonist, noggin, control SGZ lineage progression and neurogenesis. In normal aging, levels of BMP4 expression increase and levels of noggin expression decrease over a lifetime, correlating with the decline in neurogenesis and in cognition. This leads to a significant increase in BMP signaling beginning at 9 months as evidenced by increasing levels of phospho-SMAD1/5/8 in the hippocampus. Furthermore, viral mediated over-expression of BMP4 at 2 months old decreases levels of proliferation in the SGZ of adult mice and impairs hippocampus-dependent behavior, similarly to that seen in aging. Preliminary studies blocking BMP signaling in aged mice (12 months) show improvements in cell proliferation and cognitive performance. Interestingly, this increase in BMP4 expression with age is also observed in human hippocampal samples, identifying a translational therapeutic target. Thus, the balance of BMP signaling is critical for maintaining neurogenesis and cognitive function with age.

Disclosures: E.A. Meyers: None. **A.M. Bond:** None. **J.A. Kessler:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.21/TT59

Topic: F.02. Animal Cognition and Behavior

Support: University of Illinois, Center for Nutrition, Learning and Memory

Title: Performance of young and aged C57BL/6J mice on cognitive task depends on the task

Authors: *K. DU, S. D. PEREZ, J. S. RHODES;
Univ. of Illinois, Urbana, IL

Abstract: Aging has been associated with weakening of cognitive function, and nutritional supplementation has been suggested to enhance cognitive function. Prior research has

demonstrated limited effects of dietary supplementation on rodent performance on cognitive tasks. The objective of this study is to identify behavior tasks that provide the greatest degree of sensitivity in aging-associated cognitive decline for use in future studies. Young (2 months old) and aged (18 months old) female and male C57BL/6J mice were evaluated for performance on the Morris water maze, elevated plus maze, novel object recognition, passive avoidance, and active avoidance. Animals were euthanized at the end of the study to quantify proliferation of new neurons using doublecortin immunohistochemistry. The largest statistical differences in performance between age groups were seen in novel object recognition and active avoidance. This research model will lay the foundation for understanding the underlying mechanisms by which nutrition interventions may be implemented to slow the cognitive decline associated with aging.

Disclosures: K. Du: None. **S.D. Perez:** None. **J.S. Rhodes:** 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.; Abbott Nutrition.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.22/TT60

Topic: F.02. Animal Cognition and Behavior

Title: Neurogenesis-mediated forgetting of hippocampal memories

Authors: *A. GUSKJOLEN, J. R. EPP, S. A. JOSSELYN, P. W. FRANKLAND*;
Sick Kids Hosp., Toronto, ON, Canada

Abstract: Hippocampal neurogenesis occurs throughout life. As these new neurons mature and form synaptic connections, they necessarily remodel existing hippocampal circuitry. Indeed, computational models predict that high levels of hippocampal neurogenesis should lead to degradation of information stored in already established circuits (1-3). Consistent with this, we have recently demonstrated that high levels of post-learning hippocampal neurogenesis both remodel hippocampal circuitry and lead to forgetting of previously stored information (4). Here we show that P17 infant mice (in which levels of hippocampal neurogenesis are high) can learn and remember the location of a hidden platform in the MWM for 1-14d, but show complete forgetting 1month following training. This infantile forgetting is not due to poor initial memory

encoding, as overtraining infant mice does not overcome the forgetting phenotype. Interestingly, the memory is rescued by a 'reminder' trial, suggesting that infantile forgetting of spatial information stems from a deficit in memory retrieval rather than memory storage. One benefit of this forgetting phenotype is increased cognitive flexibility, as revealed by the infant groups superior reversal learning in later life. To determine the mechanism underlying hippocampal neurogenesis induced forgetting, we developed a mouse in which the Rho GTPase Rac1 can be selectively deleted from neural progenitors using a cre-loxP strategy. As a consequence, these newly generated neurons show reduced synaptic integration, as indicated by decreased dendritic growth, arborization, and spine maturation (5). Consistent with the hypothesis that forgetting is mediated by circuit remodeling caused by the synaptic integration of recently generated hippocampal neurons, inhibiting Rac1 expression in neural progenitors blocked the neurogenesis-induced forgetting effect. Together, these experiments further our understanding of the mechanisms underlying hippocampal neurogenesis induced forgetting. 1. Deisseroth et al., Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron 42, 535 (2004). 2. Meltzer et al., A role for circuit homeostasis in adult neurogenesis. Trends in Neuroscience 28, 653 (2005). 3. Weisz and Argibay, Neurogenesis interferes with the retrieval of remote memories: Forgetting in neurocomputational terms. Cognition 125, 13 (2012). 4. Akers et al., Hippocampal neurogenesis regulates forgetting during adulthood and infancy. Science, (2014). 5. Vadodaria et al., Stage-specific functions for the small Rho GTPases Cdc42 and Rac1 for adult hippocampal neurogenesis. J Neuro Sci, 33, 3 (2013).

Disclosures: A. Guskjolen: None. **J.R. Epp:** None. **S.A. Josselyn:** None. **P.W. Frankland*:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.23/TT61

Topic: F.02. Animal Cognition and Behavior

Support: SFI/RFP/NSC Grant 1298

SFI/IA Grant 1537

Title: Interactions between interleukin-1 beta and short-term exercise on hippocampal neurogenesis: implications for cognitive function in the adult mouse

Authors: *C. M. HUESTON[1], S. M. RYAN[1], B. R. AMELS[1], J. F. CRYAN[1,2], Y. M. NOLAN[1];
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Abstract: Adult neurogenesis within the subgranular zone of the hippocampus, which is integral for normal cognitive function, can be increased with exposure to exercise. Previous studies have demonstrated that chronic elevated levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) in the hippocampus has detrimental effects on memory and cognitive function, as well as a negative impact on the proliferation and survival of newly born neurons. Thus, the current study aimed to assess whether increased hippocampal IL-1β impacts upon exercise-induced neurogenesis and cognitive performance and/or *vice versa*. Adult male C57BL/6 mice (n =10) were singly housed with or without voluntary access to a running wheel. One week later, mice were bilaterally injected with a lentivirus overexpressing IL-1β into the dorsal hippocampus ($1.9x10^5$ TU) or saline as a control, and allowed to recover for 1 week while still having access to the running wheel. Following this week, mice were tested for spontaneous alternation in the Y-maze, as well as on novel object and location recognition memory. Hippocampal tissue was then collected for immunohistochemistry analysis of neurogenesis. Results demonstrate that running increased the numbers of BrdU-positive cells, DCX-positive cells and BrdU/DCX-positive cells, while overexpression of IL-1β attenuated the increase in BrdU/DCX-positive cells only. Interestingly, this deficit was not correlated with cognitive performance on the Y-maze which did not change across conditions. However, spatial memory in the object recognition tasks will be analysed as well. As increased inflammation and deficits in neurogenesis have been linked to aging as well as neurodegenerative disorders such as Alzheimer's disease, understanding the mechanisms through which exercise exerts protective effects, as well as the timing of these effects, is of importance for potential therapeutic interventions.

Disclosures: C.M. Hueston: None. **S.M. Ryan:** None. **B.R. Amels:** None. **J.F. Cryan:** None. **Y.M. Nolan:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.24/TT62

Topic: F.02. Animal Cognition and Behavior

Title: Omega-3 Polyunsaturated Fatty Acids improve mitochondrial dysfunction in brain aging - Impact of Bcl-2 and NPD-1

Authors: *G. P. ECKERT[1], S. AFSHORDEL[2], S. HAGL[2], D. WERNER[1], N. RÖHNER[3], D. KÖGEL[3], N. G. BAZAN[4];
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Abstract: The present study investigated the effects of orally administered long chain omega-3 polyunsaturated fatty acids (PUFA) on mitochondrial function and processing of the amyloid precursor protein (APP) in brains of young (3 months old) and aged (24 months old) NMRI-mice. Neuroprotective properties of fish oil (FO) (1,7 ml/kg p.o) were assessed *ex vivo* after 21 days in dissociated brain cells (DBC) and isolated mitochondria. Docosahexaenoic acid (DHA) levels were significantly lower in blood and brains of aged mice which was compensated by FO administration. Isolated DBC and mitochondria from aged mice showed significantly lower adenosine triphosphate (ATP) levels and reduced activity of complex I+II and IV of the mitochondrial respiration system, respectively. FO restored the age-related decrease in respiration and improved ATP production. Moreover, FO increased the levels of the anti-apoptotic Bcl-2 protein. Cell membrane fractions isolated from the brain of aged mice exhibited lower membrane fluidity, which was partially improved under FO treatment. In comparison to young animals, levels of neuroprotective sAPPα were significantly lower in the brain of aged mice. However, levels of sAPPα, Aβ and C-terminal APP fragments (CTF) were largely unchanged after FO treatment in aged mice. Neuroprotectin D-1 (NPD-1) represents a neuroprotective compound that is derived from unesterified DHA. Levels of NPD1-like metabolites (NPD1-like) and of unesterified DHA were significantly increased in brains of aged mice. FO treatment further strongly increased NPD1-like levels indicating an accelerated conversion rate of free DHA to NPD1-like. Our findings provide new mechanisms underlying the neuroprotective actions of omega-3 PUFA and identified FO as promising nutraceutical to delay age-related mitochondrial dysfunction in the brain and to prevent neurodegenerative diseases such as Alzheimer`s disease.

Disclosures: G.P. Eckert: None. **S. Afshordel:** None. **S. Hagl:** None. **D. Werner:** None. **N. Röhner:** None. **D. Kögel:** None. **N.G. Bazan:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.25/TT63

Topic: F.02. Animal Cognition and Behavior

Title: A mixed PUFA diet normalizes hippocampal neurogenesis and reduces anxiety in serotonin transporter knockout rats

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Abstract: The first-line treatment of depression involves the use of antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs). However, a substantial part of the patients do not respond to SSRI treatment. Among the factors that contribute to the treatment non-responsivity is the common serotonin transporter promoter polymorphism (5-HTTLPR). The low activity short (s) allelic variant of this polymorphism is associated with trait anxiety, which confers increased risk for depression in the context of early life stress, as well as non-responsivity to SSRI treatment. Epidemiological studies have revealed that omega-3 poly-unsaturated fatty acids (PUFAs) decrease the incidence of depression, and here we undertook the challenge to investigate whether a mixed diet comprising n-3 PUFA's as well as B-vitamins and phospholipids has the potential to decrease trait anxiety in adolescent and adult serotonin transporter knockout (5-HTT-/-) rats modelling the serotonin transporter polymorphism in humans. We found that 5-HTT-/- rats on control diet displayed increased anxiety-related behavioural responses, and impaired fear extinction. These effects were completely offset by the mixed PUFA diet, while this diet had no behavioural effect in wild-type (5-HTT+/+) rats. In parallel, dentate gyrus doublecortin immunoreactivity (as an index of neurogenesis) was increased in 5-HTT-/- rats fed on control diet, which was reversed by the mixed PUFA diet. Hippocampal volume was unaffected by the mixed PUFA diet in 5-HTT-/- animals, while it increased in 5-HTT+/+ rats. These data show that a mixed n-3 PUFA diet ameliorates anxiety-related symptoms in a genotype-dependent manner, potentially by normalizing neurogenesis. We suggest that such a mixed diet may serve as an attractive strategy to reduce anxiety in 5-HTTLPR s-allele carriers and thereby decrease the risk for depression across lifetime.

Disclosures: J.R. Homberg: None. **P. Schipper:** None. **A. Kiliaan:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.26/TT64

Topic: F.02. Animal Cognition and Behavior

Title: Paclitaxel reduces hippocampal neurogenesis and vesicular zinc level

Authors: *B. LEE[1], J. KIM[1], B. CHOI[1], H. KIM[1], I. KIM[1], S. LEE[2], M. SOHN[3], S. SUH[1]; [1]39 Hallym Dae Hak Gil, Dept. of Physiol. Hallym Univ., Chuncheon, Korea, Republic of; [2]Dept. of Med. biology, Hallym Univ., Chuncheon, Korea, Republic of; [3]Dept. of Nursing, Inha Univ., Incheon, Korea, Republic of

Abstract: Chemobrain is a phenomenon that includes a wide range of cognitive impairment in memory, processing speed and attention during/after chemotherapy (CTx). Little is known about the mechanism of CTx-induced impairment of cognitive function. Paclitaxel (Px) is one of the most broadly used chemotherapeutic agents for ovarian, breast, lung and several other types of cancers. Px binds to microtubules and stabilizes its structure, which reduces microtubule dynamics and limits axonal transport. Px also causes chemobrain during/after CTx. The objective of this study was to investigate the mechanisms of Px-induced chemobrain. Zinc (Zn) is the second most abundant transition metal ion in the brain following iron. Zn has been recognized as an essential element for brain development and memory functions. Zn is highly localized in the synaptic vesicle of mossy fiber terminals of the dentate granule cell sites where neurogenesis are most active in the adult brain. Our previous studies have shown that reduced vesicular Zn caused limited neurogenesis after hypoglycemia and seizure. Thus, the present study hypothesized that Px-induced chemobrain is related with vesicular Zn dyshomeostasis and impairment of neurogenesis. To investigate whether Px affects the hippocampal neurogenesis, we conducted two sets of experiment to evaluate short-term and long-term effects. To test the acute effects of Px treatment, mice were intraperitoneally administered 10mg/kg of Px every day for 7 days. To test the chronic effects of Px treatment, mice were intraperitoneally administered 10mg/kg of Px every other day for 30 days. Neurogenesis was evaluated using BrdU, Ki67 and doublecortin (DCX) immunostaining 7 days or 30 days after initial Px treatment. BrdU were injected twice a day during last four consecutive days before sacrifice. Immunohistochemistry including DCX, Ki67 and BrdU revealed that Px-treated mice showed reduced neurogenesis in the subgranular zone of hippocampal dentate compared with control group. To test whether Px treatment affects vesicular Zn in the hippocampus, zinc transporter 3 (ZnT3) was evaluated with immunohistochemistry and free Zn level was evaluated with TSQ fluorescent staining. The present study found that ZnT3 and vesicular Zn level in the hippocampus were significantly reduced in Px-treated mice, suggesting that vesicular Zn may decreased by Px. Taken together, the present study suggests that Px-induced chemobrain may be caused by reduced neurogenesis

and vesicular Zn level in the hippocampus. However, further studies are needed to elucidate how Px-induced Zn dyshomeostasis contributes to reduced neurogenesis and cognitive impairment.

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

Poster

845. Neurogenesis and Plasticity

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Program#/Poster#: 845.27/TT65

Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-74650

CIHR MOP-86762

CIHR vanier CGS

Title: Manipulating a cocaine-cue memory trace in mice

Authors: ***H.-L. HSIANG**[1,2], J. EPP[1,2,3,4], M. VAN DEN OEVER[1,2,4,3,5], C. YAN[1,2], A. RASHID[1,2,3,4], Y. NIIBORI[1,2,3,4], N. INSEL[1], L. YE[6], K. DEISSEROTH[6], P. W. FRANKLAND[1,2,3,4], S. A. JOSSELYN[1,2,3,4];
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Abstract: Drug addiction is a chronic, relapsing disorder perhaps because drugs of abuse produce powerful memories which may be resistant to behavioral extinction. Understanding how and where these memories are encoded and consolidated in the brain may allow specific manipulation of these memories. Here we identified a small population of neurons in the lateral nucleus of amygdala (LA) which constitute a critical hub in a cocaine-related memory trace. Ablating or genetically silencing this small population of neurons immediately before testing disrupted the expression of a previously encoded cocaine memory. Consistent with theories that coordinated post-encoding reactivation of a memory trace is crucial for memory consolidation, we also found that post-training suppression or non-discriminate activation of this specific population of neurons in the hours following training impaired cocaine-memory consolidation.

These findings not only reveal mechanisms underlying how the brain encodes and stores drug-related memories but may also inform the development of treatments for drug addiction in humans.

Disclosures: **H. Hsiang:** None. **J. Epp:** None. **M. van den oever:** None. **C. Yan:** None. **A. Rashid:** None. **Y. Niibori:** None. **N. Insel:** None. **L. Ye:** None. **K. Deisseroth:** None. **P.W. Frankland:** None. **S.A. Josselyn:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.28/TT66

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Functional, anatomical and effective connectivity with ca3 inhibitory interneurons underlies integration of newborn dentate gyrus cells into the adult hippocampus

Authors: ***L. RESTIVO**[1], Y. NIIBORI[1], A. L. WHEELER[1], S. A. JOSSELYN[1,2,3,4], P. W. FRANKLAND[1,2,3,4];
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Abstract: In the adult hippocampus, new neurons are generated daily in the subgranular zone of the dentate gyrus (DG). Over the course of weeks, these adult-generated granule cells (aDGCs) integrate into established hippocampal circuits, gradually forming functional input (from entorhinal cortex) and output (to CA3) connections. Using transgenic, retroviral and optogenetic approaches, here we track the maturation of anatomical, effective and functional connectivity of these aDGCs. Injection of a retrovirus expressing GFP into the DG allowed us to visualize the development of output connections of aDGCs in naïve mice. By four weeks, aDGC mossy fibers showed significantly more filopodia and enpassant CA3 boutons_ presynaptic terminals selectively innervating CA3 GABAergic cells - than both developmentally-generated and 6-week-old cells, suggesting that aDGCs transiently develop extensive anatomical connections with inhibitory CA3 cells as they mature. To test whether 4-week-old aDGCs preferentially drive activity of the inhibitory CA3 network we injected a retrovirus expressing a variant of ChR2

(ChIEF) in the dentate gyrus of adult mice. Preliminary data suggests that light-induced activation of 4-week- but not 6-week-old aDGCs induces a significant increase of the immediate early gene c-fos expression in CA3 inhibitory cells relative to control groups. Increased anatomical and effective connectivity between aDGCs and inhibitory CA3 cells suggests that these two populations of cells may also show a tight functional coupling during learning. To test this, we analyzed correlated neuronal activity among 5 different hippocampal cell populations following context fear conditioning. Post-training expression of c-fos was used as a marker of neuronal activity. Fos expression was tightly correlated between 4-week-old aDGCs and GAD+ CA3 cells across subjects, indicating that activity of aDGCs and CA3 inhibitory cells are tightly coupled. By contrast, c-fos expression showed no correlation between 6-week-old aDGCs and inhibitory CA3 cells but a strong correlation between 6-week-old aDGCs and developmentally-generated cells was detected, suggesting that by 6-weeks of age aDGCs have already developed a mature functional phenotype. Taken together these results suggest that aDGCs develop transient functional, anatomical and effective connections with the local CA3 inhibitory network and this synaptic integration may have a significant impact on behavioral and hippocampal processes.

Disclosures: L. Restivo: None. **Y. Niibori:** None. **A.L. Wheeler:** None. **S.A. Josselyn:** None. **P.W. Frankland:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.29/TT67

Topic: F.02. Animal Cognition and Behavior

Title: Effects of long-chain polyunsaturated fatty acids on cognition, vascular function and gray matter integrity in mildly obesogenic ApoE*3Leiden mice

Authors: *I. ARNOLDUSSEN[1], R. H. NOORDMAN[1], V. ZERBI[1], P. Y. WIELINGA[2], R. KLEEMANN[2], G. GROSS[3], E. A. F. VAN TOL[3], T. KOOISTRA[2], M. H. SCHOEMAKER[3], A. J. KILIAAN[1];
[1]Anat., Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [2]TNO Metabolic Hlth. Res., Leiden, Netherlands; [3]Mead Johnson Pediatric Nutr. Inst., Nijmegen, Netherlands

Abstract: Obesity is associated with impaired cognition and brain structure. We investigated wether early intervention with long-chain polyunsaturated fatty acids, arachidonic acid (ARA)

and docosahexaenoic acid (DHA), can prevent potential detrimental effects of a mild obesogenic diet on brain structure and function. Four-week old male ApoE*3Leiden mice were fed regular chow with or without a mixture of ARA (0.129 wt%) and DHA (0.088 wt%). From 14 until 26 weeks of age, mice were fed a mildly obesogenic high-fat/high-carbohydrate (HFHC) diet. At 13 and 26 weeks of age, mice performed cognitive tests, and cerebral blood flow (CBF), vasoactivity, white and gray matter integrity were examined with MRI (11.7T) using arterial spin labeling and diffusion tensor imaging (DTI). Our results showed increased CBF and vasoactivity ($P \leq 0.05$) in 13 week-old mice fed ARA&DHA. At 26 weeks, ARA&DHA supplementation seems to counteract the HFHC-induced changes in CBF and vasoactivity, overall indicating improved vascular function. All mice were able to memorize the platform position in the Morris water maze, however mice fed ARA&DHA showed an advanced cognitive search strategy ($P \leq 0.05$). Mean diffusivity levels were increased in the motorcortex of mice fed an HFHC-diet, which was prevented by ARA&DHA-supplementation ($P \leq 0.05$). This indicates that ARA&DHA pretreatment can prevent gray matter loss caused by HFHC diet. Our results suggest that ARA&DHA supplementation early in life improves cognitive and vascular function, and protects gray matter integrity in the context of mild obesity later in life.

Disclosures: I. Arnoldussen: None. **R.H. Noordman:** None. **V. Zerbi:** None. **P.Y. Wielinga:** None. **R. Kleemann:** None. **G. Gross:** None. **E.A.F. Van Tol:** None. **T. Kooistra:** None. **M.H. Schoemaker:** None. **A.J. Kiliaan:** None.

Poster

845. Neurogenesis and Plasticity

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 845.30/TT68

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

Title: Neurogenesis-mediated forgetting of recent, but not remote, contextual fear memories

Authors: *P. W. FRANKLAND[1,2,3,4], A. GAO[1], A. GUSKJOLEN[1], S. JOSSELYN[1,2,3,4]; [1]Dept Neurosci & Mental Hlth., Hosp. Sick Children, Toronto, ON, Canada; [2]Inst. of Med. Sci., [3]Dept. of Physiol., [4]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: New neurons are continuously generated in the dentate gyrus of the hippocampus throughout adulthood. These new neurons gradually integrate into hippocampal circuits and

likely contribute to the formation of new memories, once sufficiently mature. However, as they integrate into existing circuits, they may also disrupt memories already stored in those circuits. Recently we provided evidence for this idea by showing that artificially elevating levels of neurogenesis after memory formation promoted degradation (or forgetting) of hippocampal memories (Akers et al [2014]). With time, memories that are initially hippocampus-dependent may become independent of the hippocampus and dependent on cortical structures for their expression (e.g., Frankland and Bontempi [2005]). Therefore, this suggests that neurogenesis-mediated forgetting should only impact memories during their hippocampus-dependent phase and not at later time points (i.e., after ~28 days). To test this, mice were trained in contextual fear conditioning, and then hippocampal neurogenesis was elevated either immediately after training or after a 4 week delay. Only elevating neurogenesis levels immediately after training induced forgetting. In contrast, elevating neurogenesis levels after a delay did not produce any amnesia. These results suggest that as memories mature they become invulnerable to neurogenesis-mediated forgetting, perhaps because they no longer require the hippocampus for their expression.

Disclosures: **P.W. Frankland:** None. **A. Gao:** None. **A. Guskjolen:** None. **S. Josselyn:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.01/TT69

Topic: F.02. Animal Cognition and Behavior

Support: Cardiovascular Research Foundation

Title: Increased BDNF levels and improved spatial learning in mice after the treatment with Neurotropin®

Authors: *H. YANAMOTO[1,2], Y. NAKAJO[1,3], J. C. TAKAHASHI[1];
[1]Natl. Cerebral & Cardiovasc Res. Ctr., Suita, Japan; [2]Osaka Univ. Grad. Sch. of Med., Osaka, Japan; [3]Rakuwakai Otowa Hosp., Kyoto, Japan

Abstract: There is an urgent need to develop a safe compound to enhance memory due to the growing number of people who are at risk of memory dysfunction due to Alzheimer's disease or vascular dementia. An extract from the inflamed cutaneous tissue of rabbits inoculated with vaccinia virus (ERV, Neurotropin®), utilized in the clinical treatment of chronic pain, was

recently found to enhance the production of brain-derived neurotrophic factor (BDNF) in cultured neurons. As increased BDNF levels can enhance various brain functions, we examined the effect of daily oral ERV on spatial learning, and also on BDNF levels in the brain. Following the daily administration of ERV at 0.27, 0.81 or 2.43 U/kg/day, or vehicle, for 21 days, the Morris water maze (MWM) test with modifications was performed using adult C57BL/6J mice. Each animal performed four trials per day (1 session), over five consecutive days without any prior or subsequent training, setting the cut-off time at 300 sec. In each trial, the time needed to escape to the platform and the maximum velocity (Vmax) were analyzed. The rate of successful navigation, i.e. when the mouse crossed over the "exact location of the platform after being removed and reintroduced to the pool" in 1 min was also analyzed after MWM (a probe trial). The BDNF levels in the brain were determined using ELISA. Treatment with low, medium or high dose ERV improved the mean escape latencies in 2-5 sessions to 54% ($P< 0.01$), 31% ($P< 0.001$), and 41% ($P< 0.001$) respectively compared with controls (100%). Vmax significantly increased in the low dose group: 36.1 ± 3.2 cm/sec ($P< 0.001$), but did not differ in the medium or high dose groups (34.0 ± 3.8 cm/sec and 35.6 ± 3.8 cm/sec respectively) compared to the control: 32.8 ± 4.0cm/sec (mean ± SD). In the probe trial, the group treated with medium dose ERV was found to cross over the location where the platform existed more frequently (185%) than the control group ($P< 0.01$). The BDNF levels in the brain were significantly elevated in the treated groups compared with controls ($p< 0.001$). Daily oral treatment with ERV, especially at the medium dose: 0.81 U/kg/day, which is three times higher than that for humans, was found to increase BDNF levels in the brain and improve spatial learning.

Disclosures: H. Yanamoto: None. **Y. Nakajo:** None. **J.C. Takahashi:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.02/TT70

Topic: F.02. Animal Cognition and Behavior

Support: Pennsylvania Department of Health

Title: Sex differences in working memory performance after repeated exposure to Δ^9-tetrahydrocannabinol in adolescent rhesus macaques

Authors: *M. WRIGHT, JR[1], C. R. OLSON[2], D. A. LEWIS[1];
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Abstract: Nearly one in four high school seniors reports using cannabis. The rate of use among adolescents has risen steadily since 2007. This trend is of concern because repeated cannabis use during adolescence is associated with persistent memory impairment. The impact of cannabis use on memory may, however, vary by sex. Moreover, the interpretation of the findings is complicated by potential confounds involving self-selection bias and environmental history. Studies in young non-human primates afford a means by which to avoid these confounds. We report here results of a study exploring the effect of repeated exposure to the primary psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), on working memory in adolescent rhesus macaques of both sexes. The monkeys performed a spatial version of the Sternberg Memory Scanning Task 5 days each week. Half of the cohort (3 males and 3 females) were treated with Δ^9-THC immediately after each daily training session (mean dose = 0.24 mg/kg). The other half was treated with vehicle (99% saline-1% Tween 80) on the same schedule. The drug was delivered through indwelling intravenous catheters to simulate the pharmacokinetics of Δ^9-THC from smoked cannabis. The dose used during the repeated dosing phase was the minimum of Δ^9-THC required to impair response rate acutely when administered immediately before a session. After 6 months of repeated exposure, Δ^9-THC significantly reduced response accuracy and altered the response-time distribution in male monkeys. The deficit did not improve when the memory interval was shortened. In female monkeys, neither response accuracy nor the response-time distribution was affected by repeated exposure to Δ^9-THC. These results suggest that adolescent male and female monkeys are affected differentially by repeated exposure to Δ^9-THC. They are consistent with the observation of sex differences in the memory performance of human adolescent cannabis users.

Disclosures: M. Wright: None. **C.R. Olson:** None. **D.A. Lewis:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.03/TT71

Topic: F.02. Animal Cognition and Behavior

Support: NIH GrRANT DA015951

Title: Dose-dependent impairment of spatial memory performance in the morris water maze task following abuse patterns of prenatal toluene exposure

Authors: *S. E. BOWEN[1], S. P. CALLAN[2], J. H. HANNIGAN[3];
[1]Psychology, Wayne State Univ., DETROIT, MI; [2]Psychology, [3]Wayne State Univ., Detroit, MI

Abstract: Prenatal exposure to high levels of commonly abused inhalants results in a constellation of symptoms labeled Fetal Solvent Syndrome (FSS) and includes growth restriction and CNS dysfunction. In a preclinical model of the abuse of inhalants during pregnancy, timed-pregnant Sprague-Dawley rats (N = 68) were given 30-min exposures twice daily to 12,000 parts per million (ppm) toluene, 8,000 ppm toluene, or air (0 ppm) from gestation day 8 (GD8) through GD20. Beginning on postnatal day 28 (PN28), offspring were tested in a Morris Water Maze with 3 trials/day for 5 consecutive days with the goal platform in the same position for each trial. An inter-trial duration of 90 sec was used. A trial ended when the rat located the underwater platform, or when a latency of 90 secs was reached. Twelve days later, the rats were tested for 3 trials in a "reversal task" with the platform moved to the opposite quadrant. Visual cues were provided and animals were placed at a different start point for each trial. The toluene-exposed animals did not differ from the air-control cohort in velocity, thigmotaxis, average distance from the target, or distance swam. While there was no main effect of dose on latency to find the platform, a significant dose X trial interaction was observed with prenatal toluene-exposed animals showing less improvement across the trials of a given day. Survival analysis showed that rats in the 12,000-ppm group took significantly longer to show mastery of the task, defined as number of trials until the platform was found on three consecutive trials, than non-exposed rats, suggesting impaired procedural learning. These results in this animal model provide evidence that brief, repeated, high-concentration toluene exposures that mimic patterns of organic solvent abuse in pregnant women produce long-lasting cognitive deficits in offspring.

Disclosures: S.E. Bowen: None. **S.P. Callan:** None. **J.H. Hannigan:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.04/TT72

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP 2013/20378-8

Title: Proteomics analysis of the dorsal rat hippocampal formation: Neuromodulatory effects of standardized Ginkgo biloba extract on fear memory

Authors: *R. B. GAIARDO[1,2], M. M. TELLES[2], S. M. CERUTTI[1];
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Abstract: In our laboratory, we have focused efforts in describing the role of the dorsal rat hippocampal formation (DH) in the acquisition of lick suppression. Additionally we showed that acute and long-term treatments with standardized Ginkgo biloba extract (EGb) modulated the acquisition of fear memory in different ways in the DH. However, several studies have shown that, over time, the long-term consolidation involves the transfer of memories out of the hippocampus to other parts of the brain and such molecular changes may involve differential protein expression in these areas. In this sense, we investigated differential protein expression in the DH of rats submitted to acute treatment with EGb and conditioned fear memory. Adult male Wistar rats were treated with a single dose of the vehicle solution (negative control), EGb (250, 500 or 1000 mg.kg-1) or Diazepam (positive control) 30 minutes before conditioning or manipulated only. The behavioral procedure had four steps: (1) acquisition of licking response - baseline (1st to 5th day), (2) tone-shock pairings (CS-US - four times, 6th day), (3) return to baseline contingencies (7th day) and (4) re-exposition of the CS (test), performed on 2nd or on 40th day after conditioning, which evaluated the suppression of licking response calculated in ten trails of CS presentation. The latencies to complete ten licks in the presence and absence of the CS were used to calculate the suppression rate (SR). The animals were decapitated 24 hours after completion of the behavioral experiments and samples of the DH were removed for proteomes analysis by two-dimensional polyacrylamide gel electrophoresis. Analysis of SR showed that treatment with EGb 250 and 1000 mg.kg-1 resulted in higher retention of fear memory evaluated during test. Moreover, rats treated with 500 mg.kg-1 EGb exhibited a decrease in the suppression of the licking response across successive exposures of the CS, but this group had acquisition of fear memory, different that observed for Diazepam group. Additionally, the data show for the first time that rats were able to retrieval fear memory 40 days after conditioning. The proteomics analysis resulted in the detection of 338±19 spots, which 37 had significant differential expression ($p < 0.05$). Among these, 33 proteins were differentially expressed between controls and EGb groups, 12 between the different treatments with EGb and 15 between control groups. Our results provide further evidence for the efficacy of EGb in modulating fear memory, but the identification of proteins differentially expressed, by peptide mass fingerprinting method, may substantiate our behavioral findings as well as modulatory effects of EGb.

Disclosures: **R.B. Gaiardo:** None. **M.M. Telles:** None. **S.M. Cerutti:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.05/TT73

Topic: F.02. Animal Cognition and Behavior

Support: NIH/Intramural Research Program

Title: Time-dependent effects of exercise and exercise-mimetics

Authors: *D. GUERRIERI, H. VAN PRAAG;
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Abstract: Voluntary wheel running improves learning, hippocampal synaptic plasticity and adult neurogenesis. However, the peripheral triggers of the cellular and molecular cascades in the brain that may lead to cognitive enhancement are not well-understood. We aimed to test the hypothesis that skeletal muscle activation by either exercise or a pharmacological intervention may influence brain function. Exercise activates AMP-activated protein kinase (AMPK) in muscle. AMPK is a primary regulator of energy levels, and is physiologically activated by a reduction of the ATP/AMP ratio. Similar regulation occurs as a consequence of pharmacological activation using the AMPK agonist 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR). AICAR has previously been shown to mimic the effects of exercise. Administration of AICAR enhanced endurance in sedentary animals (Narkar et al., 2008) and improved memory function (Kobilo et al., 2011). In this study, we investigated time-dependent effects of exercise and AICAR administration in young C57Bl/6 male mice. Mice were housed with a running wheel or injected daily with AICAR (500 mg/kg) and tested at different time points. In particular, we compared the peripheral and central effects of running and AICAR administration, on the regulation of AMPK-dependent molecular processes. Preliminary data show an overlap in gene expression between AICAR and exercise. These findings may lead to further insight into mechanisms underlying benefits of exercise for brain function. Kobilo T, Yuan C, van Praag H. Endurance factors improve hippocampal neurogenesis and spatial memory in mice. Learn Mem. 18:103-107 Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM. 2008. AMPK and PPARdelta agonists are exercise mimetics. Cell. 134(3):405-15 This research was supported by the Intramural Research Program of the NIH, National Institute on Aging (NIA)

Disclosures: D. Guerrieri: None. **H. van Praag:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.06/TT74

Topic: F.02. Animal Cognition and Behavior

Support: NIH 1R15MH093918-01A1

Title: Grape powder supplementation prevents cognitive, behavioral and biochemical impairments in rat model of posttraumatic stress disorder

Authors: *N. SOLANKI, I. ALKADHI, F. ATROOZ, G. PATKI, S. SALIM;
Univ. of Houston -college of Pharm., Houston, TX

Abstract: Post-traumatic stress disorder (PTSD) is a condition that can result from exposure to a severe traumatic event such as those occurring during wars or natural disasters. Our previous work has suggested that single prolonged stress (SPS) induces behavioral and cognitive deficits in a rat model of PTSD. Furthermore, we have reported protective effects of grape powder (GP) on behavioral impairments in pharmacologically-induced oxidative stress. However, the protective effect of GP in PTSD is unknown. Therefore, in the present study using the SPS rat model of PTSD (2 h restrain, 20 min forced swimming, 15 min rest, and 1-2 min diethyl ether exposure), we examined the protective effect of GP on SPS-induced behavioral deficits including anxiety and depression-like behaviors and memory impairment. Male Sprague Dawley (SD) rats were randomly assigned into four groups: Naïve control, SPS, GP-SPS (3 weeks of GP followed by SPS), or GP-Control (3 weeks of GP followed by control exposure). Anxiety-like behavior tests (open-field, light-dark and elevated plus maze) suggested that GP treatment prevented SPS-induced anxiety and depression-like behavior of rats. Moreover, GP also improved SPS-induced impairment of memory function of rats, when examined using radial arm water maze test. Moreover, measurement of oxidative stress parameters in plasma suggests that GP reduced SPS-induced increased 8-isoprostane levels. However, no beneficial effect of GP was observed on plasma corticosterone levels suggesting 3 weeks of GP was not sufficient to ameliorate SPS-induced increased plasma corticosterone levels. Results suggest that grape powder ameliorates SPS-induced behavioral and cognitive deficits in rats.

Disclosures: **N. Solanki:** None. **I. Alkadhi:** None. **F. Atrooz:** None. **G. Patki:** None. **S. Salim:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.07/TT75

Topic: F.02. Animal Cognition and Behavior

Support: NSFC U1132602

Title: Memantine attenuates reinstatement of heroin seeking induced by heroin or cues in an animal model of relapse

Authors: M. LAI[1], *H. LIU[2], W. ZHOU[1];
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Abstract: **Abstract** ***Rational*** Memantine is used for the treatment of Parkinson's disease,spasticity,and dementia, characterized by antagonist activity at NMDA glutamatergic receptor. Due to the unique pharmacological property, memantine prevented acquision and expression of the preference produced by cocaine or blocked reinstatement of morphine-conditioned reward in animal model of conditioned place preference. But the effect and mechanism of memantine on reinstatement induced by heroin priming or conditioned stimuli are uncertain. ***Objectives*** The present study tested the effects of acute treatment with memantine on the heroin seeking behavior. ***Methods*** Rats were self-administered heroin under a fixed ratio 1(FR1) schedule for 14d and then extinguished for 10d. A progressive schedule (PR_{3-4}) was used to evaluate the relative motivational value of heroin reinforcement. After training, the conditioned cue or heroin priming (250 μg/kg) was introduced for the reinstatement of heroin-seeking behavior. The effects of memantine at doses 1-15mg/kg pretreatment on behaviors were examined under these schedules. ***Results*** Memantine at these doses produced a upward shift in the dose-response curve for heroin self-administration, but failed to inhibition the break point (reward value) under the PR_{3-4} schedule. In addition, memantine at doses 5-15mg/kg inhibited the reinstatement of heroin seeking induced by heroin priming in a dose-dependent manner, while at dose of 15mg/kg inhibited the reinstatement of conditioned cue-induced heroin seeking. Memantine at these doses failed to alter locomotion activity. ***Conclusions*** These data demonstrate that acute treatment with memantine inhibites the reinstatement of heroin seeking induced by heroin priming or conditioned cues and memantine may be an adunctive therapy for the treatment of heroin addiction. **Keywords:** NMDA receptor; heroin; relapse; addiction;

Disclosures: **M. lai:** None. **H. Liu:** None. **W. zhou:** None.

Poster

846. Learning and Memory: Pharmacology II

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Program#/Poster#: 846.08/TT76

Topic: F.02. Animal Cognition and Behavior

Title: Swimming forced test alteration and scanner electron microscope in cerebellar cortex from 12-days-old pup mice treated with midazolam

Authors: ***M. MARQUEZ-OROZCO**[1], G. DE LA FUENTE-JUAREZ[1], S. A. SANTIAGO-LÓPEZ[1], E. PEREZ-MENDOZA[1], J. A. JOYA-VENEGAS[1], A. FORTANEL-FONSECA[1], L. A. MORONES-SÁNCHEZ[1], J. A. SEPULVEDA SANCHEZ[3], J. A. SEPULVEDA SANCHEZ[3], A. MARQUEZ.OROZCO[2];
[1]Dept Embryol, Univ. of Mexico (UNAM), Mexico, Mexico; [2]Embryology, Univ. of Mexico (UNAM), Mexico 04510 DF, Mexico; [3]Lab. de MicroscopIa Electronica. Edificio W de la Ciencia y Tecnologia Ambiental. UAM, Iztapa, UAM-Iztapalapa, México, D.F., Mexico

Abstract: Midazolam (MDZ) is a widely prescribed benzodiazepine minor tranquilizer. MDZ is used as sedative, anxiolytic; hypnotic rapidly induces tranquility and deep sleep during brief surgical procedures performed with local anesthetic. Intravenous administration of MDZ has been widely used in pediatric patients for conscious sedation. The drug is easily transported into brain tissues after intravenous infusion of MDZ induces an anterograde amnesia that created an illusion of anesthetic in some patients. The aim of this work was investigate if MDZ administrated from postnatal 6 to 9-days old induce in 12-days old pup's mice swimming forced test alterations and ultraestructural changes in developmental neonatal cerebellar cortex. Two ICR strain pups mice group were injected daily sc from day 6 to 9. The first group (MDZ) was treated with single daily MDZ doses (1.0 mg/kg/bw) and the second group (C) with saline solution. The mice (20 MDZ and 20 C), at 12-days old were placed for 60 seg in acrylic cylindrical swimming pool (40x30 cm) filled up to 20 cm (31 to 32°C). The forced swimming test were recorded in front and dorsal position and analyzed to identified behavioral differences ($p=0.05$). The animals were killed in a CO2 atmosphere; the cerebellum fixed in 2.5 % glutaraldehyde, post-fixed in OsO4, the sections were observed under a Scanning Electron microscope (Jeol JSM-5900 LV). The MDZ mice exhibit uncoordinated movements of scapular and pelvic limbs show a clear tendency to raise, and drop the tail in a whip-like fashion, inability

to maintain a straight line swim and frequently the nose were induces introduces in the water. In the cerebellar cortex of MDZ group was observed in the outer granular layer numerous and voluminous granular cells (p=0.05). The Purkinje cell layer show areas with reduced number cell bodies of the Purkinje cells any ones had voluminous or shrink soma and other were show pseudostratified. Result show that postnatal exposure to MDZ induce in the 12-days old mice treated during 4 days delay in maturation in swimming behavior pattern such as maintenance strain direction and limb coordination. Histological alterations were attributed to deficient neural differentiation and cell neuroapoptosis produced by MDZ administration from day 6 to 9 days postnatal pups mice.

Disclosures: **M. Marquez-Orozco:** None. **G. De la Fuente-Juarez:** None. **S.A. Santiago-López:** None. **E. Perez-Mendoza:** None. **J.A. Joya-Venegas:** None. **A. Fortanel-Fonseca:** None. **L.A. Morones-Sánchez:** None. **J.A. Sepulveda Sanchez:** None. **J.A. Sepulveda Sanchez:** None. **A. Marquez.orozco:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.09/TT77

Topic: F.02. Animal Cognition and Behavior

Support: Baylor URSA Grant

Title: Repeated oral doses of aniracetam does not alter anxiety, locomotion, or learning and memory in adult C57BL/6J mice

Authors: A. PANDIAN[1], T. W. ELSTON[1], G. D. SMITH[2], A. J. HOLLEY[1], N. GAO[1], *J. N. LUGO, JR[1];
[1]Psychology and Neurosci., [2]Inst. of Biomed. Studies, Baylor Univ., Waco, TX

Abstract: There is a growing community of individuals self-administering aniracetam, a nootropic, for its purported cognition enhancing effects. Aniracetam is believed to be therapeutically useful for enhancing cognition, alleviating anxiety, and treating various neurodegenerative conditions. Physiologically, aniracetam enhances both glutamatergic neurotransmission and long-term potentiation. Previous studies of aniracetam demonstrate the cognition-restoring effects of acute administration in different models of disease. No previous studies have explored the effects of aniracetam in healthy subjects. We investigated whether

daily 50 mg/kg oral administration improves cognitive performance in naïve C57BL/6J mice by a variety of aspects of cognitive behavior. We measured spatial learning in the Morris water maze test; associative learning in the fear conditioning test; motor learning in the accelerating rotorod test; and odor discrimination. We also measured locomotion in the open field test; anxiety through the elevated plus maze test and by measuring time in the center of the open field test; repetitive behavior through marble burying. We detected no significant differences between the naive, placebo, and experimental groups across all measures. Despite several studies demonstrating efficacy in impaired subjects, our findings suggest that aniracetam does not alter behavior in normal healthy mice. This study is timely in light of the growing community of healthy humans self-administering nootropic drugs.

Disclosures: **A. Pandian:** None. **T.W. Elston:** None. **G.D. Smith:** None. **A.J. Holley:** None. **N. Gao:** None. **J.N. Lugo:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.10/TT78

Topic: F.02. Animal Cognition and Behavior

Title: Learning and memory improvement by the decoction of roots of Clerodendrum capitatum

Authors: *E. NGO BUM[1], D. ZOUHEIRA[2], J. S. K. NJAPDOUNKE[2], G. C. N. NKANTCHOUA[2], F. C. O. MOTO[3];
[2]Biol. Sci., [1]Univ. Ngaoundere, Cameroon, Ngaoundere, Cameroon; [3]Univ. of Yaoundé I, Yaoundé, Cameroon

Abstract: Clerodendrum capitatum (Verbenaceae) is used as traditional medicine in Cameroon to treat memory disorders. *In vivo* mice models (T-maze and open field) were used to evaluate the possibility of Clerodendrum capitatum to increase learning memory. Scopolamine was used to induce memory loss. Mice were divided in five or six groups. In five groups experiment, Group I received distilled water, groups II to IV: doses of the plants, group V: piraretam 200 mg/kg. In six groups experiment, Group I received distilled water, group II: scopolamine 0.3 mg/kg, groups III to V: doses of the plants and scopolamine 0.3 mg/kg, group VI: piraretam 200 mg/kg and scopolamine 0.3 mg/kg. The decoction was administered orally in a volume of 10 ml/kg of body weight. Clerodendrum capitatum like piracetam increased the number and the duration of entries into the preferred arm and reduced the latency time to enter the preferred arm

and the number of return in the initial arm. In the presence of scopolamine, the decoction reversed the effect of scopolamine. In object recognition test, Clerodendrum capitatum increased exploration time of the new object and reduced the latency time to find the new object. Finally Clerodendrum capitatum reduced cholinesterase activity in the brain. The effects of Clerodendrum capitatum suggested the presence of memory improvement activities that might show efficacy against memory loss in humans.

Disclosures: **E. Ngo Bum:** None. **D. Zouheira:** None. **J.S.K. Njapdounke:** None. **G.C.N. Nkantchoua:** None. **F.C.O. Moto:** None.

Poster

846. Learning and Memory: Pharmacology II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.11/TT79

Topic: F.02. Animal Cognition and Behavior

Support: UCLA Division of Life Sciences Recruitment and Retention Fund (Izquierdo)

Title: The role of basolateral amygdala serotonin in discrimination, retention, and reversal learning

Authors: J. G. OCHOA, *A. IZQUIERDO;
Dept. of Psychology, UCLA, LOS ANGELES, CA

Abstract: In goal-directed pursuits, the basolateral amygdala (BLA) is critical in learning about changes in the value of rewards. We recently reported that BLA-lesioned rats showed enhanced reversal learning (Izquierdo et al. 2013), a task employed to measure the flexibility of response to changes in reward. In that study, rats showed facilitated reversal learning, but also a trend for enhanced discrimination learning, suggesting that BLA may be involved in forming new stimulus-reward associations. There is a parallel literature on the importance of serotonin (5HT) in new stimulus-reward learning (Izquierdo et al. 2012) and that 5HT tone is important for reversal learning (Clarke et al. 2004; Schilman et al. 2010). Recent postulations implicate 5HT in learning from punishment: Whereas dopaminergic involvement is critical in behavioral activation and reinforcement, 5HT may be most critical for aversive processing and behavioral inhibition, complementary cognitive processes (Cools et al. 2011). Given these findings, we hypothesize a 5HT-mediated mechanism in BLA for the facilitated learning we observed previously. In the present study, we compared the effects of selective 5HT lesions in BLA using

5,7-dihydroxytryptamine (5,7-DHT; n=9) versus infusions of saline (SHAM; n=6) on discrimination, retention, and reversal learning. Rats were required to reach an 85% correct pairwise discrimination and single reversal criterion before surgery. Postoperatively, rats were then tested on 1) retention of the pretreatment discrimination pair 2) discrimination of a novel pair and 3) reversal learning performance. We found statistically comparable preoperative learning rates between treatment groups, intact postoperative retention, and unaltered novel discrimination learning and reversal learning in 5,7-DHT-depleted rats. These findings suggest that 5HT in BLA is not involved in stimulus-reward association learning, particularly when animals have already had experience with this type of learning preoperatively. Given the complementary role of orbitofrontal cortex in reward learning and its interconnectivity with BLA, ongoing studies are aimed at comparing this learning to that observed after 5HT depletions in orbitofrontal cortex.

Disclosures: J.G. Ochoa: None. **A. Izquierdo:** None.

Poster

846. Learning and Memory: Pharmacology II

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Topic: F.02. Animal Cognition and Behavior

Support: MSMT CZ LH14053

GACR 14-03627S

AS CR M200111204

GACR P304/12/G069

IGA MZ CR NT13386

Title: Task/context alternation and behavioral temporal separation as an animal model of executive functions

Authors: I. VOJTECHOVA, T. PETRASEK, A. PISTIKOVA, J. SVOBODA, K. VALES, *A. STUCHLIK;
Inst. of Physiology, Acad. of Sci. of the Czech Republic, Prague, Czech Republic

Abstract: Behavioral separation is an ability to distinguish and switch between coexisting mental representations of distinct environments, and choose the response appropriate in the particular context. We propose that it is a behavioral correlate of pattern separation, a hippocampal function enabling creating separated non-overlapping representations of similar inputs. Behavioral separation may serve as a model of executive functions in laboratory animals. We used adult male rats, which were tested in two spatial tasks tapping different hippocampal functions: the Morris water maze (testing spatial navigation) and the active place avoidance on Carousel (testing cognitive coordination). In task alternation experiment, focused on separation of different hippocampal functions, the rats were tested in the two different tasks in either alternating or non-alternating manner. In context alternation experiment, focused on separation of two similar representations, the rats solved the same task (Carousel) in two different rooms, also in alternating or non-alternating manner. In both cases, we evaluated the influence of alternation on rat performance. Alternation of neither tasks nor contexts impaired the performance of the rats in any evaluated parameter. All groups were able to learn the spatial tasks equally well. Impairments in executive functions are observed in schizophrenic patients. We applied these two experiments to test behavioral separation in adult male rats with i.p. application of MK-801 which is used as a pharmacological model of schizophrenia in laboratory rodents. MK-801 (0.08 mg/kg) caused a deficit in learning only in active place avoidance in the task alternation experiment. The rat performance in the context alternation experiment was not affected by MK-801. We can conclude that the alternation of different tasks or similar environments, requiring formation and simultaneous storage of two different spatial representations, does not impair the ability to manage the spatial task in control animals. MK-801 does not cause deficit in behavioral separation, however impairs learning selectively in active place avoidance on Carousel, but not in the Morris water maze. This work was supported by GACR grant 14-03627S and IGA MZ CR NT13386, by AS CR M200111204 and by GACR P304/12/G069 and by MSMT LH14053.

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Poster

846. Learning and Memory: Pharmacology II

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.13/TT81

Topic: F.02. Animal Cognition and Behavior

Support: CIHR Operating Grant

Title: Impaired performance on touchscreen object-location paired associates learning by acute systemic MK-801 is reversed by L-govadine but not D-govadine or CDPPB

Authors: B. R. LINS[1], A. G. PHILLIPS[2], *J. G. HOWLAND[3];
[1]Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Physiol., Univ. Saskatchewan, Saskatoon, SK, Canada

Abstract: Cognitive impairments in schizophrenia are increasingly recognized as fundamental to the illness and a great contributor to patient disability. Efforts to address these symptoms have focused on identification of novel drug targets. Tetrahydroprotoberberines, first derived from ingredients in traditional Chinese herbal medicine and recently synthesized into separate enantiomers including D- and L-govadine hold promise in treating positive, negative and cognitive symptoms of schizophrenia. Metabotropic glutamate receptors are also drug targets with the potential to modulate NMDARs, key receptors involved in both cognition and schizophrenia in general. This approach has identified CDPPB, an mGluR5 positive allosteric modulator. The present experiments assessed the effects of D- and L-govadine and CDPPB on the Paired Associates Learning (PAL) task in rats. The PAL task, part of the CANTAB battery, is impaired in patients with schizophrenia and has been adapted for use with rodents using touchscreen-equipped operant chambers. The objectives of this study were: 1) examine the effects of acute NMDA receptor antagonism with MK-801 on performance of the PAL task; and 2) test the effects of the putative antipsychotics, D- and L-govadine and CDPPB on the disruption of PAL by NMDA receptor antagonism. Two independent groups of male Long-Evans rats were trained to perform the PAL task in touchscreen-equipped operant chambers. Following training, each group was administered one of the following treatment schedules: vehicle (50% DMSO; s.c.), the NMDA receptor antagonist MK-801 (0.15 mg/kg; i.p.), D-govadine (1 mg/kg; s.c.), L-govadine (1 mg/kg; s.c.) and both MK-801 and one isomer of govadine; or vehicle (10% cyclodextrin; i.p.), MK-801, CDPPB (3.0 mg/kg, i.p.), and MK-801 with CDPPB. All treatments were administered to each rat in the appropriate group in a counterbalanced order. Acute MK-801 significantly reduced the number of trials completed, impaired accuracy, and increased the number of errors in the PAL task. Administration of L-govadine, but not D-govadine, prior to MK-801 improved accuracy and reduced errors compared to MK-801 alone. L-govadine alone, but not D-govadine, improved performance on the PAL task by reducing errors compared to vehicle. L-govadine, but not D-govadine, also increased latency to make a selection in the task. CDPPB alone had no effect on the PAL task and did not improve the MK-801 induced impairments. These data establish disruptive effects of acute MK-801 treatment on PAL task performance and suggest that L-govadine may have properties consistent with a compound useful in the treatment of the cognitive symptoms of schizophrenia.

Disclosures: B.R. Lins: None. **J.G. Howland:** None. **A.G. Phillips:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.14/TT82

Topic: F.02. Animal Cognition and Behavior

Title: Withdrawal from chronic intermittent access to ethanol alters within-session patterns of responding, but does not impair reversal learning, in rats

Authors: M. H. RAY, N. BRIGHT, M. GALLO, N. MWEBAZA, B. RODGERS, K. RUGGLE, *C. L. PICKENS;
Kansas State Univ., Manhattan, KS

Abstract: Exposure to drugs of abuse has been shown to impair reversal learning in rodent models. We determined whether chronic intermittent access to ethanol would cause impairments in go/no-go reversal learning in rats. Rats were given discrimination training with 2 levers to the right or left of the chamber. Each lever was consistently associated with a steady or flashing cuelight above the lever. Each lever-light combination was available in alternating order with presses on one lever reinforced with food pellets ("active lever") and presses on the other lever never reinforced ("inactive lever"). Each lever-light was available for 40-sec regardless of whether lever presses were made, and presses on the active lever could earn 2 food pellets/trial at predictable times within the cue. The rats then received 6 weeks of chronic intermittent access to 20% ethanol (24-h access, 3 times/week with water always available) along with a 1.5 g/kg injection of ethanol at the end of each 24-h access period, 6 weeks of ethanol access without injections, or 6 weeks of access to water alone with no injections. The group given access to ethanol without injections drank ~8 g/kg/24-h for the last 3 weeks of ethanol access. The group given ethanol injections exhibited greatly suppressed drinking (~1 g/kg/24-h for the last 3 weeks of ethanol access). The rats then received one discrimination reminder session 4 days after the final day of ethanol access, and then received 9 days with the lever-light contingencies switched and the previously non-reinforced lever-light compound reinforced and the previously reinforced lever-light compound non-reinforced. We found no evidence for impaired reversal learning in overall responding during each session or the responding during the first 8 trials of each session. However, the rats that received ethanol injections and those limited to water decreased responding on the inactive and active lever within each session (within-session extinction on the inactive lever and, possibly, a reduction in over-responding during the active lever trials). This reduction in responding within-session was delayed for inactive lever presses and absent for the active lever presses in the rats with access to ethanol without injections. This finding is accord

with previous research showing that repeated ethanol withdrawal impairs the reduction in responding to an S- seen during a different discrimination task and increases reinforced responding when rats respond on a fixed interval schedule [1]. Future work will determine the psychological and neurobiological basis of this behavioral change. 1)Borlikova et al. (2006) Eur J Neurosci. 24:205-216.

Disclosures: **M.H. Ray:** None. **C.L. Pickens:** None. **N. Bright:** None. **M. Gallo:** None. **N. Mwebaza:** None. **B. Rodgers:** None. **K. Ruggle:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.15/TT83

Topic: F.02. Animal Cognition and Behavior

Title: Swimming behavioral alteration in 21 days-old pups mice treated with midazolam

Authors: *A. MARQUEZ-OROZCO, G. DE LA FUENTE-JUAREZ, S. A. SANTIAGO-LÓPEZ, E. PEREZ-MENDOZA, L. A. MORONES-SANCHEZ, M. C. MARQUEZ-OROZCO; Dept Embryol, Univ. of Mexico (UNAM), Mexico 04510 DF, Mexico

Abstract: Midazolam (MDZ) is a hypnotic benzodiazepine that rapidly induces tranquility and deep sleep during brief surgical procedures performed with local anesthetic. The drug is easily transported in to brain tissues after intravenous infusion. MDZ induces an anterograde amnesia that created an illusion of anesthesia in some patients. Intravenous administration of MDZ has been widely used in pediatric patients of conscious sedation in procedures such as lumbar punctures, and bone marrow aspiration and epileptic status. MDZ administered postnatally alters the development of both neuronal in cerebral cortex and Purkinje cells in cerebelar cortex. The purpose of this work was investigated if 21-day-old mice postnatally treated with MDZ induce swimming behavior alterations. Two neonatal ICR strain mice groups were injected daily sc from day 6 to 9 postnatal. The first group (MDZ) was treated with single daily MDZ doses (1.0 mg/kg/bw/sc) and the second group (C) with saline solution. The pups (20 MDZ and 20 C), were placed for 60 seg in acrylic cylinder swimming of (40x30 cm) filled up to 20 cm (31 to 32°C). The forced swimming test was videorecorded in frontal and dorsal position and analyzed to identify behavioral differences ($p < 0.05$). Forced swimming activity was graded according to normal or abnormal behavioral. The MDZ animal exhibit significative abnormal swimming characteristics such as uncoordinated movements of the anterior and posterior limbs, inability to

maintain a straight line swim and a clear tendency to raise and drop the tail in a whip-like fashion. The alterations observed may be attributed to delayed neuronal differentiation and cell neurodegeneration produced in cerebral and cerebellar cortices, induces by MDZ exposition from 6 to 9 days old mice. Drugs that potentiate GABAA receptors such as MDZ and diazepam can trigger widespread apoptotic neurodegeneration. In the case of MDZ is has been previously shown the doses sufficient to maintain surgical anesthesia for 6 hr. in 7 day-old rats caused widespread apoptotic neurodegeneration in the developing brain and persisted memory/learning impairment. We found similar results, which may be explained by the histological changes in the cerebral and cerebellar cortices induced by postnatal exposure to MDZ that we found in our trial.

Disclosures: A. Marquez-Orozco: None. **G. De la Fuente-Juarez:** None. **S.A. Santiago-López:** None. **E. Perez-Mendoza:** None. **L.A. Morones-Sanchez:** None. **M.C. Marquez-Orozco:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.16/TT84

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NINDS F31NS068219-01A1

NIH/NIGMS 5T32GM008541

Title: An α5GABA$_A$ receptor negative allosteric modulator increases spatial selectivity of CA1 place cells and hippocampal-dependent spatial memory

Authors: *T. M. STEWART, M. H. RATNER, S. S. DOWNING, D. H. FARB; PHARMACOLOGY, Boston Univ. Sch. of Med., BOSTON, MA

Abstract: The use of high-density chronically implanted electrodes to monitor systems level *in vivo* electrophysiological responses to therapeutic agents is emerging as a powerful tool for the validation of targets and targeted drugs (1). In this study we sought to determine whether the spatial learning and memory enhancing effects of the α5GABA-A receptor selective negative allosteric modulator, α5IA (3-(5-methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyl-oxy]-1,2,4-triazolo[3,4-a]phthalazine) (2), may be mediated by changes in hippocampal pyramidal neuron, or "place cell," firing rates in the context of exposure to environmental

novelty. We asked whether the basic hypothesis that negative modulation of an inhibitory receptor would yield an increase in firing rate of CA1 place cells (3). We chose to record from CA1 place cells while rats explored familiar and novel environments because a previous report showed that a larger fraction of CA1 than CA3 neurons had definable place fields (by peak rate criterion), allowing more opportunities to detect pharmacological effect(s) (3). Here, we determined the effect of orally administered α5IA (1.0 mg/kg) on (a) CA1 hippocampal place cell firing rates, place field area, and spatial selectivity in a novel versus a familiar environment, and (b) spatial memory performance on the hippocampal- dependent location novelty recognition task. The results show that α5IA: i) increases peak firing rates by nearly 2-fold in familiar but not novel environments, such that the firing rate in familiar in the presence of α5IA is equivalent to novel environment, ii) increases spatial selectivity in both novel and familiar environments; iii) reduces the similarity of the place fields between the environments; iv) alters place field area depending on environment (enlarges area in a novel environment while decreasing area in a familiar environment); and v) augments the novel-environment-induced increase in place field area. In the location novelty recognition task, α5IA enhances spatial memory performance as indicated by the observation that rats spend more time exploring an object in a novel location (i.e., one displaced in location) than one that remains in the same location following treatment, in agreement with previous reports that α5IA enhances spatial learning and memory function in the rat. (1) Robbe and Buzsáki (2009). J Neurosci 29:12597-12605. (2) Dawson et al., (2006). J Pharmacol Exp Ther 316:1335-45. (3) Mizuseki et al., (2012). Hippocampus 22:1659-1680.

Disclosures: T.M. Stewart: None. **M.H. Ratner:** None. **S.S. Downing:** None. **D.H. Farb:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.17/TT85

Topic: F.02. Animal Cognition and Behavior

Title: Divergent effects of ketamine and memantine in the novel object recognition test in Wistar rats

Authors: E. KOROS, *K. A. ALLERS;
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Abstract: Patients with Alzheimer's disease (AD) display impaired explicit memory including recognition memory. Also, individuals with schizophrenia show face recognition memory deficits. In both diseases characterized by altered glutamatergic neurotransmission, the exact biochemical correlates of memory deficits have to be elucidated. It is well known that NMDA receptor activation is obligatory for memory formation whereas NMDA receptor blockade disrupts cognitive processes comprising also recognition memory. Surprisingly, it has been reported that memantine, an uncompetitive NMDA receptor antagonist approved for treating patients with moderate to severe AD, can be effective in improving certain memory deficits. Therefore, we decided to examine effects of memantine as well as ketamine, another low-moderate affinity uncompetitive NMDA receptor antagonist, on the recognition memory in Wistar rats. We utilized the novel object recognition test (NORT), as it is a widely used test for measuring recognition memory in rodents. The task is based on an animal's inherent nature to explore novel aspects of its environment, that is preferential exploration of a novel object relative to a familiar one. In the learning trial (T1) a rat was placed in an open arena with two similar objects for 5 minutes. After a defined delay (1h or 24h) the rat was re-exposed to the arena but this time with one familiar (former) and one novel (new) object. In both trials the amount of time spent exploring the objects was recorded. The results showed that ketamine, but not memantine, impaired recognition memory assessed after delay of 1 hour. Memantine was further examined in NORT using both pharmacologically-induced (1h delay) and time-dependent (24h delay) memory deficit models. Memantine did not reverse the ketamine-induced deficit in memory acquisition and showed no improving effects on time-dependent deficits in memory acquisition, retrieval and consolidation. These findings show that effects of memantine contrast strongly with the effects of ketamine on recognition memory in NORT. Possible explanations of the observed differences between these two drugs that have strikingly similar channel blocking properties will be discussed.

Disclosures: E. Koros: None. **K.A. Allers:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 846.18/TT86

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP

CNPQ

Title: H1-histamine receptors antagonist in the amygdala impair memory acquisition in mice reexposed to the elevated plus maze

Authors: *K. R. SERAFIM, C. E. M. FERNANDES, A. C. L. GIANLORENÇO, R. MATTIOLI;
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Abstract: Some evidence supports the concept that histaminergic neurons influence learning and memory via H1 histaminergic receptor activation. The aim of the present study was to verify the effects of microinjected chlorpheniramine (CPA), a histaminergic H1 receptor antagonist, on emotional memory of mice reexposed to the Elevated Plus Maze (EPM). Tests were performed on two consecutive days: Trial 1 (T1) and Trial 2 (T2). Drugs were administered prior T1 and T2. Twenty-four hours later (i.e., T2) the mice were injected again under the same experimental conditions. Before each trial, mice were microinjected with CPA (0.16 nmol/0.1μl), or saline (SAL) into the amygdala and submitted to the EPM. Anxiety was assessed in the EPM by recording the conventional measures (percentage of open arm entries - %OAE and percentage of time spent in open arms - %OAT] in T1. The decreased open-arm activity (%OAE and %OAT) in T2 was defined as learning and memory index. The data were analyzed using two-way Analysis of Variance (ANOVA) and Duncan´s tests. Post hoc test did not show significant differences between SAL-SAL and CPA-SAL or CPA-CPA groups in T1 for %OAE and %OAT ($p>0.05$), indicating that the drug did not induce changes in anxiety level. Emotional memory, as revealed by a reduction in open arm exploration between both trials, was present in the SAL-SAL group as well as in the SAL-CPA group ($p<0.05$). On the contrary, neither the CPA-CPA group nor the CPA-SAL group ($p>0.05$) exhibited this decrease in open arm activity between both trials, which reveals that CPA impaired emotional memory acquisition. No significant changes were observed in the number of enclosed arm entries (EAE), an EPM index of general exploratory activity. Taken together, these results suggest that the H(1) receptors in the amygdala are not implicated in anxiety-like behaviors but are involved in emotional memory acquisition and consolidation deficits in mice subjected to EPM testing.

Disclosures: K.R. Serafim: None. **C.E.M. Fernandes:** None. **A.C.L. Gianlorenço:** None. **R. Mattioli:** None.

Poster

846. Learning and Memory: Pharmacology II

Location: Halls A-C

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Program#/Poster#: 846.19/TT87

Topic: F.02. Animal Cognition and Behavior

Support: NICHD Grant R01-HD068388

Title: Multiple exposures to sevoflurane general anesthesia impact cognitive development in rhesus macaques

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Abstract: General anesthetics (GA) may act as neurotoxins when given early in mammalian development. In particular, children who received more than one GA before the age of 4 are at a greater risk of learning disability directly related to the number of anesthetic exposures, and negative behavior changes, such as increased social anxiety. Experiments in rodents have also demonstrated increased long-term impairments in cognition and social behavior. In nonhuman primates, GA are also neurotoxic to infants, and as we recently reported, multiple sevoflurane exposures produced lasting changes in reactivity to a social stressor. Nevertheless, the long-term effects of neonatal GA on cognition are unknown. To that end, we evaluated the effects of multiple exposures to sevoflurane, a common pediatric anesthetic, in infant rhesus monkeys over the first 5 weeks of life. Two cohorts of infants (n=20, 10 males) received either 3, 4-hour exposures to 2.5% sevoflurane 1, 3, and 5 weeks of age (Anesthesia Group), or a brief maternal separation and exposure to room air at the same ages (Control Group). The infants were returned to their dams following each procedure and were reared in large social groups at the Yerkes National Primate Research Center. Beginning at six months of age, they were tested on the visual paired comparison task (VPC) using color images of everyday objects and delays of 5, 10, 30, 60 & 120s. Results from the first cohort have yielded evidence of a mild memory impairment at the youngest testing age, that is largely captured by impaired performance in the female infants. Testing for the second cohort is underway, as well as continued cognitive testing on the first cohort, thus it remains to be seen whether this early impairment maintains or worsens with the addition of the second cohort and as the animals age.

Disclosures: M.C. Alvarado: None. **K.L. Murphy:** None. **M.G. Baxter:** None.

Poster

846. Learning and Memory: Pharmacology II

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NINDS Grant NS036654

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Lundbeck AS

Michael J. Fox Foundation

Title: Mechanism of action of a novel series of drug-like N-methyl-D-aspartate receptor positive allosteric modulators and their effects in hippocampal CA1 neurons

Authors: *R. E. PERSZYK[1], G. P. K. REDDY[2], E. C. GARNIER-AMBLARD[2], S. A. SWANGER[1], G. FERNANDEZ-CUERVO[2], L. S. LIEBESKIND[2], S. F. TRAYNELIS[1]; [1]Pharmacol., [2]Chem., Emory Univ., Atlanta, GA

Abstract: N-methyl-D-aspartate receptors (NMDA-Rs) are ionotropic ligand-gated ion channels that are activated by glutamate and glycine and mediate a slow component of excitatory synaptic currents in the central nervous system. These receptors play important roles in synaptic plasticity, neuronal development, and have been implicated in a number of neurological disorders. The role of NMDA-Rs in plasticity is postulated to underlie many learning processes involving several brain regions. For example, a mouse strain with 2-fold higher expression of the GluN2B receptor in the forebrain was shown to have enhanced learning capabilities (Tang et al. 1999, Nature). Additionally, the loss of GluN2B in similar brain structures was shown to cause learning deficiencies (Brigman et al. 2010, J Neuroscience). We have recently identified a novel series of positive modulators of NMDA-Rs. Evaluation of this series of benzamido-thiophenes compounds (1622 series) revealed that these compounds can potentiate GluN2B-containing receptors by 2 to 4 fold (EC_{50} 4-15 μM) in addition to potentiating those containing GluN2C and GluN2D by 2 to >4 fold. 1622 also enhanced glutamate potency (1.7-fold). This series potentiates NMDA-Rs by increasing the time the channel spends in the open conformation (1622 30μM, 2 fold increase in NP_o, n=3). We are currently investigating the mechanism of action of newer analogs of this class and their ability to potentiate NMDA-Rs in native tissues. In rapid solution exchange electrophysiology experiments, 1622-35 prolonged the deactivation of NMDA-Rs roughly 3-fold, as expected by enhanced glutamate potency. In hippocampal CA1 neurons, which express GluN2A and GluN2B, 1622-35 prolonged the time course of excitatory post synaptic currents (control 280 ± 30 ms, 10μM 1622-35 970 ± 220 ms, n=4) as well as increased the total charge transfer of these events (2.3 times greater than control). These modulators could be used to assess the ability of pharmacological augmentation of GluN2B-containing receptors to promote plasticity induction and facilitate learning.

Disclosures: **R.E. Perszyk:** None. **G.P.K. Reddy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emory University. **E.C. Garnier-Amblard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emory University. **S.A. Swanger:** None. **G. Fernandez-Cuervo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emory University. **L.S. Liebeskind:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emory University, NeurOp Inc. **S.F. Traynelis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Emory University, NeurOp Inc.. F. Consulting Fees (e.g., advisory boards); NeurOp Inc..

Poster

847. Learning and Memory: Aging II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 847.01/TT89

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant- R01AG029421 (JLB)

McKnight Brain Research Foundation (JLB)

McKnight Brain Institute (CAO)

Title: Aging is associated with reduced choice of risky options in Fischer 344 rats

Authors: *B. SETLOW[1], C. A. ORSINI[2], J. L. BIZON[3];
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Abstract: To date, the vast majority of cognitive and neurobiological research in aging has focused on understanding the mechanisms that contribute to impaired learning and memory. However, an emerging literature suggests that aging is also accompanied by alterations in other more integrative cognitive functions such as cost-benefit decision-making, and that these age-related alterations may not always manifest as deficits. For example, our lab previously reported that aged rats show attenuated discounting of delayed rewards in an intertemporal choice task (elevated preference for large delayed over small immediate rewards). The current study sought

to extend this work by investigating aged rats' performance on a similarly designed risk-based decision making task. In this task, young adult (6 mo) and aged (24 mo) Fischer 344 rats chose between a small (1 food pellet) "safe" reward and a large (3 food pellets) "risky" food reward that is accompanied by varying probabilities of mild footshock punishment. Aged rats exhibited a decrease in preference for the large, risky reward in comparison to young adult rats. Importantly, these age differences in choice behavior did not appear to be attributable to age-related changes in shock reactivity as young and aged rats showed comparable reactions during shock delivery. Furthermore, additional data in this rat model across a variety of control measures indicate that these differences in choice behavior are not solely attributable to global learning, motoric, or motivational deficits. This decrease in risk taking in aged Fischer 344 rats is consistent with a literature showing greater risk aversion in older humans. These findings, in conjunction with our previous work showing that aging is also associated with elevated preference for delayed rewards, could be viewed as evidence for more optimal or "wiser" decision making in aging. However, it is important to consider that extreme risk aversion and/or preference for delayed gratification can also be detrimental (for example, in pathological conditions such as anorexia nervosa). Future experiments will attempt to further elucidate the conditions under which these age-related alterations in decision making are beneficial vs. maladaptive.

Disclosures: B. Setlow: None. **C.A. Orsini:** None. **J.L. Bizon:** None.

Poster

847. Learning and Memory: Aging II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 847.02/TT90

Topic: F.02. Animal Cognition and Behavior

Support: AG029421 (JLB)

McKnight Brain Research Foundation (JLB, SNB, and BKO)

Title: Interaction between age and perceptual difficulty in olfactory discrimination learning: relationship with spatial learning impairment

Authors: *J. L. BIZON[1], W. M. YODER[3], M. LYMAN[4], O. MUNIZZA[4], S. N. BURKE[1], B. K. ORMEROD[1], B. SETLOW[2], D. W. SMITH[3];
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Cognitive Neuroscience, Psychology, [4]Interdisciplinary Studies, Neurobiological Sci., Univ. of Florida, Gainesville, FL

Abstract: Previous work from our lab has shown that a subset of Fischer 344 rats develops olfactory learning impairments in aging which strongly correlate with hippocampal-dependent spatial memory deficits. The present study employed rigorous psychophysical assessments to more explicitly characterize the relationship between age-related olfactory alterations and cognitive deficits in this aged rat model. Fischer 344 rats (n=10 young; n=8 aged) were first characterized on a Morris water maze task to assess spatial learning abilities. To evaluate olfactory acuity, rats were subsequently tested in an automated, liquid-dilution olfactometer to discriminate a series of novel, monomolecular odorant pairs, systematically arranged to vary in task difficulty. Three classes of odorants (aliphatic alcohols, aliphatic aldehydes, carboxylic acids) were tested such that each pair within a class differed solely by 5, 3 or 1 carbon atoms. Previous research from our laboratory indicates that this method provides a reliable estimate of olfactory perceptual generalization. The accuracy in discrimination performance decreased in both young and aged rats as a function of perceptual similarity between the odorants in each pair. Notably, however, across odor classes, there was a significant Age X Perceptual Similarity interaction, such that aged rats performed disproportionately less accurately on more perceptually-similar problems. Subsequent analyses compared olfactory performance relative to spatial learning performance in the water maze. Notably, aged rats with good water maze performance performed comparably to young rats on the olfactory discrimination problems whereas aged rats with poor water maze performance showed significant impairments on the olfactory discrimination problems relative to young, particularly on the problems that involved perceptually similar odorants. These differences were not attributable to general learning deficits nor were they due to an inability to detect the odorants as odor thresholds did not differ as a function of age or spatial learning ability. Together, these findings suggest that a decreased ability to encode perceptual distinctions may contribute to impaired stimulus representations and cognitive impairments in aging. Ongoing studies will test the hypothesis that both olfactory and spatial learning deficits in a subset of aged rats are associated with impaired spatial pattern separation. Supported by AG029421 (JLB) and the McKnight Brain Research Foundation (JLB, SNB, and BKO).

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Poster

847. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG029421

McKnight Brain Research Foundation

Title: Activation of basal forebrain GABAergic projection neurons alters mPFC-mediated working memory performance in young F344 rats

Authors: *C. BANUELOS, B. SETLOW, J. L. BIZON;
Univ. of Florida, Gainesville, FL

Abstract: Working memory is a prefrontal cortical (PFC) supported executive function in which information is maintained for short durations in the absence of persistent sensory input. The ability to maintain an internal representation in working memory stores is thought to be mediated by the persistent excitation of pyramidal neuronal networks within the PFC. GABAergic interneurons within these PFC circuits are thought to provide essential spatial and temporal specificity in this system. Shifts in the excitatory-inhibitory dynamics within the prefrontal cortex in conditions such as schizophrenia or aging have been directly linked to cognitive deficits, including impaired working memory. Notably, in addition to the local inhibitory circuitry, the PFC receives GABAergic projections from the magnocellular preoptic (MCPO) area in the basal forebrain. These afferents synapse primarily on GABAergic interneurons and thus are well-positioned to regulate excitatory-inhibitory dynamics in this circuitry and possibly influence working memory function. To date, however, the role of these GABAergic basal forebrain afferents in PFC-supported cognition has not been well characterized. In this study, a pharmacological approach was used to assess how modulation of GABAergic basal forebrain neuronal activity affects working memory performance in young adult male F344 rats. Rats were surgically implanted with guide cannula aimed at the MCPO and trained on an operant-based delayed response test of working memory. In this task, rats were required to remember the location of a sample lever over a delay period (0-24 s) to obtain a food reward. After reaching stable, baseline performance, rats received microinjections of the M3 muscarinic receptor agonist cevimeline (5 μg and 10 μg) or vehicle directly into the MCPO immediately prior to testing using a within-subjects design. Cevimeline was chosen because of anatomical and electophysiological data demonstrating that this M3 muscarinic cholinergic receptor agonist, selectively activates basal forebrain GABAergic projection neurons. Cevimeline significantly enhanced working memory performance compared to vehicle conditions, particularly at long delays. These data support a role for GABAergic basal forebrain-PFC projections in working memory. Future work will further delineate this circuit and the contribution of other neurochemically distinct basal forebrain projection systems to PFC-

supported cognition using pharmacological and optogenetic approaches to achieve cell type specificity.

Disclosures: **C. Banuelos:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Poster

847. Learning and Memory: Aging II

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Program#/Poster#: 847.04/TT92

Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH R01 AG029421

Title: Effects of age on excitatory inputs to pyramidal cells and interneurons in rat medial prefrontal cortex

Authors: ***K. B. KELLY**[1], H. E. CARPENTER[2], J. A. MCQUAIL[2], J. L. BIZON[2], C. J. FRAZIER[1,2];
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Abstract: Working memory is supported by the medial prefrontal cortex (mPFC) in rodents. This process, which enables short term action based on a mental representation of the environment that is no longer present, is significantly impaired by age. Persistent firing of cortical pyramidal neurons has been suggested to underlie working memory, and recent evidence suggests that maintenance of persistent firing is likely to depend on NMDAR-mediated synaptic current. A large literature supports that NMDA receptor function can decline at advanced ages and recent work from our group has shown that there is an age-related reduction in NMDAR expression in mPFC, with a particularly robust decline of the NR2A subunit that has been preferentially localized to interneurons. This project used a physiological approach to evaluate and quantify age-related changes in excitatory synaptic transmission in the rodent mPFC. Towards that end, we performed whole-cell patch clamp recordings from both pyramidal cells and interneurons in layer 2/3 of the mPFC using slices extracted from both aged (20 month) and young adult (4 month) F344 rats. Spontaneous excitatory synaptic currents (sEPSCs) were monitored in cells voltage clamped at -70 mV, and evoked excitatory postsynaptic currents (eEPSCs) were generated using a monopolar stimulator placed in layers 1 or 5 (for stimulating

afferent inputs to pyramidal neurons) or in layer 2/3 (for stimulating afferent inputs to interneurons). Isolated AMPA and NMDA receptor dependent currents were generated by subtracting mean responses obtained before and after pharmacological blockade of specific ionotropic glutamate receptors (DNQX (20 μM) to block AMPARs and APV (40 μM) to block NMDARs). Data obtained to date indicate that the AMPA/NMDA ratio in layer 2/3 pyramidal cells is unaltered in aged animals. Interestingly, however, the data suggest that layer 2/3 interneurons in aged animals exhibit an increased AMPA/NMDA ratio relative to that observed in young animals. This increase in AMPA/NMDA ratio appears to more strongly reflect decreased NMDAR current than increased AMPAR current in the interneurons. Other data support that inhibitory interneurons experience an age-related decline in spontaneous excitatory synaptic inputs. Ongoing experiments will focus on further testing the hypothesis that NMDAR hypofunction in the mPFC contributes to age-related deficits in working memory, and clarifying whether excitatory transmission to cortical interneurons is particularly sensitive to advanced aging.

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Poster

847. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation (JLB).

NIH Grant AG029421

Title: A psychophysical technique for characterizing age-associated alterations in olfactory function

Authors: *W. M. YODER[1], L. GAYNOR[1], E. WINDHAM[1], B. SETLOW[2], J. L. BIZON[3], D. W. SMITH[1];
[1]Psychology, [2]Psychiatry, [3]Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Generalization gradients are frequently used in psychophysical assessments to distinguish normal sensory perception from subtle degrees of loss. These gradients reflect

differences in stimulus parameters along a given dimension and provide a physical measure for inferring perceptual attributes. Though gradients of perceptual similarity have been studied in the olfactory system, few have been applied to investigate age-related alterations. To determine how aging affects olfactory acuity, we employed an automated, liquid-dilution olfactometer to train Fischer 344 rats (N=10 young; N=8 aged) on a two-odor discrimination task. Graded perceptual similarity was measured across a series of homologous chemical pairs (aliphatic alcohols, aliphatic aldehydes, carboxylic acids). Twenty-four odorants were arranged to produce 12 novel odorant discriminations differing in functional groups and carbon chain length (Δ1 carbon atoms, Δ3 carbon atoms, Δ5 carbons atoms, structurally unrelated); testing sessions included presentation of only one pseudorandomly assigned pair daily (200 trials). Regardless of age, results show that performance declines systematically as a function of structural similarity (ΔC atoms); accuracy universally declines as the pairs become more perceptually confusing. Moreover, this trend is most robust for aliphatic aldehydes, suggesting this particular chemical class may be ideal for maximizing task difficulty. Notably, for all functional groups tested, declines in olfactory performance were most prominent for aged rats. Importantly, these age-related alterations did not extend to the three structurally unrelated odorant pairs, suggesting impairments were related to odor perception, rather than global learning deficits. Beyond understanding the role of olfactory perception in normal aging, the present study poses interesting questions regarding the translational utility of similar olfactory measures. A critical, ongoing challenge in clinical research has been the persistent disconnect between behavioral measures used in animal models and those used for human testing. Our laboratory has previously shown that identical stimuli, procedures and equipment can be used to investigate olfactory perception in human participants. The technique described here may provide a way to integrate an objective, cross-species measure using olfactory perceptual similarity as a key behavioral correlate to study aging. Furthermore, since odor learning memory has been shown to correlate with spatial memory declines in aged rats, it may be applied as a method for detecting subtle, cognitive alternations earlier in the lifespan.

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Poster

847. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01AG029421

McKnight Brain Research Foundation

McKnight Brain Institute at the University of Florida

Title: Prefrontal cortical NMDA receptors in age-related working memory impairment

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Abstract: Working memory requires neural circuits in the prefrontal cortex (PFC) that are susceptible to the effects of advancing age. Indeed, data from delayed response tasks that tax working memory in non-human primates and rats provide a cross-species consensus that aging is associated with impaired working memory. However, the biochemical basis for age-related working memory impairments has not been fully elucidated. Evidence from primates suggests that age-related neurochemical changes that attenuate PFC pyramidal neuron excitability are likely neural correlates of working memory decline. Similarly, data from our lab has shown that alterations in GABAergic signaling contribute to impaired working memory in aged rats, and that pharmacological manipulations that facilitate neuronal excitation in the aged rat PFC restore working memory performance. This study evaluated specific markers of excitatory synapses in medial PFC homogenates prepared from young (6 months) and aged (22-24 months) F344 rats. Protein levels of NMDA receptor subunits (NR1, NR2A and NR2B) were significantly lower in aged compared to young medial PFC; however, there was no change in AMPA receptor subunit levels (GluR1 or GluR2). Similarly, levels of PSD-95, a postsynaptic scaffolding protein that organizes the NMDA receptor signaling complex, were also reduced with age. These losses in postsynaptic NMDA receptor subunits and scaffolding proteins were not accompanied by loss of markers of presynaptic inputs, including the pan-synaptic vesicle associated glycoprotein synaptophysin or the cortex-specific neuronal vesicular glutamate transporter (VGluT1). Broadly, these data are consistent with the notion that aging modulates specific aspects of excitatory signaling, and such changes may underlie age-related working memory impairment. Ongoing work from our lab is determining whether these changes in NMDA receptors are reliably associated with working memory impairment in aged rats, and testing whether pharmacological compounds targeting NMDA receptors in the PFC are sufficient to manipulate working memory performance.

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Poster

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McKnight Brain Research Foundation

Title: Prefrontal cortical GABA(B) and NMDA receptor interactions in working memory

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Abstract: Working memory is an executive function that depends critically on the prefrontal cortex and that declines with age. Most models of working memory suggest that the ability to maintain information in the absence of sensory input requires coordinated and sustained firing of glutamatergic pyramidal neurons. Previous work from our laboratory has shown that GABA(B) receptor antagonists can robustly facilitate pyramidal neuron excitability in aged prefrontal cortex and can enhance working memory performance in aged rats. The focus of the current study was to further elucidate the signaling mechanisms whereby GABA(B) receptor antagonists enhance working memory functions. As prior studies have shown both that voltage-dependent NMDA receptor signaling is necessary for normal working memory and that NMDA receptor function can decline with age, this study specifically tested the hypothesis that the cognitively-enhancing effects of GABA(B) receptor antagonists may be mediated by restoration of NMDA receptor function. Aged (22 mo.) male F344 rats were implanted with guide cannula in the medial PFC and then characterized in operant test chambers on a delayed response task that assesses working memory. In this task, rats had to recall the position of the "sample" lever over a delay period which ranged from 0-24 seconds, to obtain a food reward. Upon achieving five days of stable performance, rats received mPFC microinjections of the GABA(B) receptor antagonist CGP55845 (0.6 μmol), NMDA receptor antagonist, MK-801 (1.0 and 3.0 μg) or vehicle, using a within-subjects design. In agreement with previous work from our lab, performance was significantly enhanced following GABA(B) receptor blockade. In contrast, NMDA receptor

blockade impaired performance at the 3.0 µg but not the 1.0 µg dose. Subsequently, the subthreshold dose of the MK-801 (1.0 µg) was co-administered with the GABA(B) receptor antagonist. Despite the 1.0 µg dose of MK-801 producing no effects on its own, it completely blocked the enhanced performance produced by the GABA(B) receptor antagonist. These data suggest that GABA(B) receptor antagonists enhance working memory via modulation of NMDA receptor signaling.

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Poster

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Title: The effect of PPAR alpha/gamma modulator DSP-8658 on reversing cognitive decline in aging

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Abstract: Prior work from our labs has identified a role for insulin sensitizers (i.e., pioglitazone) in alleviating depressive symptoms in two small clinical pilot studies of major depressive disorder and bipolar disorder (Kemp et al., 2014, 2012), as well as reducing a major electrophysiological Ca2+-dependent biomarker of aging in an animal model of aging (Blalock et al., 2010), and improving learning and memory in a mouse model of Alzheimer's disease (AD) (Searcy et al., 2012). While different types of thiazolidinediones have been used in animal models for the treatment of cognitive decline in aging/ AD, the poor blood-brain barrier penetration has limited the use of this class of drugs to the treatment of peripheral insulin insensitivity in the clinic. Recently, however, a novel modulator of PPAR alpha and PPAR

gamma (DSP-8658) with improved CNS penetration has been identified. Following 3 months treatment, the drug was shown to improve spatial learning in APP/PS1 transgenic mice (Yamanaka et al., 2012). Here, we tested the hypothesis that DSP-8658 could offset cognitive decline in a well-characterized animal model of aging, the F344 rat. Diets were complemented with 0.25% DSP-8658 (2.5 g DSP-8658/Kg diet). Based on food intake measurements, DSP-8658 dose attained was ~90 mg/Kg body weight. All animals (21 months old males) were given the control diet for 6 days, after which one half of the animals (n=8) was placed on the DSP-8658 diet while the other half (n=8) remained on the control diet. Diet duration lasted for 19 days during which animals were tested for changes in performance on the Morris water maze (days 3-9) and the active avoidance tasks (days 13-15). DSP-8658 treatment reduced learning performance on the Morris water maze but had no effect on the memory component of the task. The treatment did not alter avoidance learning on the active avoidance task but a significant dark side preference was noted. Based on a significant reduction in serum lipids, it was concluded that physiologically-relevant levels of DSP-8658 were attained. Our study has limitations, including use of a non-Abeta producing animal model of aging, lack of inflammatory biomarker measurement, and use of a relatively short drug exposure. Still, the data suggest DSP-8658 was unable to reduce cognitive decline in aging on 2 standard behavioral tests. Our results may underscore the negative impact of overzealous serum lipid reduction on cognitive brain function. Indeed, based on prior work in the literature and some controversies over statin use to alleviate memory problems in AD, it appears greater, rather than lesser serum lipids are associated with improved cognitive function.

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Poster

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Title: Cognitive deficits of early aging are prevented by vitamin D3: Underlying mechanisms from the hippocampal transcriptome

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Abstract: We have been studying the effects of vitamin D on neuronal physiology and genomic expression and examining the relationship between circulating levels of vitamin D and cognitive aging. Low vitamin D levels are common among the elderly and are correlated with a greater risk for cognitive decline. Our previous studies have found that 1,25 dihydroxyvitamin D3, the active form of the hormone, reverses two prominent electrophysiological markers of brain aging, an increase in both L-type calcium channel activity and the calcium-dependent afterhyperpolarization. Reversal of such age-related changes can improve synaptic function and facilitate neurotransmission. We have also directly tested whether vitamin D3 can offset age-related cognitive decline and found that middle-aged rats fed high vitamin D3 for 5-6 months could perform a more challenging version of the Morris water maze than rats on lower vitamin D3. Here we examined whether higher dietary vitamin D3 induced underlying changes in hippocampal gene expression which could account for the improved performance in the Morris water maze. Gene expression microarrays and DAVID pathway analysis revealed that higher vitamin D3 enhanced expression of genes involved in synaptic transmission, G-protein receptor coupled activity, cell communication, calcium binding and cation homeostasis. We also examined whether any of the significantly upregulated hippocampal genes in these functional categories contained a potential or putative vitamin D response element (VDRE). The VDR typically forms a heterodimer with the RXR (VDR-RXR) and the VDRE commonly consists of two, slightly variable, 6 base pair repeats separated by three random nucleotides with binding sites for both the VDR and the RXR. We used *in silico* analysis for VDRE identification and probed for a motif (5'GGGTCA-NNN-GGTTCA3') that identified sequences within the promoter regions having at least 70% homology. Our analysis showed that approximately 5% of the hippocampal genes upregulated by higher dietary vitamin D3 ($p \leq 0.001$) contained a putative VDRE. Among the genes were synaptojanin 1 and synaptogamin II suggesting that vitamin D3 may selectively target the molecular machinery of the synapse. Ongoing analyses are determining whether similar VDREs are found in corresponding human genes. Our results demonstrate a causal relationship between vitamin D3 and cognitive function and suggest that

changes in the hippocampal transcriptome induced by dietary vitamin D3 may prevent or slow cognitive aging.

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Title: Intranasal adiponectin versus insulin on behavioral and electrophysiological biomarkers of aging and memory decline

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Abstract: Prior work from our lab identified an age-dependent reduction in adiponectin levels in the hippocampus of aged F344 rats (Pancani et al., 2013). Because this reduction was seen in the same animals displaying robust cognitive decline, this suggested that CNS adiponectin could modulate cognition with age. Also, this CNS reduction coincided with elevated circulating adiponectin levels. The present study tested whether CNS adiponectin levels would follow a similar profile as that seen in type 2 diabetes, where elevated peripheral insulin levels mediate a reduction in available insulin levels in the brain. Also, because intranasal insulin improves cognition in the same animal model of aging, we tested the impact of intranasal adiponectin on learning and memory (Morris water maze) as well as on several electrophysiological Ca^{2+}-dependent markers of aging and memory decline. We tested the impact of daily intranasal adiponectin on cognitive function in 20 months old F344 rats. Eighteen aged animals received either daily doses of adiponectin (1.2 ug/animal/day) or saline. Treatment lasted 11-15 days with training on the Morris water maze task starting on the fifth day of intranasal delivery. Compared to intranasal insulin (Humalog® or Levemir®) intranasal adiponectin was unable to reverse or

attenuate age-dependent cognitive decline. Further, analyses of adiponectin actions *ex vivo* (400 ng/mL) on hippocampal physiology using intracellular and extracellular techniques revealed little to no effect of the peptide in slices from young-adult Sprague-Dawley rats. As a monomer, adiponectin is ~30 KDa, and based on prior pharmacokinetic profiles showing that relatively large peptides (i.e., VEGF ~38 KDa, ovalbumin ~45 KDa) can be detected in the brain following intranasal delivery, we anticipated adiponectin would enter the brain. However, adiponectin appears to prefer a trimeric conformation in solution (~ 70 KDa) which may have hindered entry into the CNS. Still, direct application of the peptide onto hippocampal slices was unable to alter electrophysiological markers of aging. Together, these results suggest adiponectin may have very little impact on hippocampal function irrespective of entry into the CNS via the intranasal delivery route.

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Title: Age-related emergence of HCN channelopathies in hippocampal neurons is independent of of intraneuronal amyloid and tau pathology in Alzheimer's disease transgenic mice

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is pathologically characterized by the presence of β-amyloid (Aβ) plaques and tau-containing

neurofibrillary tangles. In addition, AD patients present with deficits in learning and memory early in disease onset, and harbor neuron loss the hippocampal CA1 region. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are expressed at high levels in CA1 pyramidal neurons and help govern temporal summation of synaptic potentials and excitability via their influence on resting membrane potential and input resistance. Using mouse models harboring allelic variants of genes that produce autosomal dominant familial forms of AD (FAD), we set out to determine whether alterations in HCN function have an impact on synaptic function, and whether such alterations are linked to deposition of intraneuronal Aβ. Whole-cell patch-clamp recordings revealed that spontaneous excitatory postsynaptic potentials (EPSPs) in CA1 pyramidal neurons from 3xTg and 5XFAD mice harboring either gain-of-function or loss-of-function HCN channelopathies were similar to those from their non-transgenic littermate controls at ages ranging from 30 days to 24 months. We also combined single cell recording and neuronal immunohistochemistry, using intraneuronal amyloid staining as a proxy to detect transgene-expressing neurons. Notably, we found that HCN channelopathies were present in CA1 pyramidal neurons with or without intraneuronal Aβ or tau, indicating that neurons presumably lacking the AD-linked transgenes also develop age-related AD-linked HCN channelopathies. These data are consistent with the idea that high circulating levels of amyloid in the cellular milieu may drive some changes in the expression or localization of ion channels important for neuronal function.

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Shapiro Foundation

Title: Synaptic compensation among aged rats is associated with successful cognitive aging

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Abstract: Many aged individuals present with impairments in hippocampus-dependent forms of learning and memory, without ever being diagnosed with dementia. Previous studies on the young adult rodent hippocampus have shown that voltage attenuation is partially offset by distance-dependent synaptic scaling along the apical dendrite of the CA1 pyramidal neuron. The current work is a detailed synaptic study of the dorsal CA1 of the aged rat (AU rat), and furthermore in the aged CA1 of learning-impaired rat (AI rat). The aged rats were behaviourally categorised using two hippocampal-dependent tasks; Morris water maze and trace eyeblink conditioning. We estimated the total number of axospinous synapses, and we also determined the synaptic expression levels of AMPA and NMDA receptor in the proximal and distal stratum radiatum (pSR and dSR, respectively), and the stratum lacunosum-moleculare (SLM) of CA1 using conventional and immunogold electron microscopy and unbiased stereology. The total number of synapses and distance-dependent synaptic scaling of perforated synapse number remained constant across chronological aging. AI rats, however, had a greater percentage of synapses expressing undetectably low levels of AMPARs. Those synapses lacking AMPAR immunoreactivity were smaller than any other synapses throughout the entire study. Interestingly, the AMPAR content of synapses in the AI rats did not differ from that of the young rats. Notably, nonperforated synapses from AU rats, the “successful” cognitive agers, expressed significantly more AMPARs than those in both the young adult and AI groups. We are currently examining synaptic NMDAR expression in all 3 groups. These results suggest synaptic compensation is one adaptation to aging that segregates those aged rats with intact learning and memory from those with impairments.

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Shapiro Foundation

Title: Potential sources of aberrant activity in CA3 region of the hippocampus in a rat model of cognitive aging

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Abstract: Aging is often associated with neurological dysfunction in the cognitive domain of learning and memory. Particularly sensitive to aging and age-related dementias are forms of learning and memory that depend on the hippocampus. An ample body of evidence suggests that aging-related learning impairments are linked to synaptic alterations in subregions of the hippocampus that affect its overall function. Recent evidence suggests that CA3 hyperactivity contributes to cognitive status; however, results from our lab suggest that mossy fiber-thorny excrescence (MF-TE) synapses display hypoactivity as suggested by decreased AMPA receptors and reduced synapse sizes. This suggests that synaptic changes may be compartment specific. Consequently, we are looking at other possible sources of hyperactivity. Synaptic input to the basal and apical dendrites of CA3 pyramidal neurons comes mostly from autoassociational connections within CA3 and aberrant feedback dynamics in these compartments may cause increases in excitatory activity, thereby providing a substrate for CA3 hyperactivity. This notion is supported by our preliminary immunogold electron microcopy data, which shows increased connectivity amongst autoassociational connections in aged-impaired rats.

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Title: Overexpression of Kv4.2 and Kv4.3 channels in CA3 pyramidal neurons may contribute to age-related cognitive impairments

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Abstract: Age-related cognitive decline has been attributed to various morphological and physiological modifications of hippocampal neurons. Among these phenomena of interest, is an age-related increase in calcium-dependent potassium currents that are responsible for the postburst afterhyperpolarization (AHP) in CA1 pyramidal neurons (Disterhoft and Oh, 2006). The CA3 region of the hippocampus has been of interest, as place cells within this region have been shown to exhibit higher firing frequencies in aged rodents with cognitive impairments (Wilson et al., 2005). Previously it has been reported through whole-cell patch-clamp analyses that the fast AHP (fAHP) was greater in aged CA3 neurons as compared to young adults, thereby expediting repolarization after an axonal action potential (Simkin et al., 2013). Among the conductances activated during the fAHP are those conducted by A-type potassium conductance channels and calcium-dependent BK channels. We wanted to elucidate which of these may play a role in this age-related increase in the fAHP, and where on the neuron they are located. Here we studied the expression of Kv4.2 and Kv4.3 channels utilizing free-floating immunofluorescence (IF) microscopy and immunofluorescence array tomography. Slices from young (2-4 month) and aged (28-32 month) old naïve male Fischer 344xBrown Norway rats were processed for IF and AT using Kv4.2, Kv4.3 and BK antibodies (neuromab) and Alexa 647 secondary antibodies (life technologies). Regions of interest selected for analysis in area CA3 were strata oriens, pyramidale, lucidum, and radiatum. Fluorescence intensity per region of interest was calculated using Fiji software. Results indicated an increase in Kv4.2 and Kv4.3 expression with age overall, but particularly concentrated in the perisomatic region of area CA3. There were no differences in BK expression with age using both experimental approaches. These data are in agreement with previous physiological findings in area CA3 (Simkin et al., 2013), where A-type potassium currents, and not, calcium-dependent potassium currents, were demonstrated to drive the age-related increase in the fAHP.

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Title: Alzheimer's disease-linked synaptic alterations in mouse and human hippocampal CA1 pyramidal neurons

Authors: *T. F. MUSIAL[1], K. NEUMAN[1], E. MOLINA-CAMPOS[1], E. BUSS[1], K.-J. OH[1], S. SCHEFF[2], E. MUFSON[1], D. A. NICHOLSON[1];
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Abstract: Alzheimer's disease (AD) is associated with numerous alterations in hippocampal neurons including the distribution, number, and size of synaptic contacts. Some changes are believed to be compensatory responses to maintain stable circuitry, whereas other changes resemble a plasticity-like response with the remaining inputs reactively innervating available dendritic regions. We provide evidence that both responses are at work in the axospinous synapses of mice harboring AD-linked genetic mutations (the 5XFAD line) as well as in human AD cases, in the form of synapse loss and changes in strength with the remaining synapses. Using array tomography, quantitative conventional electron microscopy, immunogold electron microscopy for AMPARs, and whole-cell patch-clamp physiology, we find that hippocampal CA1 pyramidal neurons in transgenic mice undergo an age-related cortico-hippocampal disconnection, and that the remaining synapses express more AMPARs. Furthermore, the number of axonal boutons that synapse with multiple spines is significantly reduced in the transgenic mice. With serial section electron microscopic analyses of human hippocampal tissue, we further show that compensatory changes in synapse strength are also detectable in intra-

hippocampal and cortico-hippocampal synapses in human AD cases, and that their multiple synapse boutons may be more powerful than those in non-cognitively impaired humans. Our findings are consistent with the notion that the pathophysiology of AD is a multivariate product of both neurodegenerative and neuroplastic processes, which may produce both adaptive and maladaptive compensatory changes in hippocampal synaptic strength and plasticity.

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Poster

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Title: Longitudinal evaluation of Tau-P301L transgenic mice reveals cognitive impairments in old age

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Abstract: Tau is the microtubule-associated binding protein implicated in neurodegenerative tauopathies, including frontotemporal dementia (FTD) and Alzheimer's disease (AD). These neurodegenertive diseases show intracellular accumulation of hyperphosphorylated tau as neurofibrillary tangles, which are associated with cognitive deficits. In this study, we behaviourally evaluated transgenic mice expressing the longest isoform of human tau-2N/4R with the P301L mutation [TgTau(P301L)23027] (Murakami et al., 2006). P301L is the mutation most frequently observed in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). This model has been previously shown to recapitulate the progressive development of glial fibrillary and neurofibrillary tangles, and associated cerebral atrophy

observed in patients. This may therefore be a promising model for studying the effects of P301L mutant tau on behavior and allow for identification of cognitive alterations at early stages of pathology, which could be translated to have early diagnostic value in patient populations. We conducted a longitudinal study to provide a cognitive profile of the TgP301L mouse across multiple ages. We examined frontal cortex-dependent executive function and attention with the touchscreen version of the 5-choice serial reaction time test (5-CSRTT) at 4, 7, 12, and 16 months of age and detected no between-group differences. Similarly, no differences were detected when we examined perirhinal cortex-dependent recognition memory using an object recognition (OR) task at 5, 8, 13, and 17 months of age. Surprised by this apparent lack of phenotype, at 18-20 months of age we turned to hippocampus-dependent t-maze and spontaneous location recognition memory tasks. The results were variable but suggestive of mild spatial memory impairment in the aged TgP301L mice. At 21 months of age, we identified a robust recognition memory deficit using OR. Histological analysis of these mice was undertaken to assess the extent of pathology development in the brain regions associated with the behavioural assays performed. These findings suggest that the TgP301L mouse recapitulates the relatively extended age of onset of behavioural symptoms often associated with neurodegenerative diseases such as FTDP-17. Surprisingly, there were no apparent changes in executive function or attention in these animals. However, robust memory impairments were observed on at least one task, consistent with a dementia-like phenotype in these mice when aged.

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NARSAD, Brain & Behavior Research Foundation

Title: Genetic interaction of disease-relevant genes modulates cognitive components assayed using touchscreens in mice

Authors: *J. NITHIANANTHARAJAH[1,2], N. H. KOMIYAMA[2], L. M. SAKSIDA[3], T. J. BUSSEY[3], S. G. N. GRANT[2];
[1]Univ. of Melbourne, Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia; [2]Ctr. for Clin. Brain Sci. and Ctr. for Neuroregeneration, Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Cognitive deficits are a core feature of most neurological and psychiatric disorders, however unravelling the genetic basis of cognitive disorders is complex due to the involvement and interaction of multiple genes, which manifest in overlapping cognitive impairments. Human genetic studies are increasingly identifying that many of the mutations implicated in cognitive disorders converge upon genes associated with the synapse - the connection between neurons that form the most fundamental information-processing units in the nervous system. Little is known about the genetic basis of distinct aspects of higher cognitive functions such as complex forms of learning and memory, attention and executive functions (including cognitive flexibility and response inhibition). Moreover, there is negligible evidence exploring the involvement of epistasis or non-additive gene interactions in the context of cognitive functions. Towards this, using mice with mutations in Dlg2/PSD-93 and Magi2/S-SCAM, two key synaptic scaffold genes implicated in cognitive disorders, we examined the role of these genes and gene interactions on different components of cognition. Exploiting the emerging technology of the touchscreen assays, a useful behavioural tool for modelling higher cognitive functions in rodents, we observe evidence for complex genetic interactions whereby the Dlg2/Magi2 double mutants show a genetic suppression or enhancement (double mutants have a less or more severe phenotype than one predicted by the additive effects of the single mutants) in selective cognitive functions. These findings provide novel evidence for gene interactions modulating different components of cognition, and pave the way forward for elucidating how mutations in multiple susceptibility genes gives rise to distinct and overlapping cognitive phenotypes, and influence disease susceptibility.

Disclosures: J. Nithianantharajah: None. **N.H. Komiyama:** None. **L.M. Saksida:** Other; Campden Instruments Ltd. **T.J. Bussey:** Other; Campden Instruments Ltd. **S.G.N. Grant:** None.

Poster

847. Learning and Memory: Aging II

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 847.18/UU14

Topic: F.02. Animal Cognition and Behavior

Support: Intramural Research Program of the National Institute on Aging

Title: Regionally selective decline in hippocampal somatostatin-immunoreactive neuron number in aged rhesus monkeys with memory impairment

Authors: *A. M. SPIEGEL, E. J. PEREZ, J. M. LONG, P. PARK, P. R. RAPP;
Lab. of Behavioral Neurosci., Natl. Inst. on Aging, BALTIMORE, MD

Abstract: Accumulating evidence indicates that hippocampal interneuron integrity is compromised in aged rats with memory deficits. Specifically, we previously reported that aged rats with memory loss exhibit a selective vulnerability of somatostatin-expressing hilar interneurons (Spiegel et al., 2013). The decline in hilar interneuron integrity may represent a marker of hippocampal circuitry change related to cognitive decline in older individuals. To better understand how interneuron integrity contributes to cognitive impairment in normal aging, here we determined the total numbers of somatostatin immunoreactive neurons in the hippocampus of young and aged behaviorally characterized monkeys using design-based stereological techniques. Male and female young adult (n=7, mean age=4.4 years) and aged (n=13, mean age=26.8 years) rhesus monkeys (*Macaca mulatta*) were tested on a battery of neuropsychological tasks assessing medial temporal lobe memory, including the delayed nonmatching-to-sample (DNMS) test of visual recognition memory and an object discrimination task. Aged monkeys were classified as impaired or unimpaired relative to young adults based on their scores from DNMS. For each monkey a 1 in 20 series of histological sections (50μm nominal thickness) through the rostro-caudal extent of the hippocampus was prepared for the immunocytochemical visualization of somatostatin using a commercially available antibody (#SC7819, Santa Cruz). Total numbers of immunoreactive neurons were estimated using design-based stereological quantification methods (StereoInvestigator, MBF Inc.). Independent of age and cognitive status, the total number of somatostatin-immunoreactive neurons averaged 194,488. Group-wise comparisons revealed a selective and statistically significant decrease in somatostatin-positive neuron number in the CA1 hippocampal subregion in aged monkeys with memory deficits compared to both young adults and aged animals with preserved memory function. The number of somatostatin-expressing neurons was unchanged across all other hippocampal subregions in relation to age and cognitive status. Together these data highlight an age-related vulnerability among somatostatin interneurons that is circuit specific in the primate hippocampus and linked to individual differences in cognitive outcome.

Disclosures: A.M. Spiegel: None. **E.J. Perez:** None. **J.M. long:** None. **P. Park:** None. **P.R. Rapp:** None.

Poster

847. Learning and Memory: Aging II

Location: Halls A-C

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Program#/Poster#: 847.19/UU15

Topic: F.02. Animal Cognition and Behavior

Support: NIH 4R00MH086615-03

Ellison Medical Foundation New Scholar in Aging

Whitehall Foundation

Title: Rejuvenating the dentate gyrus with stage-specific expansion of adult-born neurons to enhance memory precision in adulthood and aging

Authors: K. MCAVOY[1], K. N. SCOBIE[2], S. BERGER[3], N. GUO[1], H. VEGA-RAMIREZ[1], S. MIAKE-LYE[1], M. WHALEN[4], M. NELSON[5], M. BERGAMI[6], B. BERNINGER[7], D. BARSTCH[3], R. HEN[8], *A. SAHAY[1];
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Abstract: Neural stem cells in the dentate gyrus (DG) generate dentate granule neurons throughout life, a process exquisitely sensitive to the environment. Adult-born dentate granule neurons contribute to encoding functions important for minimizing interference during storage of episodic memories such as pattern separation. These observations suggest that adult hippocampal neurogenesis represents an adaptive mechanism of encoding by which generation and integration of new neurons is governed by environmental demands on hippocampal circuitry to maintain memory precision. However, the underlying mechanisms by which mature dentate granule neurons sense and transduce changes in activity to dictate lineage homeostasis are poorly understood. Here, we interrogated the impact of decreasing synaptic inputs onto mature dentate granule neurons on their competition for perforant path inputs with adult-born dentate granule neurons and also on neural stem cell activation. Using a novel genetic system by which we reversibly eliminate a subset of dendritic spines on mature dentate granule neurons, we found that adult-born dentate granule neuronal integration and activation of neural stem cells are bidirectionally sensitive to these alterations. We have harnessed this strategy to determine how

rejuvenating the DG with expanded cohorts of adult-born dentate granule neurons at distinct stages of maturation impacts encoding and memory precision in adulthood and in aging. Our studies indicate that multiple feedback loops within the adult neurogenic lineage mediate nuanced adaptation to changes in activity of mature dentate granule neurons and uncover differential effects of stage-specific expansion on encoding and memory in adulthood and in aging. Importantly, targeting adult hippocampal neurogenesis is sufficient to reverse impairments in pattern separation and memory precision in aging. Funding **Support:** NIMH, Ellison Medical Foundation and Whitehall Foundation.

Disclosures: **K. McAvoy:** None. **K.N. Scobie:** None. **S. Berger:** None. **N. Guo:** None. **H. Vega-Ramirez:** None. **S. Miake-Lye:** None. **M. Nelson:** None. **M. Bergami:** None. **B. Berninger:** None. **D. Barstch:** None. **R. Hen:** None. **A. Sahay:** F. Consulting Fees (e.g., advisory boards); Consultant to PsyBrain LLC.. **M. Whalen:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.01/UU16

Topic: F.02. Animal Cognition and Behavior

Support: Whitehall Foundation Research Grant

Alfred P Sloan Research Fellowship

Dean's Postdoctoral Fellowship from Stanford University School of Medicine

Title: Postsynaptic HCN channel deletion viaTRIP8b knockout causes expansion of grid scale and reduces malleability of grid cells

Authors: *R. G. MUNN[1], M. CAMPBELL[1], C. S. MALLORY[1], D. CHETKOVICH[2], L. M. GIOCOMO[1];
[1]Neurobio., Stanford Univ., Stanford, CA; [2]Med. Sch., Nortwestern Univ., Chicago, IL

Abstract: The activity of grid cells in the medial entorhinal cortex is thought to be central to the ability of animals to navigate in space (Hafting et al., 2005). Grid cells display a regular, hexagonal firing pattern across spatial environments, suggesting that they may serve as a type of fixed coordinate system (Hafting et al., 2005). The grid coordinate system has the potential to represent environments at many different scales, with grid size and spacing increasing along the

dorsal to ventral axis of layer II (Hafting et al., 2005). Recent findings have shown that when the dimensions of an environment are suddenly reduced along one axis, the nodes of the grid cell pattern temporarily re-scale , suggesting that the grid network is at least transiently malleable in response to changes in environmental geometry (Barry et al., 2007; Stensola et al., 2013). Investigation of the cellular properties of grid cells initially suggested that I(h), conducted by HCN channels, may control of grid scale. Forebrain-restricted deletion of the HCN1 subunit of the HCN channel causes an expansion of grid scale, but the gradient in grid scale is preserved (Giocomo et al., 2011). While this demonstrated that HCN1 tunes grid scale, it also suggests that channels other than HCN1 are responsible for the gradient in grid scale of. Since previous research has focused on HCN1 deletion, it is of interest to examine the effect of deletion of other subunits of the HCN channel, such as HCN2. The auxiliary subunit TRIP8b is central to the trafficking and insertion of both HCN1 and HCN2 channels postsynaptically (Huang et al, 2012). In these experiments, we investigate the effect of TRIP8b deletion on grid cell activity. First, we find that deletion of TRIP8b produces an expansion in grid size and spacing with preservation of the dorsal-ventral grid scale gradient, similar to HCN1-specific deletion, suggesting that postsynaptic HCN1 function is largely responsible for the expansion in grid scale. Secondly, we find that grid cells in wildtype mice undergo re-scaling when the dimensions of an environment are suddenly reduced along one axis, as has been previously reported in rats. The grid cells of mice lacking TRIP8b, however, show no such malleability. These findings suggest a specific role for postsynaptic HCN channels in the rapid adaption of the spatial navigation network to novel changes in environment.

Disclosures: R.G. Munn: None. **M. Campbell:** None. **C.S. Mallory:** None. **D. Chetkovich:** None. **L.M. Giocomo:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

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Program#/Poster#: 848.02/UU17

Topic: F.02. Animal Cognition and Behavior

Support: Alfred P Sloan Research Fellowship

Whitehall Foundation Research Grant

Title: Voltage-gated Na current kinetics vary systematically along the dorsal-ventral axis of the MEC

Authors: *J. S. BANT, L. M. GIOCOMO;
Neurobio., Stanford Univ., Stanford, CA

Abstract: As a rat moves through an environment, neurons in the medial entorhinal cortex (MEC) fire in regular repeating locations in a hexagonal grid pattern (Hafting et al., 2005). Grid scale expands systematically along the dorsal-ventral MEC axis (Sargolini et al., 2006). This change in grid scale correlates with a gradient in the kinetics and density of hyperpolarization activated cyclic nucleotide (HCN) channels (Garden et al., 2008; Giocomo & Hasselmo, 2008 & 2009). In behaving animals, the loss of HCN1 channels results in an increase in grid scale, suggesting that grid cells use ion channel kinetics for spatial scaling. However, the loss of HCN channels does not eliminate the dorsal-ventral gradient in grid scale (Giocomo et al., 2011). This suggests additional, as of now unidentified, ionic conductances must work in concert with HCN1 to control the gradient in grid scale. Computational work has proposed that Na channels work alongside HCN channels to endow MEC cells with their unique electrophysiological profile (Fransen et al., 2004). However, the organization of Na currents kinetics along the dorsal-ventral axis has never been investigated. Using *in vitro* whole-cell voltage clamp recordings, we examined the expression of Na currents along the dorsal-ventral MEC axis of mice (P19 - P30). We used several strategies to provide reliable slice physiology measurements: reduced Na gradients, sub-saturating TTX, and voltage protocols designed to minimize loss of clamp. We measured TTX-sensitive resurgent and persistent Na currents and normalized these amplitudes to the amplitude of the transient Na current. We found that Na current kinetics were graded along the dorsal-ventral MEC axis, with layer 2 cells in the more dorsal end of the MEC characterized by larger amplitude resurgent and persistent Na currents ($n = 18$; resurgent Na current $R2 = 0.46$, $p < 0.03$; persistent Na current $R2 = 0.32$, $p < 0.10$). Moreover, the resurgent and persistent currents significantly covaried ($R2 = 0.87$, $p < 0.01$), raising the possibility that the molecular or biophysical determinants of these current components are homeostatically regulated to scale together. Combined, our data potentially identifies Na currents as a new ionic substrate that could be involved in the organization of grid scale.

Disclosures: J.S. Bant: None. **L.M. Giocomo:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

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Program#/Poster#: 848.03/UU18

Topic: F.02. Animal Cognition and Behavior

Support: NIH 2T32MH020016-16

Alfred P Sloan Fellowship

Whitehall Foundation Research Grant

Title: Error accumulation and landmark-based error correction in grid cells

Authors: *K. HARDCASTLE[1], S. GANGULI[2], L. M. GIOCOMO[1];
[1]Neurobio., [2]Applied Physics, Stanford Univ., Palo Alto, CA

Abstract: Spatial navigation utilizes neural circuitry found in medial entorhinal cortex (MEC) that translates external space into an internal map. In particular, grid cells form a neural metric of space by firing only at the vertices of equilateral triangles that tile an open arena available to the animal (Hafting et al., 2005). Grid cells are thought to perform continuous path integration through accumulation of self-motion cues, in which an estimate of the animal's position depends only on the previous estimate of position and a current velocity input (Hafting et al., 2005). However, due to a finite number of neurons and stochastic spiking dynamics, this procedure necessarily results in error accumulation (Burak & Fiete, 2009). Thus, a corrective mechanism is imperative for the observed accuracy in grid pattern stability (Hafting et al., 2005; Burak & Fiete, 2009). Although many have hypothesized that sensory cues from environmental landmarks may provide a method for error correction (Burgess, 2008), experimental evidence for this phenomenon has been lacking. In this work, we hypothesize that activation of border cells, neurons located in MEC that are active along boundaries of an environment, correct the neural map through preferential activation of grid cells. We present experimental evidence of error accumulation over time and distance traveled by a behaving mouse during time periods from exit to entry of regions near the border. Consistent with predictions from an attractor network model (Burak & Fiete, 2009), this error accumulation results from drift in the grid pattern. Furthermore, this error is corrected through encounters with the border in a direction-dependent manner, where an encounter with a wall of the enclosed arena results in decreased error in the direction perpendicular to the wall. All results were replicated in a separate dataset from rat grid cells (recorded by Sargolini et al., 2006). In addition, we implemented a mechanism for error correction by the boundary in an attractor network model (Burak & Fiete, 2009), and replicated all experimental results through identical analysis of simulated data. These results strongly suggest that noisy path-integration in grid cells gives rise to error accumulation that can be corrected through mechanisms involving sensory cues from environmental landmarks, and more broadly point to the corrective role of landmarks in accurate spatial navigation.

Disclosures: K. Hardcastle: None. **S. Ganguli:** None. **L.M. Giocomo:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: National Science Foundation Graduate Research Fellowship

Whitehall Foundation Research Grant

Title: Increased grid spacing and impaired spatial navigation following down-regulation of entorhinal HCN1

Authors: *C. S. MALLORY[1], J. DICKINSON[2], L. M. GIOCOMO[2];
[1]Stanford Univ., Portola Valley, CA; [2]Stanford Univ., Stanford, CA

Abstract: The medial entorhinal cortex (MEC) possesses several types of spatially-modulated cells that act in concert to translate the external environment into an internal representation of space. A neural metric for this cognitive map is provided by grid cells, whose firing fields tile the environment of an exploring animal in a lattice of equilateral triangles (Fyhn et al., 2004; Hafting et al., 2005). It was recently demonstrated that grid spacing, the distance between firing fields, increases following reduction of the hyperpolarization-activated current I(h) (Giocomo et al., 2011). As this effect was achieved through genetic deletion of the HCN1 subunit across the entire forebrain, it remained unclear as to whether the expansion in grid scale resulted from changes within MEC, or in regions providing sensory input to MEC. To determine the degree to which processes within the MEC modulate grid scale, we reduced HCN1 expression locally through targeted injections of a Cre recombinase-carrying AAV virus into the MEC of floxed HCN1 mice (fKO). We found that bilateral MEC-specific knockdown of HCN1 also results in an expansion in grid scale, suggesting that mechanisms intrinsic to the MEC regulate the scale of grid cell spatial representations. Furthermore, these results indicate that processes regulating grid scale remain plastic in the adult animal. We additionally found that running speed modulation of the EEG-measured theta frequency was unaffected by local HCN1 knockdown. This contrasts with the reduction in speed-modulation of theta observed following forebrain-restricted HCN1 deletion (Giocomo et al., 2011). Together, these data suggest that changes in grid scale cannot be attributed to alterations in a speed signal carried by theta frequency, posing a challenge to oscillatory interference models of grid cell formation. Finally, we took advantage of the expanded grid scale of fKO mice to examine how loss of the smallest grid scale (20-30 cm) impacts spatial learning and memory. fKO mice performed significantly worse than wildtype

littermates on a Barnes Maze task of spatial navigation, suggesting that the loss of small grid cells impairs spatial cognition. These findings are consistent with mathematical analyses which posit that small-scale grid cells are required for precise spatial representations (Towse et al., 2014).

Disclosures: C.S. Mallory: None. **L.M. Giocomo:** None. **J. Dickinson:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.05/UU20

Topic: F.02. Animal Cognition and Behavior

Support: European Research Council grant no. 282091 - DEVSPACE

Title: Head direction cells recorded in the anterior dorsal thalamus of preweanling rats

Authors: *J. P. BASSETT, T. J. WILLS, F. CACUCCI;
Univ. Col. London, London, United Kingdom

Abstract: Neurons that fire as a function of an animal's head direction in the horizontal plane, known as head direction (HD) cells, have been identified in a number of interconnected sites within the classic Papez circuit. As one of several classes of neuron whose firing patterns exhibit spatial behavioral correlates (e.g., place cells and grid cells), HD cells are believed to contribute information about orientation around the yaw axis to a functional network for spatial representation in the brain. As part of the effort to determine how representations of space are assembled by this network, research has recently turned to its development in young animals. Preweanling rats spend their first two weeks of life blind and with limited mobility, which restricts their ability to learn about the spatial environment around them. Yet, by post-natal day 16 (P16), HD cells in the dorsal presubiculum and medial entorhinal cortex fire with spatial tuning comparable to adults', preceding the emergence of mature firing patterns in other spatially tuned cell classes. The early maturation of HD cell firing properties suggests that some features of spatial representation may be inherent in the network's architecture, independent of learning. To explore this possibility, we recorded single-unit activity in the anterior dorsal thalamus, a subcortical site known to contain HD cells in adult rats, and which is presynaptic to the cortical areas where HD cells have been recorded in rat pups thus far. Prior to eye-opening (which typically occurs at P14) we found cells during recording in the open field whose firing was

consistent with unstable HD representations, as if preferred firing directions were drifting extensively within a trial. On the first day after eye-opening, HD cell activity was stable within trials but subject to drift in the dark, and control by distal landmark cues was poor. By the second day after eye opening, HD cell firing was stable in the dark and cue control was robust. The appearance of HD-like "drifty" activity in the HD cell network before eye-opening suggests that some part of its network architecture is in place before visual feedback is available to stabilize tuning. The early emergence of adult-like cue-control in anterior thalamic HD cells suggests that the HD cell signal may be foundational to cortical and hippocampal spatial representations that mature later in development.

Disclosures: J.P. Bassett: None. **T.J. Wills:** None. **F. Cacucci:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.06/UU21

Topic: F.02. Animal Cognition and Behavior

Title: The scale of grid cell firing patterns is modulated by spatial uncertainty

Authors: *D. MANSON[1,2], F. CARPENTER[3], J. O'KEEFE[1,4], N. BURGESS[5,3], C. BARRY[1]; [1]Cell and Developmental Biol., [2]Ctr. for Mathematics and Physics in the Life Sci. and Exptl. Biol., [3]Inst. of Neurol., [4]Sainsbury Wellcome Ctr., [5]Inst. of Cognitive Neurosci., UCL, London, United Kingdom

Abstract: The representation of self-location by grid cells has variously been described as "densely packed" or "hexagonal"; leading to hypotheses about the role of a grid cell network in providing a metric for space[1]. However, recently, it was shown that exposure to environmental novelty provokes a temporary increase in grid scale, which attenuates with increasing familiarity[2]. This differs from evidence that grids are anchored to sensory cues, and can be rotated or stretched by changes to sensory cues, and suggests that grid cells do not simply provide a fixed metric for space that becomes attached to environmental cues. The exact neural mechanisms driving grid scale expansion and its functional role are currently unknown. However, it has been hypothesised[3] that grid scale varies inversely with the precision with which the animal's self-location can be determined (spatial uncertainty), so as to encode location more accurately. Thus grid scale would be expected to increase *in situ*ations with few spatial cues or unknown configurations of cues, such as in a novel environment, compared to situations in

which multiple cues were accessible. To test this hypothesis, we recorded medial entorhinal grid cells while rats foraged in two types of environment: one rich in local cues and the other lacking any such cues. It was hypothesised that grid scale would be smaller in the cue rich environment, reflecting higher certainty in the representation of self-location, similar to that observed in familiar environments. We recorded cells in both environments from their first experience of it and throughout the familiarisation process. We found that grid patterns had a smaller scale in the cue rich environment, supporting the hypothesis, and were also more regular. This confirms that the grid cells do not simply encode a fixed metric for space, rather the encoding is sensitive to additional factors such as environmental novelty and the availability of cues. 1.Moser, E. I. & Moser, M.-B. A metric for space. *Hippocampus* **18,** 1142-56 (2008). 2.Barry, C., Hayman, R., Burgess, N. & Jeffery, K. J. Experience-dependent rescaling of entorhinal grids. *Nat. Neurosci.* **10,** 682-684 (2007). 3.Towse, B. W., Barry, C., Bush, D. & Burgess, N. Optimal configurations of spatial scale for grid cell firing under noise and uncertainty. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **369,** 20130290 (2014).

Disclosures: D. Manson: None. **F. Carpenter:** None. **J. O'Keefe:** None. **N. Burgess:** None. **C. Barry:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Program#/Poster#: 848.07/UU22

Topic: F.02. Animal Cognition and Behavior

Support: BBSRC Studentship BB/F015968/1

BBSRC grant BB/J009792/1

Axona Ltd.

Title: Updating of head direction cells in a three-dimensional environment

Authors: *J. J. WILSON, H. J. SPIERS, K. JEFFERY;
Exptl. Psychology, Univ. Col. London, London, United Kingdom

Abstract: Head direction (HD) cells are neurons that are maximally active when an animal faces a particular direction in an environment. These cells provide directional information to other spatially modulated neurons, which work together to form a cognitive map of the

environment. In two-dimensional horizontal environments, rodent HD cells are only modulated by rotations around the dorso-ventral axis of the head (yaw rotations). However, the real world is more complex, and many animals regularly navigate through environments with vertical as well as horizontal components. We investigated how HD cells maintain orientation when animals move between differently oriented vertical surfaces. When an animal moves from the floor onto a wall the directional firing of HD cells remains unchanged, for example, a HD cell that is firing as the rat approaches the wall continues to fire as the rat transitions onto the wall and is then subsequently modulated as usual by yaw rotations (Taube, Wang, Kim, & Frohardt, 2013), now occurring in the vertical plane. The preservation of firing direction across the transition from floor to wall might be because the cell is trying to maintain its firing in a room-centred reference frame, or it might be because the system simply failed to detect the rotation that carried the rat onto the wall. Our experiment aimed to distinguish these possibilities by having the rat transition directly from one wall to another differently oriented wall. If the system was failing to detect these rotations, cells on different walls should maintain the same local directional firing preferences (e.g. firing when the rat faced towards the right edge of the wall) but have different room-centred ones; if the system did detect the rotation then the firing direction of the cells relative to the local surface should change, while their direction relative to room-centred co-ordinates should remain the same. Our data show the latter; that is, active updating of HD cells during rotations between vertical planes, so as to maintain a room-centred frame of reference. These results indicate that the HD system is able to integrate information relating to complex rotations in three-dimensional space, thus providing some of the information required to allow animals to accurately navigate the three-dimensional world. Taube, J. S., Wang, S. S., Kim, S. Y., & Frohardt, R. J. (2013). Updating of the spatial reference frame of head direction cells in response to locomotion in the vertical plane. Journal of Neurophysiology, 109(3), 873-888.

Disclosures: J.J. Wilson: None. **K. Jeffery:** None. **H.J. Spiers:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.08/UU23

Topic: F.02. Animal Cognition and Behavior

Title: Grid cell representations in connected, perceptually identical compartments

Authors: *F. CARPENTER[1], D. MANSON[2,3], K. J. JEFFERY[4], N. BURGESS[4,1], C. BARRY[2]; [1]Inst. of Neurol., [2]Cell & Developmental Biol., [3]Ctr. for Mathematics and Physics in the Life Sci. and Exptl. Biol., [4]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: The firing fields of grid cells form a triangular array which tessellates the environment[1]. The periodicity and regularity of these fields, together with the fixed relative properties of pairs of grid cells across environments, has led to the suggestion that they provide the functional basis of path integration. That is, grid cells are thought to form a cumulative representation of the angle and distance travelled based on self-motion inputs[2]. However, grid cells also receive non self-motion inputs, and are known to be anchored to the environment by sensory cues[1]. To test the relative contribution of sensory and self-motion inputs to grid cell firing we recorded from medial entorhinal grid cells in rats foraging in a multicompartment environment containing two perceptually identical boxes. Each 90x90cm box had a trapezoidal doorway at its northern side and a light at its southern side. A corridor connecting the doorways allowed animals to move freely between the two boxes. The boxes were constructed such that between trials their positions could be switched and the floor rotated to control for unidentified olfactory or somatosensory cues. Initial recordings emphasised the contribution of sensory inputs to grid cell firing: spatial firing patterns tended to replicate between the two boxes and were highly correlated. That the grids initially had similar spatial offsets to the local boundaries suggests a role for local environmental boundaries in anchoring grid cell firing patterns. Further, grid regularity (gridness) was significantly reduced in the multicompartment relative to a similar sized screening environment; perhaps due to the ambiguity inherent to the apparatus. However, with prolonged experience, self-motion inputs allowed grid cell firing to increasingly disambiguate the compartments and a strong negative correlation was observed between experience and representation similarity in the two boxes. The representation of individual cells was seen to gradually diverge, reminiscent of the gradual divergence of place cell firing between two similar boxes[3]. Even with extensive experience however, grid cell firing fields did not form a single global pattern that overlaid the entire environment. Though cells maintained similar scale and orientation in each compartment, their fields were phase shifted so as to form a non-continuous representation across the two boxes. 1. Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Nature 436. 801-6 (2005) 2. McNaughton BL, Battaglia FP, Jensen O, Moser EI, Moser MB. Nat Rev Neurosci 8. 663-78 (2006) 3. Lever C, Wills T, Cacucci F, Burgess N, O'Keefe J. Nature 416. 90-4 (2002)

Disclosures: F. Carpenter: None. **C. Barry:** None. **D. Manson:** None. **K.J. Jeffery:** None. **N. Burgess:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Principal Research Fellowship to NB

Sir Henry Dale Fellowship to CB

Title: How to use grid cells for high-precision large-scale vector navigation

Authors: *C. BARRY[1], D. BUSH[2,3], N. BURGESS[2,3];
[1]Cell and Developmental Biol., [2]Inst. of Cognitive Neurosci., [3]Inst. of Neurol., UCL, London, United Kingdom

Abstract: Grid cells, which appear to be common to mammals, including humans, have multiple spatial firing fields distributed in a regular hexagonal array[1]. This striking and regular metric has prompted suggestions that grid cells are a core component of a network responsible for path integration; their repetitive firing fields being a cumulative representation of self-motion cues[2,3]. Numerical and analytical analyses indicate that a network including grid cell modules of different scales can support an extremely accurate representation of self-location, the range of which outstrips the scale of the individual components. In particular the capacity of such a system is believed to increase combinatorially with each additional grid module, comparable to a residue number system[4,5]. Finally, grid cells are found throughout a network of brain regions traditionally corresponding to both input and output targets of the hippocampus. In light of these points it has been suggested that grid cells might also support goal directed navigation[6]. Such a network could calculate the allocentric vector between the current location and a spatial goal in large-scale space; performing vector based navigation - in essence the inverse of path integration. Here we present a computational level network based on grid cells which calculates the vector between any two locations in grid space over scales larger than the largest grid scale. Next we present several plausible algorithmic implementations of this network which are able to direct navigation between locations separated by distances exceeding the largest grid scale. These calculations are performed rapidly and accurately without exhaustive searching of the numerous possible vectors, and provide experimental predictions to test for the proposed navigational mechanisms. 1. Hafting, T., Fyhn, M., Molden, S., Moser, M.-B. & Moser, E. I. Microstructure of a spatial map in the entorhinal cortex. *Nature* **436,** 801-806 (2005). 2. Burgess, N., Barry, C. & O'Keefe, J. An oscillatory interference model of grid cell firing. *Hippocampus* **17,** 801-812 (2007). 3. McNaughton, B. L., Battaglia, F. P., Jensen, O., Moser, E. I. & Moser, M. B. Path integration and the neural basis of the "cognitive map."*Nat. Rev. Neurosci.* **7,** 663-678 (2006). 4. Fiete, I. R., Burak, Y. & Brookings, T. What grid cells convey about rat location. *J. Neurosci.*

28, 6858-71 (2008). 5. Mathis, A., Herz, A. V. M. & Stemmler, M. Optimal Population Codes for Space: Grid Cells Outperform Place Cells. *Neural Comp.* **24,** 2280-2317 (2012). 6. Erdem, U. M. & Hasselmo, M. A goal-directed spatial navigation model using forward trajectory planning based on grid cells. *Eur. J. Neurosci.* **35,** 916-931 (2012).

Disclosures: C. Barry: None. **D. Bush:** None. **N. Burgess:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Bernstein Center for Computational Neuroscience, Berlin

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Gottfried Wilhelm Leibniz Prize, DFG

Title: Medial entorhinal cortex architecture across evolution and development

Authors: *S. RAY[1,2], R. NAUMANN[1,2], M. BRECHT[1,2];
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Abstract: Layer 2 of the medial entorhinal cortex contains a large fraction of grid cells in several mammalian species. We compared entorhinal architecture in layer 2 across five mammalian species (Etruscan shrews, mice, rats, Egyptian fruit bats and humans) to understand the structural basis of grid cell activity. We differentiate layer 2 principal neurons by calbindin immunoreactivity, which identifies a class of pyramidal neurons, but not stellate cells. We confirm the existence of patches of calbindin+ pyramidal cells across species, which were arranged in a regular and often hexagonal grid according to spatial autocorrelation analysis, grid scores and statistical assessment. Cholinergic innervation targeted calbindin patches in rats and

mice, avoided such patches in humans and was uncorrelated to the calbindin pattern in bats. The organization of stellate and calbindin+ pyramidal cells showed marked differences in entorhinal sub-regions. Layer 2 of rodent medial and human caudal entorhinal cortex was similar, however, in that patches of calbindin+ pyramidal cells were superimposed on scattered stellate cells. The number of calbindin+ neurons in a patch increased from ~70 in Etruscan shrews to ~800 in humans, i.e. only ~11 fold over a 20000 fold difference in brain size. Investigating the developmental aspect of calbindin patches, we observed the clustering of calbindin+ cells in patches in postnatal day 4 rats and perhaps even earlier. The early appearance of the calbindin-patches, their conserved grid-like layout and size and the correlation of cholinergic innervation of calbindin patches with theta rhythmicity of grid cells across different mammalian species, makes us wonder if they are involved in generating grid cell activity.

Disclosures: S. Ray: None. **R. Naumann:** None. **M. Brecht:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

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IPFW RESP Research Grant

Title: Otolith dysfunction impairs dead reckoning in mice

Authors: *S. N. BROCKMAN[1], E. A. GOEBEL[1], J. R. KOPPEN[2], P. A. BLANKENSHIP[2], D. G. WALLACE[2], R. M. YODER[1];
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Abstract: Navigation in non-visual environments is impaired by damage to the vestibular system. However, no studies have tested whether the critical vestibular signals originate in the semicircular canals, which detect angular head acceleration, or the otolith organs, which detect linear head acceleration and static pitch/tilt relative to gravity. We therefore evaluated the navigation performance in otoconia-deficient *tilted* mice and their littermate controls on a food-hoarding task. In light, food-restricted mice were trained to exit a start box in search of a single

food item, and to carry the food directly back to the visible start box for consumption. After training was complete, a test phase was conducted in darkness, forcing animals to rely on idiothetic cues (e.g., proprioception, motor efference copy, vestibular) experienced during the outward journey in order to return directly to the start box. Motion capture software was used to quantify movement characteristics during a food hoarding trip. Each trip was divided into outward (i.e., departing the refuge to locating the food item) and homeward (i.e., locating the food item to contacting the refuge) components. Groups did not differ in outward segment path circuity, peak speed, or movement segmentation. However, the homeward segment for control mice was relatively direct, whereas the homeward journey for *tilted* mice was significantly more circuitous. These results suggest the otolith organs contribute to dead reckoning in darkness.

Disclosures: S.N. Brockman: None. **E.A. Goebel:** None. **R.M. Yoder:** None. **J.R. Koppen:** None. **P.A. Blankenship:** None. **D.G. Wallace:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DC012630

INSGC Research Initiation Grant

Title: The place-specific activity of hippocampal place cells does not require signals from the otolith organs

Authors: *R. M. YODER[1], S. A. RUTAN[1], J. J. SIEGEL[2];
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Abstract: Hippocampal place cells represent perceived location within an environment, and are thought to contribute to spatial memory. This representation is typically controlled by visual information, but is abolished by damage or inactivation of the vestibular system. This vestibular contribution may originate in the semicircular canals or otolith organs, or both, but no studies have directly tested the contribution of each vestibular component to place cell function. We investigated the otolithic contribution to place cell function by recording hippocampal cells in

freely moving *tilted* mice, which have dysfunctional otolith organs. Male homozygous *tilted* mice and their heterozygous littermates were implanted with 4 moveable tetrodes to record the activity of single neurons within the hippocampus. Following a 1-2 week recovery period, neuronal activity was recorded across 5 trials (1-Standard, 2-Cue Rotation, 3-Standard, 4-Darkness, 5-Standard), after which the electrode bundle was lowered ≈50μm to record a putatively different subset of cells on the following day. Single-neuron spike activity was isolated offline. For each isolated neuron, the position of the mouse at the time of each action potential was used to produce a Firing Rate X Place map of the arena. Spatial information content and coherence values were calculated for each rate map to determine whether that cell's activity was significantly modulated by location under the different conditions. These measures, along with spatial correlation between trials, were used to determine whether place cell activity remained stable across trials or were differentially modulated by the conditions. Numerous place cells were identified in both control and *tilted* mice. Spatial information content and spatial coherence values did not change significantly across trials, and place field position was dominantly controlled by the visual cue card for both groups. Both groups also had many cells that maintained their place-specific activity in darkness. These results suggest that signals from the otolith organs are not necessary for the location-specific activity of hippocampal place cells in light or in darkness.

Disclosures: R.M. Yoder: None. **S.A. Rutan:** None. **J.J. Siegel:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Support: NIH Grant NS039456

Johns Hopkins University Brain Science Institute

Title: Population coherence along the CA3 transverse axis

Authors: *H. LEE, S. DESHMUKH, J. KNIERIM;
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Abstract: The hippocampal subfield CA3 is proposed to act as an attractor network because of its recurrent collateral system. Anatomically, there seems to be a gradient in recurrent network density along the CA3 transverse axis (Ishizuka et al., 1990). We hypothesized that this gradient in density of recurrent collaterals would lead to a gradient in coherence of neuronal activity. To test this hypothesis, we recorded CA3 neurons along the transverse axis during the double rotation cue-shift experiment. Rats were trained to run around a circular track containing salient local cues (textures on the track) in a room containing salient global cues (landmarks along the curtains). Three standard sessions were interleaved with two mismatch sessions, in which the local cues on the circular track were rotated counterclockwise (CCW) and the set of distal cues was rotated clockwise (CW) by an equal amount. Total mismatch angles between the local and the distal cue sets varied between 45°, 90°, 135°, or 180°. We segregated neurons from CA3a, CA3b, and CA3c regions. However, due to the difficulty of dissociating CA3c neurons from hilar cells, our CA3c ensemble is likely to include hilar neurons. Thus we refer to neurons from this region as CA3c/hilus neurons. Observation of rate maps showed that cellular responses to the environment manipulation seemed to be different in the CA3 subregions. Comparing the rate maps between a standard and the following mismatch session from 19 rats, approximately 37% of CA3a neurons (n=112/302) and 39% of CA3b neurons (n=114/289) remapped (place fields either appearing or disappearing in one of the two sessions), while 60% of CA3c/hilus neurons (n=82/136) remapped ($\chi 2 = 22.36$, $p < 0.0001$). However, our preliminary population correlation analysis did not reveal a strong difference in coherent rotation of neuronal ensembles along the transverse axis in response to cue rotation. Further analyses will test if other measures reveal differences in population ensemble coherence in the CA3 subregions.

Disclosures: H. Lee: None. **S. Deshmukh:** None. **J. Knierim:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: R01 MH079511

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HFSP Fellowship LT00683/2006-C

Title: A case of disrupted grids by reference frame rotational dissociation

Authors: *F. SAVELLI, J. D. LUCK, J. J. KNIERIM;
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Abstract: The regular, periodic firing pattern of grid cells is presumed to reflect an internally generated spatial framework that underpins hippocampal maps. The interaction between the endogenous pattern generation and sensory spatial information remains largely unexplored. While grid scaling and elliptical compression have been documented, only preliminary evidence has suggested that the grid hexagonal structure can be outright disrupted by conflicting external inputs in unobstructed open space (Song et al, SfN 2012, 293.05). We report on one experimental manipulation that repeatedly caused a loss of hexagonal structure in grid cells of one rat over the course of 18 days. Recordings were performed during a series of sessions in which a foraging platform was shifted or rotated in a room endowed with prominent visual cues, thereby producing a dissociation between the proximal and distal reference frames. We previously described how grids can anchor to either reference frame in 7 rats, without loss of gridness, other than occasional elliptical compression (Savelli & Knierim SfN 2012, 812.13; Savelli et al SfN 2013, 670.28). In one rat, however, we observed that in the 70° rotation of the box, hexagonal gridness, but not the multi-field structure, was often lost even though it was prominent in previous and following sessions. We concentrated our preliminary inspection of this phenomenon on well-isolated units that exhibited a striking degree of gridness (autocorrelogram-based gridness score adjusted for possible elliptical compression > 1.0, with additional geometry checks) in at least one session (22 cells). We characterized the loss of hexagonal gridness in a different session of the same day as the gridness-score falling below 0 or its calculation failing because a suitable configuration of correlation fields could not be found in the autocorrelogram. Out of 21 cells that were exposed to the 70° rotation, 13 lost hexagonal gridness. In contrast, only 4 out of 18 did so in the 20° rotation, and only 3 out of the 22 exposed to any of the standard, repeated standard, or shift condition. Of the 9 cells that were exposed to multiple sessions that included both 70° and 20° rotations and lost hexagonal gridness in only one session, 7 did so in the 70°, up to the last recording day by which the rat had experienced this rotation on 18 consecutive days. Selective loss of hexagonal gridness therefore persisted in spite of considerable familiarity with the experimental manipulation. These observations suggest that the internal consistency of grid-generation mechanisms can be disrupted by external spatial inputs, and the disruption can be incorporated into a stable spatial representation.

Disclosures: F. Savelli: None. **J.J. Knierim:** None. **J.D. Luck:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH094146

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Title: Potentiation of place fields along the CA1 transverse axis by investigatory head-scanning behavior

Authors: *C.-H. WANG, G. RAO, J. D. MONACO, S. S. DESHMUKH, J. J. KNIERIM; Krieger Mind Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: During exploration in both familiar and novel environments, potentiation of hippocampal place fields is significantly correlated with cell firing in locations where the animal has recently paused to execute investigatory head movements (Monaco et al, 2014). Characterizing how scanning behavior may modulate the firing of place cells across the CA1 transverse axis is an important step in understanding how information about the environment could be incorporated into the hippocampal spatial code. The hippocampus receives cortical input from two major pathways: the medial entorhinal cortex (MEC), a predominantly spatial input reflecting path integration, and the lateral entorhinal cortex (LEC), an object-related or non-spatial input that may be primarily driven by external sensory cues. LEC preferentially innervates the CA1 distal portion and MEC innervates the CA1 proximal portion. If object or other information about the environment during pauses is being conveyed by LEC, but not MEC, during scanning behavior, scan-elicited potentiation may be most prevalent in distal CA1 (the portion of CA1 receiving direct LEC input), relatively weaker in intermediate CA1 (receiving mixed input from LEC and MEC), and weakest in proximal CA1 (the portion receiving direct MEC input). Data were analyzed from 35 rats trained to run clockwise 15-17 laps on a circular track inside a curtained enclosure for randomly placed food reward. CA1 place cell firing during scanning behavior was observed across all CA1 subregions. Contrary to our hypothesis, however, the proportion of place field potentiation events predicted by scanning on the previous lap was not different along the CA1 transverse axis (scan predictive index= number of predicted potentiation events/total number of potentiation events: CA1 prox 20.7%, CA1 intermediate 22.2%, CA1 distal 18.2%). The presence of scan-predicted potentiation even in the portion of CA1 receiving primarily spatial input underscores its importance in the construction and maintenance of a spatial map during exploration. Further analyses will investigate whether subtle differences in these phenomena exist along the CA1/CA3 transverse axis.

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Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Title: A closed-loop virtual reality apparatus for investigating place and grid cell formation

Authors: ***R. P. JAYAKUMAR**[1], M. S. MADHAV[2], H. T. BLAIR[4], J. J. KNIERIM[2], N. J. COWAN[3];
[2]The Zanvyl Krieger Mind/Brain Inst., [3]Dept. of Mechanical Engin., [1]Johns Hopkins Univ., Baltimore, MD; [4]Behavioral Neuroscience, Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Spatial navigation involves the reconciliation of external landmarks with self motion cues to form a coherent representation of one's position and orientation within an environment. Thus, the ability to perturb an animal's perception of these categories of information is essential to understand how they are integrated by the nervous system. This question has led to the development of a number of virtual reality systems that involve constraining and suspending a rodent atop a freely rotating sphere, allowing the animal's legs to rotate the sphere as it attempts to walk. Such systems enable precise control of visual feedback based on real-time measurement of the sphere rotation. Furthermore, these systems allow for the application of invasive physiological measurements that require head-fixed preparations. However, a serious drawback is that the animal's movement is highly non-naturalistic and the restraints eliminate or profoundly alter self-motion cues such as inertial or proprioceptive signals. We designed and constructed a virtual reality arena where an animal can locomote under minimal physical constraints, while maintaining the ability to precisely control the visual scene in response to the animal's locomotor output. In this setup, the rat is restricted to run on a circular track by means of a freely rotating radial boom arm. The animal fully supports its own weight and experiences naturalistic motion cues, inertial forces, and proprioceptive feedback. An optical encoder connected to the boom precisely measures the angular position of the rat relative to the track

(0.02 deg resolution at 1 KHz sampling rate). A hemispherical dome is positioned over the track, and custom optics project a visual image onto the inner surface of the dome. The visual scene spans the entire 360 degrees of azimuth and approximately 175 degrees of elevation of the dome. Importantly, this visual scene can be modified in real-time based on the angular position of the rat. Simultaneous, tethered, multi-electrode recordings from the brain are synchronized with the visual scene. We believe this setup will enable the investigation of questions about the neural encoding of position and orientation, and will be especially powerful for understanding the contribution of self-motion information to navigation.

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Poster

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Topic: F.02. Animal Cognition and Behavior

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AI-HS studentship

Title: Reactivation of rate remapping in CA3

Authors: *C. SCHWINDEL, K. ALI, M. TATSUNO, B. MCNAUGHTON;
Canadian Ctr. For Behavioural Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: The hippocampus supports memory possibly by incorporating sensory information onto a spatial framework to provide a contextual link to data stored in neocortex. Place fields of CA1 and CA3 cells are thought to be generated initially by path-integration mechanisms and are reactivated in slow-wave sleep after behavior; however, traversing the same field from two directions results in different rates within the same fields (rate remapping), induced by external sensory input during behavior. Are these secondary rate differences preserved during reactivation? Recently, Colgin et al. (2010) found that CA3 exhibits attractor dynamics for different places (global remapping), but not for cues experienced in the same place. This suggests that there might be remapping of the initial path-integration based patterns but without the firing rate differences due to rate remapping. Place cells in CA3 were recorded from 5 rats

running on a circular track in alternating 15-min sessions of clockwise (A) and counter clockwise (B) direction and in the intervening sleep (A-sleep-B). As shown previously (Navratilova et al., 2012), there was pronounced rate remapping in A vs. B. There was significant reactivation of track activity patterns during sleep measured by the Explained Variance (EV) (Kudrimoti et al., 1999). Reactivation was strongest on the first day and got weaker over days. We investigated the rate difference reactivation by both the EV method and by calculating the log-firing rate correlations between maze and sleep sessions (Battaglia et al., 2005). The critical comparison was the similarity between the sleep (S) and the first (preceding) running session (A) and the second (subsequent) running session (B). The null hypothesis is that the activity patterns in A provide no greater information about the subsequent sleep patterns than the activity in B, which the animal had not yet experienced, since both A and B are different random perturbations of the original path-integration-based activity distribution. We found that A showed a higher prediction than B for activity in sleep for the log-firing rate correlations ($p = 0.0015$, t-test) and for the EV method ($p = 0.07$, t-test). We conclude that the effects of rate remapping are preserved in CA3 reactivation during sleep, in spite of the lack of attractor dynamics in CA3 for rate-remapping. It is of course possible that the rate differences are reactivated in lateral entorhinal cortex and secondarily imposed on CA3. The lack of significance in the EV measure likely reflects the lower S/N ratio of this second-order measure.

Disclosures: **C. Schwindel:** None. **K. Ali:** None. **M. Tatsuno:** None. **B. McNaughton:** None.

Poster

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Title: Automated whole brain cortical connectivity maps reveal anatomically distinct areas in rat posterior parietal cortex

Authors: *A. A. WILBER[1,2,3], B. J. CLARK[1,3], A. J. DEMECHA[1], L. MESINA[1], J. M. VOS[1], B. L. MCNAUGHTON[1,2];
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Abstract: Current theory suggests that allocentric representation of landmark direction could be accomplished from neurons that encode the conjunction of egocentric landmark x head-direction. Landmark direction and distance representations could be produced by combining these cells with distance encoding cells. Recently, we described a parietal-hippocampal network that seems to utilize these cell types to translate landmark representations from egocentric to allocentric coordinates. Further, we demonstrated that the initial stages in this process occur in a region of the rodent "posterior parietal cortex" (PPC) that has previously been characterized as the rostral tip of medial secondary visual cortex (i.e., V2MM and V2ML). However, there is very little information reported on the connectivity of this region of the rat cortex. Therefore, we set out to map the connectivity of the entire anterior to posterior span of this region of the rat medial isocortex that we refer to as PPC based on its functional similarity to primate PPC, including the regions sometimes characterized as secondary visual cortex (i.e., V2MM and V2ML). To do this we have used anterograde and retrograde tracers (Fluoro-Gold, Cholera Toxin Subunit B, and high molecular weight Biotinylated Dextran Amine) in conjunction with open source neuronal segmentation and tracer detection tools (FARSIGHT; www.farsight-toolkit.org) to generate whole brain connectivity maps of parietal inputs and outputs (see the Demecha et al., 2014 poster for details). Our present results show that bidirectional connectivity between PPC and hippocampus is indirect and likely achieved largely via dysgranular retrosplenial cortex. In addition, we found that inputs to the PPC vary along the rostral-caudal axis of the rat PPC. For example, inputs to PPC from dysgranular retrosplenial cortex is adjacent to the location of the injection (i.e., rostral PPC is connected to rostral retrosplenial cortex). Dysgranular retrosplenial cortex tends to receive more visual inputs, suggesting one possible route of visual input to PPC. Alternatively, though no portion of rat PPC received dense inputs from primary visual cortex, the density of higher order visual inputs (e.g., secondary lateral, temporal cortex) to caudal PPC is higher, compared to rostral PPC. Thus, similar to primates, PPC in rats may have functionally distinct areas; though, the precise function of these rat PPC areas is likely to differ compared to primates.

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Poster

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AIHS polaris award to BLM

NERF

Title: Methodology for automated unrolled cortical connectivity maps of the rodent brain

Authors: *A. J. DEMECHA, L. MESINA, B. J. CLARK, A. A. WILBER, B. L. MCNAUGHTON;
Canadian Ctr. For Behavioural Neurosci., Lethbridge, AB, Canada

Abstract: Understanding the neurobiological basis of cognition and behaviour, and disruptions to these processes following brain injury and disease, requires a large-scale assessment of functional and anatomical connectivity. For example, recent research implicates a posterior parietal-hippocampal circuit for encoding landmark position and this same circuit is likely involved in consolidation and retrieval of this information; however, no direct network connections have been observed between the parietal cortex and hippocampus in rodents (see the Wilber et al., 2014 poster for details). We present custom analysis software that allows for large-scale investigation of the connectivity of cortical circuits. Rats first received small injections of Fluorogold or Cholera Toxin-Alexa Fluor conjugates. Following recovery, brains were extracted and sectioned on a custom vibratome with a camera mounted above the specimen thereby allowing the acquisition of block-face images in the coronal or sagittal plane. Sections were collected, and three parallel series were stained with fluorescent NeuN (a selective neuronal marker), Parvalbumin, and Cresyl Violet. Rapid image acquisition of the sections was conducted using NanoZoomer whole-slide scanning microscopy (Hamamatsu), which is capable of automatically capturing wide-field multispectral fluorescent images over entire brain sections at high resolution, in a matter of several hours. A custom software platform has been developed in Matlab/Fiji to accommodate an automated analysis pipeline that generates unfolded cortical maps illustrating the anatomical position of identified projection neurons and can be adapted for functional markers. First, high-resolution images are automatically split into large, but

manageable 40X magnification tiles. Second, the xy position of tracer positive and negative neurons are identified using open source segmentation tools (FARSIGHT; www.farsight-toolkit.org) and mapped onto a manually drawn line that runs through the cortical sheet boundary between layers IV and V (i.e., distance from the line is retained). This allows the creation of layer specific maps for comparisons of neuronal populations originating from superficial (II to IV) vs. deep layers (V/VI). Data are aligned to the lateral border of the cingulate cortex and the location of the rhinal sulcus is marked for an additional reference point. This alignment method can be applied to any brain image collected in this manner across studies and atlases. This tool will facilitate a structural assessment of the entire cortex, and will form the basis of a large-scale understanding of whole brain connectivity.

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Poster

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HFSP - RGY0089/2012

Title: Impact of objects on dentate gyrus firing activity

Authors: ***D. JUNG**[1], T. GEILLER[3], D. KIM[2], S. ROYER[3];
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Abstract: The hippocampus plays a critical role in episodic memory, which is believed to require an integration of object and place information. While most studies on 'place cells' have been carried in the CA1 and CA3 regions, much less is known about neural correlates of the dentate gyrus (DG). It has been shown that DG neurons activity is more sensitive than CA1 and CA3 to small environmental changes, consistent with its hypothesized role in pattern separation. Both spatial and non-spatial information converge on DG neurons. In order to investigate how these different modalities are represented by DG activity, we trained mice to run for water

rewards on a long treadmill belt on which small objects could be attached or removed at will. Typically 4 types of objects with contrasting color and texture were used: 1) a 'forest' of ~3 cm long spines made with hot glue, 2) a 20 cm long 'field' of scattered shrink tubes, 3) a 20 cm long 'field' of scattered pieces of Velcro, and 4) a single big ~0.8 cm diameter shrink tube. The objects layout consisted of a regular alternation between big shrink tubes (BT) and 20 cm long objects (LO). Each LO type was repeated in at least 2 locations of the belt. A ~5ul sweet water reward was delivered through a lick port at a specific belt position on every trial. After 3 weeks of training, two 64-channels silicon probes held in stereotaxic manipulators were inserted (under isoflurane anesthesia) into the DG area. Recordings started ~30-60 min after insertion, when the mice fully recovered from anesthesia and started running. A total of 10 recording sessions were performed in a total of 7 mice. We observed that a large fraction of DG neurons exhibited sharp and stable firing fields across many trials. Among these cells, most had multiple fields (up to 7), while only a minority of cells displayed 1 or 2 fields. The fields of individual cell were tightly coupled with the objects on the belt, as their positional relation with the objects was in most case maintained. However, as opposed to what we observed in CA1, DG neurons did not differentiate the objects identity, repeating the fields with equal chance and intensity for dissimilar LO objects. The periodicity of the fields corresponded mostly to the periodicity of the BT (or LO) objects, with a few cells exceptions which repeated the firing either at both BT and LO objects or at lower frequency than BT objects. These findings indicate a different treatment of object identity in DG compared to CA1, and might be interpreted as BT and LO objects providing reference frames for entorhinal grid cells, which in turn are inducing a regular object -anchored but not -specific activity pattern in DG.

Disclosures: **D. Jung:** None. **T. Geiller:** None. **D. Kim:** None. **S. Royer:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.21/UU36

Topic: F.02. Animal Cognition and Behavior

Support: NERF funding through imec, KUL, and VIB to VB and BMcN

Alberta Innovates Health Solutions Polaris Award to BMcN

Alberta Innovates Health Solutions Graduate Studentship to DM

Title: Task-related signals in mouse primary visual cortex during virtual path integration

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Abstract: Successful navigation and spatial memory requires the integration of multiple information sources into a coherent representation of external sensory features and an animal's position in space. This convergence is thought to occur in the hippocampal formation, where information about self-motion (path-integration) is used to create a metric representation of space onto which sensory information is superimposed (Navratilova et al., 2012). Recent studies, however, indicate that navigation-related signals are also present at the level of primary sensory areas, possibly reflecting signals from higher processing levels. The functional role of these signals, however, is poorly understood. We studied the encoding of visual and non-visual information in the mouse primary visual cortex (V1) in a 'virtual' spatial paradigm comprised of both path-integration and local sensory cues. We developed a head-fixed treadmill assay (Royer et al., 2012) that reliably drives place field activity in the hippocampus and in which visual stimuli can be precisely controlled. We trained mice to move a linear treadmill belt covered with tactile cues and to interrupt their movement at regular intervals for a water reward. We studied how calcium responses of V1 populations to brief visual noise stimuli presented in closed-loop were influenced by task-relevant variables. V1 neurons showed pronounced task-related activity during navigation, even in the absence of visual inputs. Consistent with previous reports, V1 responses to visual stimulation were strongly modulated by movement, with most neurons showing increased responsiveness during motor activity. During visual stimulation, V1 neurons showed reward- and position-related modulations that could not be explained by motor activity and other potentially confounding factors. In the absence of visual inputs, V1 neurons showed signals that reflect distinct aspects of the animal's behavior, the environment, and the task. These response properties were robust to behavioral variations within imaging sessions and showed even constancy across sessions. We hypothesize that neurons in mouse primary visual cortex encode the conjunction of visual and contextual information, which may be particularly useful in guiding navigational behavior.

Disclosures: **S. Kandler:** None. **D. Mao:** None. **B. McNaughton:** None. **V. Bonin:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.22/UU37

Topic: F.02. Animal Cognition and Behavior

Title: Place recognition and heading retrieval are dissociable in mice (and possibly men)

Authors: *J. B. JULIAN, A. KEINATH, I. MUZZIO, R. A. EPSTEIN;
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Abstract: A navigator who becomes lost must identify her current location and recover her facing direction in order to restore her bearings. We tested the idea that these two tasks-place recognition and heading retrieval-might be mediated by distinct cognitive systems. Previous work has shown that both rodents and young children rely primarily on the geometric shape of navigable space to regain their sense of direction after disorientation, often ignoring non-geometric visual cues even when they are informative (Cheng, 1986; Hermer & Spelke, 1994). Notably, these experiments are almost always performed in single-chamber environments in which there is no ambiguity about place identity. We examined the navigational behavior of disoriented mice presented alternately with two rectangular chambers that were geometrically identical but distinguishable by horizontal or vertical stripes along one wall. Thus, the stripes could be used both to identify the chambers (place recognition) and also to disambiguate directions within the chambers (heading retrieval). In one chamber, mice were rewarded whenever they searched in the left corner nearest the striped wall, and in the other chamber, whenever they searched in the right corner nearest the striped wall. We found that in each chamber mice searched in the correct corner for that chamber or its geometrical equivalent (diagonally opposite) corner with equal frequency, and did so significantly more often than at the other corners. Thus, mice used the stripes to identify the chamber in which they were located, but not to disambiguate between the two geometrically-equivalent facing directions. These results suggest the existence of separate systems for place recognition and heading retrieval in mice that are differentially sensitive to geometric vs. non-geometric visual cues. We speculate that a similar cognitive architecture may underlie human navigational behavior.

Disclosures: J.B. Julian: None. **A. Keinath:** None. **I. Muzzio:** None. **R.A. Epstein:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: GACR 14-03627S

IGA MZ CR NT13386

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GACR P304/12/G069

MSMT LH14053

Title: Contributions of posterior parietal, medial prefrontal and anterior cingulate cortices to navigation in dynamic environment in rats

Authors: *J. SVOBODA, M. VODICKA, K. BLAHNA, P. TELENSKY, T. PETRASEK, I. VOJTECHOVA, K. VALES, S. KUBIK, A. STUCHLIK;
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Abstract: Several neocortical areas, including posterior parietal (PPC), medial prefrontal (mPFC), and anterior cingulate cortex (ACC) have been implicated in place navigation in rats. To better understand their role we evaluated their contribution to navigation in dynamic environment, in which its parts moves with respect to each other, reflecting higher cognitive demands of our naturally ever-changing environments. Rats of Long Evans strain were trained to avoid an unmarked to-be-punished sector on a slowly rotating arena called Carousel. Position of the sector either rotated with the arena (arena frame condition) or remained stable according to reference frame of the experimental room (room frame condition). This task requires segregating spatial cues into two coherent sets according to the correspondent frame, navigating by the appropriate one and abandoning the misleading cues in the other frame (this is referred to as cognitive coordination). Cognitive coordination under room frame cognition has been found to be critically dependent on functional hippocampus. We examined an effect of thermocoagulation PPC lesion, mPFC lesion by NMDA, and ACC lesion by quinolinic acid on acquisition and reversal in both room frame and arena frame condition. We observed the largest deficit in PPC lesioned rats in room frame condition, particularly during reversal. Lesions aimed to mPFC or ACC disrupted acquisition and reversal in either frame only marginally. Neither lesion affected locomotor activity. Collectively these data underscore the importance of PPC in coordinating room-bound, i.e. distal landmark navigation and show minor contribution of ACC and mPFC to arena or room based navigation. PPC therefore might communicate with hippocampus to process dynamic distal visual information. The additional role of mPFC and ACC in the navigation in dynamic environment remains to be elucidated in more detail. This work was supported by GACR grant 14-03627S and IGA MZ CR NT13386, by AS CR M200111204 and by GACR P304/12/G069 and by MSMT LH14053.

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Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 848.24/UU39

Topic: F.02. Animal Cognition and Behavior

Support: ERC 250047

Title: Preserved grid cell spatial firing pattern despite impaired place cell spatial selectivity in Connexin-36 KO mice

Authors: ***O. TOADER**, K. ALLEN, H. MONYER;
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Abstract: Mammals possess spatially selective neurons in the hippocampal and parahippocampal region that enable them to navigate through their environment. Grid cells in the medial entorhinal cortex fire at multiple locations arranged in a regular hexagonal pattern, covering the entire environment. The firing rate of grid cells is thought to be controlled by the integration of self-motion (or "idiothetic") cues, a process prone to accumulation of errors. Errors in the grid cell network, which cause the grid cell representation to drift relative to the environment, can be minimised provided a spatial input anchored to external cues in the environment. This input could be provided by place cells in the hippocampus that fire when the animal is at one or a few specific locations in the environment. Indeed, computational work as well as pharmacological experiments suggest that place cell activity is important for anchoring grid cell representation, by providing information related to external cues. Consequently, a reduction in the spatial selectivity of the place cell signal could destabilize grid cell firing patterns. To test this hypothesis, we recorded the activity of grid cells in mice lacking connexin 36, the major neuronal gap junction protein. It was previously shown that place cells have a lower spatial selectivity and reduced stability in these mice. Surprisingly, we found the spatial activity of grid cells in Cx36-/- mice to be indistinguishable from that of control mice. Grid cells in Cx36-/- mice had normal grid scores and their firing activity showed no reduction in spatial information content. Other functional properties, such as mean firing frequency, theta modulation and number of firing fields were also not changed. However, theta oscillations were slower in Cx36-/- mice, and the firing of putative interneurons was less modulated by theta

oscillations. These results suggest that normal place cell spatial selectivity is not essential for anchoring the grid cell spatial representation in a familiar environment.

Disclosures: **O. Toader:** None. **K. Allen:** None. **H. Monyer:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: Israeli Science Foundation

Foundation Adelis

Human Frontier Science Program long-term fellowship

Title: The dynamics of global remapping in a network model of hippocampal circuit

Authors: ***S. ROMANI**[1], S. MARK[2], M. TSODYKS[2];
[1]Ctr. for Theoretical Neurosci., Columbia Univ., New York, NY; [2]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Hippocampal place cells represent different spatial environments with distinct activity patterns. Following a sudden switch between two configurations of local cues, place cells activity transitions almost immediately to the correct representation. Surprisingly, during a transient period immediately following the switch, occasional reactivations of activity patterns representing the pre-switch environment were observed. These reactivations are paced by the ongoing theta rhythm. Here we show that an attractor neural network model of place cells activity whose synapses are endowed with short-term activity-dependent synaptic depression and facilitation can account for this phenomenon. In the network model, two overlapping populations of neurons encode the two environments. Synapses connecting neurons that represent the same environment are strengthened. External input to either one of the populations determines which environment is active. The two populations compete via feedback inhibition. Before the switch, synapses connecting active neurons in the population corresponding to the pre-switch environment are facilitated. This allows the maintenance of a memory trace of that environment for a time dictated by the time-scale of short-term synaptic facilitation. Following the switch, there is a competition between the reactivation of the pre-switch representation, due to the

presence of a memory trace, and the current representation, due to the external input. The model predicts that the amplitude of theta rhythm and the animal velocity, both influencing network activity and hence the state of the synapses, affect the tendency of the network to produce such behavior. We test these predictions with novel analysis of existing experimental data. Our results suggest a prominent role of short-term synaptic plasticity in shaping hippocampal activity in behaving animals.

Disclosures: **S. Romani:** None. **M. Tsodyks:** None. **S. Mark:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant 5R01MH083686

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NIH grant 5RC1NS068148

Helen Hay Whitney postdoctoral fellowship (DA)

Title: Using 2D virtual reality in rats to study neural representation of spatial reference frames

Authors: *D. ARONOV, D. W. TANK;
Princeton Univ., Princeton, NJ

Abstract: Cognitive control requires the brain to select a relevant subset of possibly conflicting cues and to execute appropriate behavioral responses. In navigation, this process can be studied by presenting animals with conflicting spatial frames of reference - e.g., by rotating 2D reference frames relative to one another. Virtual reality (VR) enables real-time control of sensory cues and is potentially a valuable tool for manipulating reference frames. However, neural activity in rodents has only been studied on virtual linear tracks, and the ability of VR to engage the spatial navigation system in 2D environments is unknown. It is also not known how the navigation system represents VR reference frames that are in conflict with the real world (laboratory). We studied the feasibility of using VR to manipulate reference frames by building a VR apparatus for 2D navigation in rats. Rats navigated in a square virtual environment using a spherical

treadmill. The environment was displayed on a 360° screen, and a commutator allowed turning and walking in any direction. We used tetrodes to record in the hippocampus and the medial entorhinal cortex - two brain areas involved in cognitive control and spatial representation. Neurons in both areas, including place cells, grid cells, head direction cells and border cells, showed 2D patterns of activity similar to those in the real world. In one experiment, we continuously rotated the image of the virtual environment relative to the laboratory, thus creating uncoupled VR and real-world reference frames. Activity patterns of all cell types preferentially followed the VR reference frame during this manipulation. In the second experiment we recorded place cells on two sessions, as the orientation of the VR environment relative to the real world was discretely changed between the sessions. In most cases, place fields on the second session aligned to their prior location in VR. Yet in other cases, place fields coherently rotated relative to the VR environment and aligned better to their prior orientation in the real world. In these cases the rotation was always in multiples of 90°, corresponding to the angle between walls of the virtual environment. Place fields that aligned to the real world at the start of the session proceeded to track the VR environment as it was slowly rotated. Thus, the conflict of VR with the real world caused coherent remapping of place fields relative to salient cues, while maintaining alignment to environment geometry. Our results indicate that the navigation system is engaged in 2D VR. They further suggest a potential use of this technique in studying cognitive control and remapping across multiple spatial reference frames.

Disclosures: D. Aronov: None. **D.W. Tank:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Agence Nationale de la Recherche - ANR-REG-071220-01-01

HABOT project funded by the Emergence(s) program of the Ville de Paris

Title: Unraveling the neural circuitry of sequence-based navigation using a combined Fos imaging and computational approach

Authors: *B. M. BABAYAN[1], A. WATILLIAUX[1], C. TOBIN[1], B. GIRARD[2], L. RONDI-REIG[1];
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Abstract: Spatial navigation is a complex function requiring the combination of external and self-motion cues to build a coherent representation of the external world and drive optimal behavior directed towards a goal. This multimodal integration suggests that a large network of cortical and subcortical structures interacts with the hippocampus, a key structure in navigation. Studying navigation through this global approach, we focused on sequence-based navigation, which consists in remembering a sequence of turns. This navigation specifically relies on the temporal organization of movements at spatially distinct choice points thus requires manipulating information in a spatio-temporal framework. Our aim was to identify the functional network underlying sequence-based navigation using Fos imaging and computational approaches. We trained mice in a multi-intersection maze to learn a path without environmental cues. This learning required distinguishing locations by the order in which they were met. The functional networks dynamically changed across early and late learning stages. The early stage network was dominated by a highly inter-connected cortico-striatal cluster. The hippocampus was activated alongside structures known to be involved in self-motion processing (cerebellar cortices), in topographical representation manipulations (retrosplenial, parietal, entorhinal cortices) and in goal-directed path planning (prefrontal-basal ganglia loop). The late stage was characterized by the emergence of correlated activity between the hippocampus, the cerebellum and the cortico-striatal structures. Conjointly, we explored whether path integration, model-based or model-free reinforcement learning algorithms could explain mice's learning dynamics. Only the model-free system, as long as a retrospective memory component was added to it, was able to reproduce both the group learning dynamics and the individual variability observed in the mice. These results suggest that a unique model-free reinforcement learning algorithm was sufficient to learn sequence-based navigation and that the multiple structures this learning required adapted their functional interactions across learning.

Disclosures: B.M. Babayan: None. **A. Watilliaux:** None. **C. Tobin:** None. **B. Girard:** None. **L. Rondi-Reig:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Program#/Poster#: 848.28/UU43

Topic: F.02. Animal Cognition and Behavior

Support: FP7-ERC “GABA Networks” grant (# 242842)

Title: Encoding of distance by recurrent hippocampal cell sequences in the absence of external landmarks

Authors: *V. VILLETTE, A. MALVACHE, T. TRESSARD, R. COSSART;
INSERM, Marseille Cedex 9, France

Abstract: The hippocampal formation is central for spatial cognition and navigation. Here, we analyzed spatial tuning under the sole influence of self-motion cues by using chronic calcium imaging of the activity of hundreds of hippocampal CA1 neurons simultaneously from head fixed mice allowed to self-regulate their motion in the dark on a treadmill. While place cells are activated in the presence of tactile cues, sequences of neuronal activation ordered by the run distance are observed when cues are removed. In contrast to location, distance is encoded at ensemble- but not single cell-level. These sequences rely on strong functional links between the neurons they engage as they can be transiently held during second-long immobility periods and as they recruit similar cells in the same order across consecutive days. Importantly, the intrinsic distance extent covered by these sequences correlates with spontaneous mouse behavior. This finding indicates that not only landmark-based but also idiothetic navigation can be encoded at the behavioral timescale within the hippocampal map in the firing of self-motion triggered but internally hardwired neuronal sequences.

Disclosures: V. Villette: None. **A. Malvache:** None. **T. Tressard:** None. **R. Cossart:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Program#/Poster#: 848.29/UU44

Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Grant

Title: Grid cell symmetry is shaped by the geometry of the environment

Authors: *J. KRUPIC[1], M. BAUZA[1], S. BURTON[1], C. BARRY[1], J. O'KEEFE[1,2];
[1]Univ. Col. London, London, United Kingdom; [2]Univ. Col. London, Sainsbury Wellcome Ctr., London, United Kingdom

Abstract: Grid cells represent an animal's location by firing in multiple fields arranged in a striking hexagonal symmetry [1]. It was originally believed that the main properties of the grid cell firing pattern (its symmetry, orientation and scale) are preserved across multiple testing conditions suggesting that it represents a universal and environment-invariant metric system for navigation [1]. Subsequent experiments showed however that grid scale changes in response to stretching of familiar enclosures by rescaling in the same direction [2] and also increases in novel environments [3]. In both cases the changes in grid pattern are temporary, reinforcing the view that a 'canonical' pattern is determined by intrinsic properties (either on a single cell or network level). Currently this notion dominates all mainstream theoretical grid cell models.However, more recent studies suggest that the boundaries of the environment may play a key role in shaping grid cell symmetry [4-6], but the precise mechanism remains unknown. Here we show that grid orientation, scale, symmetry and homogeneity are permanently affected by the geometry of the environment. We found that grid orientation tends to cluster in polarised enclosures such as squares and trapezoids but not in circles suggesting that input from boundary cells shapes grid cell symmetry and does not simply provide an anchoring of grid offset as previously assumed. Furthermore, the hexagonal grid symmetry is permanently broken in highly polarised 2-dimensional environments such as trapezoids where grid cell fields become more elliptical in comparison to more symmetrical environments such as squares and circles. Moreover, the grid pattern becomes non-homogeneous as the grid structure elongates towards the narrower part of the trapezoid. Finally, different grid modules are unequally affected by the shape of the enclosure, tending to preserve their relative orientations and scale ratios across different symmetrical enclosures such as circles, hexagons and squares but not in trapezoids. 1. Hafting et al., Nature, 2005. 2. Barry et al., Nature Neurosci., 2007. 3. Barry et al., PNAS, 2012. 4. Brun et al., Hippocampus, 2008. 5. Krupic et al., Science, 2012. 6. Stensola et al., Nature, 2012.

Disclosures: J. Krupic: None. **M. Bauza:** None. **S. Burton:** None. **C. Barry:** None. **J. O'Keefe:** None.

Poster

848. Cortical and Hippocampal Circuits: Spatial Navigation III

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust

Gatsby Charitable Foundation

European Research Council

Title: Synaptic mechanisms of sparse activity in hippocampal granule cells during mouse navigation

Authors: *C. SCHMIDT-HIEBER, H. WEI, M. HAUSSER;
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Abstract: The hippocampus must form distinct neuronal representations of memory items in order to differentiate between similar events. Granule cells of the dentate gyrus, the first stage of the hippocampal circuit, have been proposed to perform this task in a process termed pattern separation (Neunuebel and Knierim, Neuron, 2014). It is unclear how synaptic input patterns are decorrelated and converted into action potential output during this process. Moreover, it is unknown what fraction of the granule cell population is recruited during memory tasks, such as during spatial navigation, and it has been speculated that only a small part of the population is excitable at all, with most mature granule cells being "retired" (Alme et al., Hippocampus, 2010). To address these questions, we performed whole-cell patch-clamp recordings from mature hippocampal neurons in head-restrained mice navigating in a virtual-reality environment on a treadmill (Schmidt-Hieber & Häusser, Nat. Neurosci., 2013). Granule cells were identified by their characteristic electrophysiological properties, as well as via biocytin filling and subsequent anatomical reconstruction. Most granule cells (n = 18 of 21 cells) did not fire any action potentials throughout the recording duration (up to 18 runs along a 1−2 m long linear track). Under the same conditions, we found that CA1 pyramidal cells exhibited robust place fields during extra- and intracellular recordings. When firing was induced in silent granule cells by injecting steady depolarizing current, 4 of 9 neurons showed significant spatial modulation of firing with multiple spatial firing fields. Although granule cells were mostly silent, voltage-clamp experiments revealed that they received prominent excitatory inputs in both resting and navigating animals (mean EPSC frequency during navigation: 32 ± 3 Hz; mean EPSC amplitude: 22 ± 4 pA at −70 mV; mean ± SEM; n = 3). During running periods, the mean excitatory current input increased, while the frequency of large multicomponent EPSCs decreased. Thus, our results provide direct evidence that identified granule cells are silent, but not retired during navigation, and will require highly specific and coincident inputs to drive firing.

Disclosures: C. Schmidt-Hieber: None. **H. Wei:** None. **M. Hausser:** None.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: JSPS Grant 25291071

JSPS Grant 12J06407

Title: Foraging effort and its social facilitation in the domestic chick: double dissociation of medial striatum and substantia nigra

Authors: *Y. OGURA[1,4,2], Q. XIN[1], T. MATSUSHIMA[3];
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Abstract: Social facilitation generally denotes increments in frequency / intensity of behaviors in the presence of conspecifics (Crawford 1939). As the facilitation is reported in a variety of animals including humans (Zajonc 1965, Clayton 1978), common biological backgrounds have been assumed. In domestic chicks, foraging efforts are socially facilitated even without resource competition (Ogura & Matsushima 2011), suggesting that the facilitation could be separable from the behavioral processes responsible for reward gain and efforts. As a step to reveal the neural substrates, we focused on the two major dopaminergic pathways, i.e., (1) ventral tegmental area (VTA) projecting to nucleus accumbens (NAc) and (2) substantia nigra (SN) projecting to medial and lateral striatum (MSt and LSt). Despite the functional dissociation reported in human (O'Doherty et al. 2004; but see Morris et al. 2006), few studies have been made in birds. We made localized electrolytic lesion or dopamine depletion in VTA / SN, and examined the behavioral effects. First of all, we revealed topography of the dopaminergic projections by tyrosine hydroxylase (TH) immunostaining. Microinfusion of 6-hydroxydopamine in SN caused impaired staining exclusively in LSt, MSt, arcopallium and globus pallidus. On the other hand, infusion that included VTA caused additional impairments in NAc core/shell, ventral pallidum and septum. Our results thus confirmed the homology of subpallial structures between birds and mammals (Metzger et al. 1996, Reiner et al. 2004). Secondly, we examined the lesion effects on foraging effort and its social facilitation. In an I-shaped maze equipped with a pair of feeders on both ends, chicks foraged by running back and forth between the feeders. The running activity during the initial session in isolation (single condition) was assumed to represent the basal foraging effort. Increment in the running activity in the presence of a companion (paired

condition) was assumed to represent the social facilitation. Electrolytic lesion to MSt/NAc suppressed the basal foraging effort, whereas the social facilitation remained unimpaired. In contrast, electrolytic lesion to SN suppressed the social facilitation, but the activity in the single condition did not change. On the other hand, dopamine depletion had no effects on the running activities in both groups of chicks that received depletory injections in MSt/NAc and in SN. The neural substrates responsible for reward-based foraging effort could be doubly dissociated from those of the social facilitation.

Disclosures: Y. Ogura: None. **Q. Xin:** None. **T. Matsushima:** None.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 849.02/UU47

Topic: F.03. Motivation and Emotion

Title: Analysis of neural/molecular mechanisms of mate-guarding behavior in small fish, medaka

Authors: *S. YOKOI[1], T. OKUYAMA[1], Y. KAMEI[2], Y. TANIGUCHI[3], S. ANSAI[4], M. KINOSHITA[4], T. KUBO[1], H. TAKEUCHI[1];
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Abstract: In various animal species ranging from insects to vertebrates, males exhibit mate-guarding behavior to prevent other males from mating with their potential or former mates. Although mate-guarding behavior has been studied extensively in the field of behavioral ecology, the underlying neural/ molecular basis remains largely unknown. We previously reported that medaka fish (Oryzias latipes), a model animal for molecular genetics, exhibited mate-guarding behavior. We found that, when one female and two males were placed in a single tank, the two males competed each other for the female and, the dominant male prominently approached the female and interrupted the other subordinate male (mate-guarding behavior). The dominant male had significantly higher mating success rate than the other subdominant male. Interestingly medaka males exhibit this behavior, irrespective of mating period. Next we show that the long-lasting mate-guarding led to high mating success of the dominant male via female preference, where female medaka tend to choose familiar (visually learned) males rather than

unfamiliar males as mating partners (Okuyama et al. Science 2014). It strongly suggested that medaka female actively choose the dominant male, that can prominently approached and become familiar with the female. Finally, to analyze the neural basis of mate-guarding behavior, we generated several medaka mutants by Tilling and TALEN methods and identified some mutant strains with defect in the behaviors.

Disclosures: **S. Yokoi:** None. **T. Okuyama:** None. **T. Kubo:** None. **H. Takeuchi:** None. **Y. Kamei:** None. **Y. Taniguchi:** None. **S. Ansai:** None. **M. Kinoshita:** None.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 849.03/UU48

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01DC008962

Title: Optogenetic activation of accessory olfactory bulb mitral cells differentially modulates the attraction of male mice to female and male urinary odors

Authors: *T. KUNKHYEN[1], D. A. DOCTOR[2], W. J. KORZAN[1], E. A. MCCARTHY[2], M. J. BAUM[2], J. A. CHERRY[1];
[1]Psychological and Brain Sci., [2]Biol., Boston Univ., Boston, MA

Abstract: We asked whether optogenetic activation of the accessory olfactory bulb (AOB) was rewarding in its own right and whether AOB stimulation would modify the attraction of male mice to different pheromonal as well as food odors. Adult male transgenic protocadherin21-Cre mice in which expression of cre recombinase in the OB is limited to mitral cells, received bilateral infections of the AOB with a cre-dependent channelrhodopsin-2 and mCherry vector (AAV5/EF1a-DIO-hChR2H134; UNC Gene Therapy Center). Histology confirmed that AOB mitral cells selectively expressed mCherry. A single optical fiber was fixed to the skull on the midline, just above the AOB. Males were tested in an apparatus with two adjacent nose-poke ports that provided nasal access to odorants soaked on filter paper. Optogenetic stimulation of the AOB with a blue laser (20 Hz, 5 mW power at the tip) was automatically triggered during the time that a male inserted his snout into a port. Testing with different odorants (or water) was conducted in 10-min trials given on separate days. Prior to optogenetic stimulation, males showed a significant preference to investigate 100% estrous female urine (EFU) vs water, but

showed no preference for 5% EFU vs water. By contrast, males investigated 100% testes-intact male urine (IMU) vs water equally, but spent more time investigating 5% IMU than water. Males also displayed a significant attraction to a cookie odor (when food deprived). Subsequently optogenetic stimulation significantly augmented the time males spent investigating a dilute (5%) solution of EFU whereas optogenetic stimulation of the AOB significantly reduced the time males spent investigating a 5% solution of male urine (vs water in the opposite port). Optogenetic AOB stimulation failed to affect males' investigation of a port containing either water alone or food odor. Thus optogenetic activation of the AOB modulated males' investigation times only when conspecifics' urinary odors were present. Our results suggest that the vomeronasal-accessory olfactory system is a motivational circuit that selectively controls males' attraction as well as aversion to sex-specific pheromonal odorants.

Disclosures: **T. Kunkhyen:** None. **D.A. Doctor:** None. **W.J. Korzan:** None. **E.A. McCarthy:** None. **M.J. Baum:** None. **J.A. Cherry:** None.

Poster

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Program#/Poster#: 849.04/UU49

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01DC008962

Title: DREADD-induced silencing of the medial olfactory tubercle disrupts opposite-sex odor preference in estrus female mice

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Abstract: Rodent courtship behaviors are mediated by pheromonal signals released from opposite-sex conspecifics. Previous research has shown that the anterior medial amygdala (MeA; a limbic structure which receives pheromonal input from both the main- and accessory olfactory systems) is necessary for the display of these critical behaviors. Additionally, the MeA sends relatively dense monosynaptic axonal projections to the medial olfactory tubercle (mOT) - an integral part of the ventral striatum. The mOT was previously implicated in the reinforcing effects of both natural hedonic stimuli and drugs of abuse and was also linked to pheromone processing (Ikemoto 2012; Agustin-Pavon et al., 2014). To further explore the role of the mOT

in the processing of pheromonal odors, we first performed a functional neuroanatomical tracing study. Both the MeA and ventral tegmental area (VTA) were found to send direct projections to the mOT; a significant subset of these projections were selectively activated (expressed Fos) by male- (but not female- or clean) volatile odors emanating from soiled bedding. These projections likely drive the augmented Fos expression observed in the mOT of female mice exposed to male odors. Next, we bilaterally expressed the inhibitory DREADD receptor, hM4Di, in the mOT of female mice by way of viral infection and later subjected these animals to odor preference tests after i.p. injection of either vehicle or clozapine-N-oxide (CNO), which binds to the hM4Di receptor to hyperpolarize and effectively silence infected neurons. When the mOT was silenced, female subjects (in estrus) lost their preference to investigate male over female urinary odors (both volatiles only and volatiles+nonvolatiles) and showed a significant reduction in both the number of approaches toward male urinary odors as well as in overall odor investigation times. These effects were not observed when the same subjects were treated with saline in counterbalanced tests and subjects' ability to discriminate between the odors tested remained intact after receiving CNO. Furthermore, subjects showed no deficits in locomotor activity or preference for food odors when given CNO injections prior to testing. The mOT appears to be a critical segment of the pheromone-reward pathway in mice.

Disclosures: B. Dibenedictis: None. **A.O. Olugbemi:** None. **M.J. Baum:** None. **J.A. Cherry:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01NSO77769

NIH grant NS055215

Title: Effect of stroke on depressive behavior and sociability outcome in mice

Authors: *R. VERMA, B. D. FRIEDLER, N. M. HARRIS, J. CRAPSER, L. D. MCCULLOUGH;
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Abstract: Social isolation (SI) has been linked epidemiologically to high rates of morbidity and mortality following stroke. By contrast, strong social and emotional support enhances recovery and lowers stroke recurrence. However, the mechanism by which social support influences stroke recovery has not been adequately explored. The goal of this study was to examine the effect of post-stroke pair housing and SI on behavioral phenotypes and chronic functional recovery in mice. Young male mice were paired for 14 days before a 60 minute transient middle cerebral artery occlusion (MCAO) or sham surgery and assigned to various housing environments immediately after stroke. Post-stroke mice paired with either a sham or stroke partner showed significantly higher ($p<0.05$) sociability after MCAO than their isolated animals. Sociability deficits worsened over time in isolated littermates. Pair-housed mice showed restored sucrose consumption ($p<0.05$) and reduced immobility in the tail suspension test compared to isolated group. Pair-housed stroked mice demonstrated significantly reduced cerebral atrophy after 6 weeks ($17.5 \pm 1.5\%$ in PH vs. $40.8 \pm 1.3\%$ in SI; $p<0.001$). Overall, our findings suggest that 1) pair housing hastens recovery from histological damage and behavioral deficits after stroke, 2) SI and stroke can either independently or additively alter at least 3 major aspects of the depressive phenotype: sociability, avolition and anhedonia, 3) progressive SI hinders recovery while pair-housing facilitates it, and 4) social interaction reduces post-stroke depression and improves functional recovery.

Disclosures: R. Verma: None. **B.D. Friedler:** None. **N.M. Harris:** None. **J. Crapser:** None. **L.D. McCullough:** None.

Poster

849. Social Behavior: Neural Mechanisms

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Program#/Poster#: 849.06/UU51

Topic: F.03. Motivation and Emotion

Title: Multi-unit activity in the medial prefrontal cortex during social interaction in rats

Authors: C. MINAMI[1], T. SHIMIZU[1], S. MOMMA[1], T. MIKAMI[2], *A. MITANI[1];
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Abstract: The medial prefrontal cortex (mPFC) has direct connection with subcortical limbic areas including amygdala and is thought to play an important role in regulating social behavior. However, the role of the mPFC in social behavior is not understood. In the present study, we recorded the mPFC neuronal activity in pairs of unfamiliar male rats in which novelty of the

partner remained as the principal anxiogenic stimulus, and investigated whether there were any firing activities related with social interaction. Adult male SD rats were used. Stainless steel wires (two wires of 50 μm in diameter) were implanted in the infralimbic division (IL) or the prelimbic division (PL) of the mPFC. Animals were allowed to recover for one week. On the day before the experiment, each animal was habituated for 15 min to an open field box (100 x 100 x 50 cm) placed in a dark room illuminated by red lighting. On the test day, pairs of rats were placed in the open field box for 15 min, and the multi-unit activities in the IL or PL were recorded in the freely behaving rats using a wireless recording system. The activities were amplified, filtered and continuously sampled, and the behaviors were also monitored with a video camera fixed above the box. The neuronal and behavioral data were continuously acquired over the experimental periods and stored for further analyses. The rats showed exploratory behavior and then engaged in social interaction that included a variety of behaviors (approach, aggressive grooming, submissive posture, moving away, chasing, fleeing). PL neurons showed increase in firing when the rats engaged in approaching the partner and aggressive grooming. In contrast, IL neurons showed increase in firing when the rats saw moving away of the partner and showed decrease in firing when the rats engaged in submissive posture. The PL may be related to active interaction whereas the IL may be related to relief from aversion caused by the presence of a novel partner.

Disclosures: **C. Minami:** A. Employment/Salary (full or part-time):; naya clinic. **A. Mitani:** A. Employment/Salary (full or part-time):; kyoto university. **T. Shimizu:** A. Employment/Salary (full or part-time):; Kyoto university. **S. Momma:** A. Employment/Salary (full or part-time):; Kyoto university. **T. Mikami:** A. Employment/Salary (full or part-time):; Biotex Research Laboratory.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 849.07/UU52

Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Altering the development and expression of play behaviour in rats

Authors: ***S. M. HIMMLER**, J. M. LEWIS, S. M. PELLIS;
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Abstract: During play fighting, rats attack and defend the nape of the neck, which if contacted is nuzzled with the snout. A variety of defensive tactics are used to defend against nape contacts, including running away and rotating to supine. While all strains of rats can use all defensive tactics to protect the nape, there are strain-typical preferences for using particular tactics. Two hypotheses were posited to account for this difference: (1) that each strain has strain-specific neural based thresholds for each tactic or (2) that each strain attacks differently which leads to strain differences in defense tactics used. Juvenile Long-Evans (LE) and Sprague-Dawley (SD) males, raised in same strain quads, were tested with unfamiliar same strain partners (experiment 1) and LE and SD raised in mixed LE-SD quads were tested with both familiar (experiment 2) and unfamiliar same-strain and different-strain partners. SD rats tested with unfamiliar SD or LE defended in SD-typical manner, and LE rats defended in LE-typical patterns when tested with unfamiliar SD or LE partners, supporting hypothesis 1. However, when raised with opposite strain partners, the thresholds for defense changed to a pattern intermediate between the two strains, irrespective of the strain of the attacking partner, suggesting it is possible that, during the early juvenile period, play fighting experiences shape strain-typical patterns of use of defensive tactics.

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Poster

849. Social Behavior: Neural Mechanisms

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Program#/Poster#: 849.08/UU53

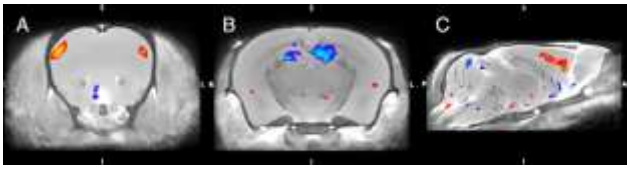
Topic: F.03. Motivation and Emotion

Title: Neuroanatomical correlates of dominant and submissive behaviour in mice

Authors: *J. SCHOLZ[1], E. A. OPALA[2], J. P. LERCH[3];
[1]Mouse Imaging Ctr. (mice), Hosp. For Sick Children, Toronto, ON, Canada; [2]Human Biol., Univ. of Toronto, Toronto, ON, Canada; [3]Mouse Imaging Ctr., Toronto, ON, Canada

Abstract: Objective In mice a range of dominant and submissive behaviours communicate standing in the colony's hierarchy. Here we aim to investigate how dominant and submissive behaviours are related to brain anatomy. **Methods** *Animals:* Male CD-1 mice (N=16) were divided into 4 cages. Dominant behaviours (mounting, thrusting, attacking, tail rattling) and submissive behaviours (fleeing, freezing) were observed and recorded. *Perfusion and MRI imaging:* Mouse brains were perfusion fixated and scanned using MR imaging at 56 μm isotropic

resolution. *Statistical analysis:* Counts of dom./subm. behaviours were modelled using Bayesian zero-inflated Poisson regression. Briefly, the model assumes that counts y were Poisson distributed, y ~ Pois(l). Zero inflation was modelled using a Bernoulli distribution Bern(t). Both the lambda parameter l and the zero-event bias t were derived from a linear model consisting of intercept (subm.) and slope (dom.-subm.). Model parameters were correlated with local volumes estimated from MR images. **Results** Parameters stemming from the Poisson process, i.e. the propensity of the individual mouse to engage in dominant or submissive behaviour (once it is active) showed diffuse correlations across the brain. However, parameters stemming from the zero-inflation part of the model showed a clear dissociation between how brain volume might be related to subm./dom. behaviours. These parameters could be interpreted as being a measure of having active periods of a certain behaviour. At a liberal threshold ($p<0.05$, uncorrected) we found that a decrease in submissive behavioural periods is related to larger volumes in the frontal cortex (Fig 1, A). A smaller hippocampus is related to a bias toward submissive behavioural periods (B). A larger cingulate cortex is related to a decrease in dominant behavioural periods (C). **Conclusions** We have presented first evidence that brain anatomy might be related to social behaviours. This raises the question if these brain structures and thus behaviour could be genetically determined or the results of social learning and experience.



Disclosures: **J. Scholz:** None. **E.A. Opala:** None. **J.P. Lerch:** None.

Poster

849. Social Behavior: Neural Mechanisms

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Klingenstein Foundation

Title: Neural encoding dynamics in the amygdala during observational fear learning

Authors: *S. A. ALLSOP[1,2], A. C. FELIX-ORTIZ[1], R. THOMAS[1], E. H. NIEH[1], K. M. TYE[1]; [1]Dept. of Brain Cognitive Sci., M.I.T., Cambridge, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: The ability to engage in appropriate social interaction is a critical component of daily life that requires integration of multiple neural processes and can be perturbed in numerous psychiatric diseases. One approach to begin understanding how the brain supports a complex array of social behaviors is to study fundamental, evolutionarily conserved social behaviors. Observational learning is one such social behavior that offers a distinct advantage for survival and is thus highly conserved across various species including rodents, monkeys, and humans. Observational learning has been studied under experimental conditions in various animal species. In studies that have looked at observational learning behavior, the basolateral amygdala (BLA) and the anterior cingulate cortex (ACC) have been repeatedly implicated. In mice, observational fear learning was ablated by injection of lidocaine into the BLA or ACC while in humans it was shown that the amygdala and ACC were recruited when subjects acquired a fear to a conditioned stimulus through observation with no direct experience of the aversive event. These studies provide evidence that the amygdala is necessary for observational fear learning. However, the temporal dynamics of neural activity in these regions during observational learning and the manner in which they support observational learning remains unknown. To elucidate the coding dynamics of the amygdala during observational learning, we designed an experiment where observer mice learn to freeze during a cue that predicts delivery of shock to a demonstrator. We performed single unit recordings in the amygdala of the observer and show that neurons in the amygdala respond to a variety of task elements, including delivery of shock to the demonstrator. Lastly, we demonstrate that neurons in the amygdala of the observer show conditioned responses to the predictive cue during learning. This data provides evidence that the amygdala responds acutely to the distress of a conspecific and that conditioned responses to the predictive cue may be a neural correlate of observational learning.

Disclosures: S.A. Allsop: None. **A.C. Felix-Ortiz:** None. **R. Thomas:** None. **E.H. Nieh:** None. **K.M. Tye:** None.

Poster

849. Social Behavior: Neural Mechanisms

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Simons Center for the Social Brain

JPB Foundation

Title: Effects of non-social stressors on the modulation of social behavior by photostimulation of dopamine neurons

Authors: *R. WICHMANN[1], J. P. H. VERHAREN[1,2], S. SRIDHARMA[1], C. P. WILDES[1], K. M. TYE[1];
[1]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [2]Master's program Neuroscience, Ctr. for Neurogenomics and Cognitive Res., VU Univ. of Amsterdam, Amsterdam, MA

Abstract: Impaired social interest and social interaction are observed in many major psychiatric disorders such as autism, schizophrenia, social anxiety disorder or depression. However, little is known about the neurobiological underpinnings regulating social behavior and social deficits associated with these disorders. Dopamine (DA) is an important neuromodulator that exerts an essential role in the regulation of social behaviors. Disruptions in dopaminergic neurotransmission can have profound effects on mood and behavior and as such are known to be implicated in various neuropsychiatric behavioral disorders, such as autism and depression. It is known that ventral tegmental area (VTA) DA neurons respond differently to different stressors. To investigate how acute non-social stressors modulate the influence of the mesolimbic dopamine system on same-sex social interaction behavior we used different time points of a non-social stressor (forced swim) combined with optogenetic activation of VTA DA neurons. To selectively target DA neurons, we injected a cre-dependent adeno-associated virus (AAV5-DIO-ChR2-EYFP), into the VTA of Tyrosine Hydroxylase (TH)::Cre mice. Temporally precise

phasic stimulation of these ChR2-expressing VTA DA neurons induced a significant increase in time spent in social interaction during a juvenile-intruder and a 3-Chamber sociability paradigm. However, after a stress regimen at different time points animals showed divergent effects to the same phasic stimulation of VTA DA neurons. Whereas stress exposure during adulthood seemed to invert the effect of stimulation of dopamine neurons, stress exposure early in life differentially affected social interaction behaviors. Importantly, general locomotion was not altered by the stimulation parameters used. These findings suggest that non-social stressors play an important role in the regulation of social behaviors by the mesolimbic dopamine circuitry.

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Poster

849. Social Behavior: Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

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Title: Social relevance drives viewing behavior independent of low-level salience in rhesus macaques

Authors: *J. A. SOLYST[1,4], E. A. BUFFALO[2,3,5];
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Abstract: Quantifying attention to social stimuli during the viewing of complex social scenes with eye tracking has proven to be a sensitive method in the diagnosis of autism spectrum disorders years before average clinical diagnosis. Rhesus macaques provide an ideal model for understanding the mechanisms underlying social viewing behavior, but to date no comparable behavioral task has been developed for use in monkeys. Using a novel scene-viewing task, we monitored the gaze of three rhesus macaques while they freely viewed over 500 well-controlled

composed social scenes and analyzed the time spent viewing objects and monkeys in the scene. In each of six behavioral sessions, the monkey viewed a set of 90 images (540 unique scenes) with each image presented twice. The image remained on the screen until the monkey accumulated 10s of viewing time for novel images and 6s of viewing time for repeated images. In two-thirds of the repeated scenes, either a monkey or an object was replaced with a novel item (manipulated scenes). Eye movements were recorded using a noninvasive infrared eye-tracking system (ISCAN) and were sampled at 200 Hz. The monkey was not rewarded during the scene presentation, but received rewarded trials on an unrelated task between scene viewing trials. When viewing a repeated scene, monkeys made longer fixations and shorter saccades, shifting from a rapid orienting to global scene contents to a more local analysis of fewer items. This is consistent with previous findings of scene viewing in humans (Smith et al., 2006). In addition to this repetition effect, in manipulated scenes, monkeys demonstrated robust memory by spending more time viewing the replaced items. By analyzing attention to specific scene content, we found that monkeys strongly preferred to view objects of social relevance and that this was not related to their salience in terms of low-level image features. A model-free analysis of viewing statistics found that monkeys who were viewed earlier and longer had direct gaze and red sex skin around their face and rump, two important visual social cues. These data provide a quantification of viewing strategy, memory and social preferences in rhesus macaques viewing complex social scenes, and provide an important baseline with which to compare to the effects of therapeutics aimed at enhancing social cognition.

Disclosures: J.A. Solyst: None. **E.A. Buffalo:** None.

Poster

849. Social Behavior: Neural Mechanisms

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Topic: F.03. Motivation and Emotion

Support: NSERC Grant 40058

Title: Effects of the absence of 50-kHz ultrasonic vocalizations on the playful interactions among unfamiliar juvenile and adult rats

Authors: *S. M. PELLIS, T. M. KISKO, D. R. EUSTON;
Dept Neurosci, Univ. Lethbridge, Lethbridge, AB, Canada

Abstract: We know from previous research that juvenile rats will play similarly whether they are interacting with familiar or unfamiliar partners and that during playful interactions rats emit many 50-kHz ultrasonic vocalizations (USVs). Based on recent findings in pairs of devocalized rats there appears to be a significant reduction in the frequency of play in the absence of 50-kHz USVs and that the same reduction in play can be seen in pairs of vocal rats housed with devocalized rats. The present study used rats that were able to vocalize and paired them with either an unfamiliar vocal or an unfamiliar devocalized rat. The overall frequency of play and relative use of the various types of playful defense strategies were scored to evaluate any differences in the pattern of play. In addition, cortisol levels were examined as a measure of stress. The rats were tested both when juveniles (30-35 days post birth) and again when adults (80-85 days). Given that the absence of USVs depresses play among familiar partners, it was hypothesized that the outcome would be even worse among unfamiliar pairs and that in adulthood, such an absence could lead to aggression. Preliminary data support this hypothesis.

Disclosures: S.M. Pellis: None. **T.M. Kisko:** None. **D.R. Euston:** None.

Poster

849. Social Behavior: Neural Mechanisms

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Topic: F.03. Motivation and Emotion

Support: MH09716

Silvio O. Conte Center (NIH P50MH100023)

Title: Saccadic eye-movements induce an oscillatory phase-reset in the amygdala of monkeys freely viewing videos of macaque social behaviors

Authors: *C. P. MOSHER[1], P. E. ZIMMERMAN[2], C. TANG[2], K. M. GOTHARD[2]; [1]Grad. Interdisciplinary Program in Neurosci., The Univ. of Arizona, TUCSON, AZ; [2]Physiol., The Univ. of Arizona, Tucson, AZ

Abstract: During natural vision, saccadic eye-movements segment the visible world in space and time. From discrete sampling of the visual scene, the brain creates a seamless perception of the visible world. Recent evidence suggests that eye-movements might facilitate visual perception by synchronizing local processing in anatomically distant areas of the brain. Indeed,

during natural viewing, eye-movements reset the phase of ongoing oscillations in the local field potential recorded from primary visual cortex (Ito et al., 2011), temporal association areas (Turesson et al., 2012), the striatum (Courtemanche et al., 2003), and hippocampus (Hoffman et al., 2013; Jutras & Buffalo, 2013). Our data indicate that eye-movements also synchronize neural activity in the amygdala, perhaps to facilitate processing of social and emotional stimuli. During visual exploration of video stimuli, the firing pattern of single neurons in the monkey amygdala are entrained by an ongoing 8-14 Hz oscillation in the local field potential. However, when monkeys saccade to faces or other socio-emotional stimuli, the ongoing 8-14 Hz oscillation undergoes a phase-reset. The reset in oscillatory phase emerges in advance of the saccade but reaches its greatest pair-wise phase consistency 100 ms after achieving fixation on a social stimulus. The consistency of the phase-reset varies along the dorso-ventral axis of the amygdala with the most reliable reset occurring in the centromedial nuclei of the amygdala. Given the role of the centromedial nuclei in modulating attention and emotional arousal, these data suggest that the amygdala might be requisite for allocating attention to social stimuli during natural vision. To test this conjecture, ongoing experiments locally manipulate neural activity within the amygdala while monkeys engage in naturalistic social interactions with video stimuli (see poster by Putnam P.T., et al.).

Disclosures: C.P. Mosher: None. **P.E. Zimmerman:** None. **C. Tang:** None. **K.M. Gothard:** None.

Poster

849. Social Behavior: Neural Mechanisms

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Program#/Poster#: 849.14/UU59

Topic: F.03. Motivation and Emotion

Title: Prosocial behavior in rats depends on the delivery of food to another rat displaying food seeking behavior

Authors: *C. MARQUEZ, S. RENNIE, D. COSTA, M. MOITA;
Champalimaud Neurosci. Programme, Champalimaud Ctr. For the Unknown, Lisbon, Portugal

Abstract: Animals often are prosocial, displaying behaviors that result in a benefit to another even in the absence of self-benefit. Several factors have been proposed to modulate these behaviors, namely familiarity or display of seeking behaviour. Rats have been recently shown to be prosocial, however what drives prosociality in these animals remains unclear. To address this

issue, we developed a two choice task, where prosocial behaviour did not yield a benefit or a cost to the focal. We used a double T-maze (one for the focal and the other for the recipient rat) in which only the focal rat controlled access to the food-baited arms of both mazes. In this task, the focal rat could choose between one side of the maze that yielded food only to itself (selfish choice) or the opposite side that yielded food to itself and a recipient rat (prosocial choice). Rats showed a high proportion of prosocial choices. By manipulating reward delivery to the recipient and its ability to display a preference for the baited arm, we found that both the display of food-seeking behavior and the delivery of rewards were necessary to drive prosocial choices. This study shows that rats provide access to food to others in the absence of direct self-benefit, while bringing new insights into the factors that drive prosociality thus contributing to the study of the neural mechanisms underlying these behaviors.

Disclosures: C. Marquez: None. **S. Rennie:** None. **D. Costa:** None. **M. Moita:** None.

Poster

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Program#/Poster#: 849.15/UU60

Topic: F.03. Motivation and Emotion

Support: NIH Grant MH08864

Title: Nucleus accumbens' modulation of periaqueductal gray inputs mediate isolation-induced vocalizations in the infant rat

Authors: *H. MOORE[1,3], M. O. CHOHAN[1], J. MULLER[2], H. N. SHAIR[1];
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Abstract: Mammalian infants vocalize when socially isolated. Vocalization guides the return of the caregiver and thereby maintains an environment critical to the infant's survival. Although the role of the periaqueductal gray area (PAG) in these vocalizations is established, other aspects of the relevant neural circuitry remain under-studied. Here we report that output from the nucleus accumbens (Acb) is necessary for social isolation-induced vocalizations in infant rats aged postnatal day [P] 12-14. Local inhibition via infusion of the GABAA agonist muscimol (0.8 μg/side) of the Acb, but not the dorsolateral striatum, blocked isolation-induced vocalizations, an effect that persisted when isolation occurred in a cold (10ºC) environment. Candidate

neurocircuitry was examined with anterograde and retrograde tract tracers deposited into the Acb and PAG, respectively in pups at P12-14 A small direct projection from the Acb to the ventrolateral (vl) PAG and adjacent mesopontine reticular formation (MRN) was observed. More notable however was that major terminal fields of the Acb within the ventral pallidum, substantia innominata, lateral bed nucleus of stria terminalis, the lateral hypothalamus, and the ventral tegmental area/substantia nigra pars reticulata overlapped with dense populations of neurons projecting to the vlPAG and MRN. In conclusion, Acb efferents are critical for social isolation-induced vocalizations in the infant rat and may exert this effect via modulation of basal forebrain and midbrain inputs to the vlPAG and MRN. These findings highlight a possible anatomical macrosystem mediating the mammalian infant response to social separation and, more generally, to the development of social attachment.

Disclosures: **H. Moore:** None. **M.O. Chohan:** None. **J. Muller:** None. **H.N. Shair:** None.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 849.16/UU61

Topic: F.03. Motivation and Emotion

Support: NIH Grant MH086947

Title: Impact of ventromedial prefrontal cortex lesions on macaque emotional face processing

Authors: *L. E. MURPHY[1], T. FENG[1], K. GOTHARD[2], J. BACHEVALIER[1];
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Abstract: A fundamental component of social interaction is the ability to recognize conspecifics and evaluate their emotional state. A complex network of regions is involved in the recognition of basic emotions. This network includes the amygdala, the anterior insula, the superior temporal sulcus, and the prefrontal cortex (PFC). Within the PFC, damage to the orbitofrontal cortex (ORB) and ventromedial PFC (vmPFC) in humans negatively affects the ability to identify emotions in facial stimuli (Hornak et al., 2003; Zald & Adreotti, 2010) and damage to vmPFC yields an inability to distinguish between different static exemplars of positive and negative emotional valence (Tsuchilda & Fellows, 2012). Yet, interpretations from brain-damaged human cases have limitations, given that the lesions are not restricted and may include several fields of the orbitofrontal cortex (ORB). To assess the specific ORB field(s) most involved in these

cognitive processes, we prepared adult monkeys with selective lesions of ORB fields 12, 13, or 14, as well as sham-operated controls, and measured their visual exploration patterns toward conspecific face stimuli depicting negative, neutral, or positive content. Here, we report data from three animals with area 14 (ventromedial, vmPFC) lesions and a control animal. During testing, subjects were seated in a primate chair and head movements were minimized using a thermoplastic helmet. Subjects freely watched 10-second videos of unknown macaques displaying stereotyped expressions of neutral, negative (threat) or positive (lip smack) emotional valence. Their eye movements were recorded using a Tobii eye tracker. For each video, the number of fixations made on the monkey stimulus was compared to the number of fixations made on the background. The data showed that monkeys with area 14 lesions spent as much time as the control exploring stimulus monkeys displaying neutral and positive emotional valence, but spent less time than the control exploring stimulus monkeys displaying negative valence [Group X Emotion: $F(2, 84) = 4.859$, $p = 0.01$]. These results demonstrate that damage to ventromedial area 14 weakens attention towards negative emotional stimuli. The findings are consistent with similar changes reported in patients with ORB damage (Zald & Adreotti, 2010) and with perceptual and cognitive processing biases towards negatively valenced information associated with altered vmPFC activation in individuals with depression (Laxton et al., 2013). The data suggest that the vmPFC plays a critical role in processing unpleasant or negatively valenced information.

Disclosures: **L.E. Murphy:** None. **T. Feng:** None. **K. Gothard:** None. **J. Bachevalier:** None.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 849.17/UU62

Topic: F.03. Motivation and Emotion

Support: NIMH R37 MH065334 (Jan)

Randolph College Internal Grants

Title: Assessing emotional prosody in mouse socio-sexual induced ultrasonic vocalizations

Authors: ***K. SCHENK**[1], H. Y. LEE[2], S. GRISSOM[1], G. SOUTHWICK[1], M. GIBSON[1], L. Y. JAN[2,3], Y. N. JAN[2,3], Y. J. KWON[1];

[1]Physics and Astronomy, Randolph Col., Lynchburg, VA; [2]Physiol., Univ. of California, San Francisco, San Francisco, CA; [3]Howard Hughes Med. Inst., San Francisco, CA

Abstract: "Many neuropsychiatric diseases are associated with communication and/or social deficits. Mouse Ultrasonic Vocalizations have been used by many investigators as an assay for these deficits in mouse models of disease. However, all of these assays suffer from the same fundamental ambiguity, the lack of a connection between mouse ultrasonic calls and behavioral and emotional significance. For instance, in our 2010 PNAS paper [Young et al, 2010] we analyzed the ultrasonic vocalizations (USVs) of mice with a genetic mutation that causes Tuberous Sclerosis, a disease highly associated with autism. Our assay consisted of quantifying several spectral and temporal quantities of the calls as well as classifying the calls into several categories according to their visual morphology on a spectrogram. Although we found differences in several parameters including call type between the mutant and wild-type mice, these differences may not have represented real differences in social or communication abilities. For example, if mutant mice are found to make more of a certain type of call than wild-type mice it may be because these overrepresented calls signify a different emotional state than other calls. If this were true, then this difference should be taken into consideration in our attempts to look for high level social or communication processing or production deficits. To gain a clearer understanding of any differences one finds between mouse models of disease and their wild-type counterparts one must understand the emotional context and behavioral meaning of mouse USVs. We feel that progress can be made on this issue if one seeks the answers to three questions: 1) What is the mouse doing when he is emits a call with a certain property? (Behavioral Context Assessment, BCA) 2) What is the emotional content of a call? (Emotional Prosody Assessment, EPA) 3) What can one learn from the interaction between behavioral context and emotional prosody, (BCA X EPA)? Valid and robust methods of answering these three questions would be highly relevant to assays for communication and social deficits. Here we present progress on the development of these assays using a synced video/USV record of male FVB mice interacting with female mice. Young, D., Schenk, A., Yang, S.-B., Jan, Y. & Jan, L. Altered ultrasonic vocalizations in a tuberous sclerosis mouse model of autism. Proceedings of the National Academy of Sciences of the United States of America 107, 11074-9 (2010)."

Disclosures: K. Schenk: None. **H.Y. Lee:** None. **S. Grissom:** None. **G. Southwick:** None. **M. Gibson:** None. **L.Y. Jan:** None. **Y.N. Jan:** None. **Y.J. Kwon:** None.

Poster

849. Social Behavior: Neural Mechanisms

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 849.18/UU63

Topic: F.03. Motivation and Emotion

Support: BLAN-SVSE4-023-01

Title: Behavioural correlates of socio-emotional states in macaques

Authors: *S. BALLESTA, M. POZZOBON, G. REYMOND, J.-R. DUHAMEL;
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Abstract: Primates live in highly social environments, where altruism is believed to contribute to group fitness. To test the ability of macaques to form representations of others' emotions, several pairs were involved in social or non-social decision tasks, using positive (juice) or negative (airpuff) outcomes to the actor, the partner or nobody. Results show that, according to the identity of their partner, monkeys may behave benevolently, indifferently, or malevolently, as defined by their rate of prosocial decisions. Anticipatory eye blinks and mutual gazes correlate with these social decision profiles, and thus represent efficient proxies of emotional responsiveness. Moreover, correlations of decisions across sessions reveal a social motivation independent of the outcome valence, and also show that altruistic tendency is based on personal experience. We demonstrate that macaques are able to take into account the welfare of their peers while making social decisions, and propose cognitive and emotional mechanisms motivating altruism in primates.

Disclosures: S. Ballesta: None. **M. Pozzobon:** None. **G. Reymond:** None. **J. Duhamel:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

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Program#/Poster#: 850.01/UU64

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant R01HD31476

NIH Grant T32HL105355

Title: Evaluation of intramuscular interstitial fluid flow using fluorescent microspheres

Authors: *L. Q. EVERTZ, S. M. GREISING, D. A. MORROW, G. C. SIECK, K. R. KAUFMAN;
Mayo Clin., Rochester, MN

Abstract: The force within a single muscle may be assessed using intramuscular pressure measurements (IMP). However, the limiting factor to implementing this technology in the clinic is the variability of IMP measurements that has been observed. Factors such as sensor location, muscle geometry and architecture, as well as anatomical boundary conditions have been shown to affect IMP measurements. These pressure variations may be due to the flow of interstitial fluid. The purpose of this study was to develop a technique for tracking interstitial fluid flow during muscle lengthening using fluorescent microspheres and evaluate the effect of bead diameter on dispersion. Fluorescent microspheres were injected into the rat tibialis anterior (TA) either before (right limb) or after (left limb) lengthening to 10% strain (n = 5). Two microsphere diameters, 0.1 and 0.2 µm, were injected into individual muscles post-dissection. To help discriminate the effects of bead size, two colors of beads were injected simultaneously, 0.1 µm in sky blue and 0.2 µm in yellow green. Once frozen, 20 µm muscle cross-sections were stained and imaged using confocal microscopy to evaluate the microsphere's axial dispersion. The microsphere dispersion was normalized to the muscle length. The fluorescent microspheres were concentrated at the injection site (Fig. 1a) and flowed with the interstitial fluid appearing to not penetrate the muscle fiber, (Fig. 1b). Bead size did not appear to affect the dispersion during lengthening (Fig. 1c). The beads were dispersed axially 19% (0.1 µm beads) and 29% (0.2 µm beads) for muscles lengthened pre-injection and 44% (0.1 µm beads) and 45% (0.2 µm beads) for muscles lengthened post-injection. This study demonstrated that fluorescent microspheres may be used to evaluate intramuscular interstitial fluid flow. The beads remain in the interstitial space without penetrating the muscle fiber. The expanded dispersion of beads in the post-injection lengthened specimens indicates interstitial fluid flow occurs during muscle lengthening. Bead size does not affect dispersion with lengthening.

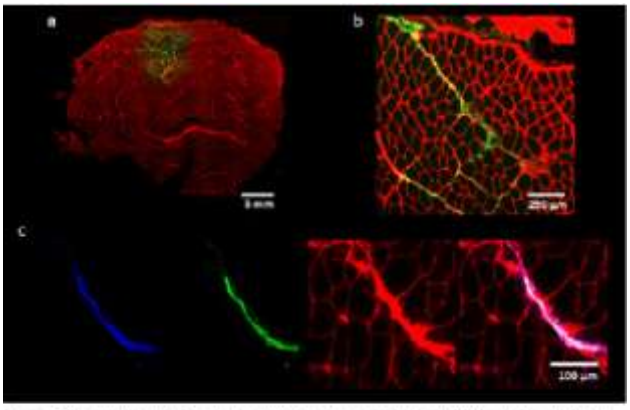

Figure 1: The muscle cross-section at the injection site. (a), illustrates the microspheres (green) superficial injection site and the resulting radial spread throughout the extracellular space (red). A cross-section taken about 5 mm away from the injection site. (b), illustrates that the microspheres travel within the interstitial fluid axially down the length of the muscle. Microspheres do not appear to penetrate the muscle fiber. (c) A difference in dispersion was not seen for the two sizes, 0.1 µm (blue) and 0.2 µm (green).

Disclosures: **L.Q. Evertz:** None. **S.M. Greising:** None. **D.A. Morrow:** None. **G.C. Sieck:** None. **K.R. Kaufman:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.02/UU65

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Tohoku Univ. IIARE Research Grant

Title: Deleting G protein of rabies virus vector enhances transgene expression level via decreased cell toxicity and increased RNA polymerase expression

Authors: ***S. SATO**, S. OHARA, K.-I. TSUTSUI, T. IIJIMA;
Div. of systems neuroscience, Tohoku Univ., Miyagiken-Sendaishi, Japan

Abstract: The rabies virus (RV) selectively infects neurons from axon terminals and spreads retrogradely to presynaptic neurons. The glycoprotein-deleted rabies vector (ΔG-RV) variants are an especially useful tool for investigating the detailed morphology of infected neurons. Recently, we demonstrated that deleting the G gene of the RV vector increases the transgene expression level and decreases cytotoxicity (Ohara et al., PLOS ONE, 2013) by comparing two rabies virus vectors, rHEP-5.0-CVSG-mRFP and rHEP-5.0ΔG-mRFP. The rHEP-5.0-CVSG-mRFP was derived from an avirulent HEP-Flury strain. The G gene was substituted with that of the CVS strain, and a monomeric red fluorescent protein (mRFP) was inserted into its genome. The rHEP-5.0ΔG-mRFP was created by deleting the entire G gene of rHEP-5.0-CVSG-mRFP. The mRFP expression level of rHEP5.0-ΔG-mRFP was much higher than that of rHEP5.0-CVSG-mRFP *in vitro* and *in vivo*. There are two possible causes of the increase of transgene expression level caused by deleting the G gene. The first one is the cytotoxicity of the G protein. We also demonstrated that deleting the G gene decreases the cytotoxicity of the RV vector. The cytotoxicity of rHEP5.0-CVSG-mRFP may cause some dysfunction in the transcription and replication system of the infected cells. The second possible cause is the differences in the expression level of the L gene, which encodes the viral polymerase. The expression levels of the rabies viral genes decrease monotonically as the distance increases from the start (3' end) of the genome. The L gene is originally located at the end of the genome; hence, deleting the G gene decreases the distance between the start of the genome and the L gene and may increase the transgene expression level. To verify these two possibilities, we created a third RV vector

variant. To evaluate the effect of the cytotoxicity of the G protein, the CVS-G of rHEP5.0-CVSG-mRFP was replaced with two nontoxic blue fluorescent proteins and a self-cleaving 2A peptide that has approximately the same length as that of CVS-G (rHEP5.0-ΔG-mRFP-BFP-P2A-BFP). The mRFP expression level of rHEP5.0-ΔG-mRFP-BFP-P2A-BFP was lower than that of rHEP5.0-ΔG-mRFP and higher than that of rHEP5.0-CVSG-mRFP. This result suggests that deleting the G protein of the rabies virus vector enhances transgene expression via both decreased cell toxicity and increased RNA polymerase expression.

Disclosures: **S. Sato:** None. **S. Ohara:** None. **K. Tsutsui:** None. **T. Iijima:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant NS033506

NIH Grant NS060699

NIH Grant P40RR018604

Title: A set of herpes simplex virus type 1 recombinants for use in neural circuit tracing

Authors: ***J. N. BROOKS**[1], E. A. ENGEL[2], L. W. ENQUIST[2];
[1]IDA - Sci. and Technol. Policy Inst., WASHINGTON, DC; [2]Mol. Biol., Princeton Univ., Princeton, NJ

Abstract: Two powerful tools for the visualization of neurons and neural circuits have recently been developed: modified α Herpesviruses, and the Brainbow genetic cassette. The neuroinvasive property of the α Herpesvirus subfamily has permitted their use as cellular markers, since infected neurons can be distinguished by the expression of genes carried by the viral genome. Unlike labelling dyes, these viruses are self-amplifying, spread only between synaptically connected neurons, and can be used to selectively label a genetically-defined population of cells. In addition, the identification of unidirectional α Herpesvirus strains, which are defective for either anterograde or retrograde transport from the soma, offer neuroscientists the ability to answer direction-specific questions. The Herpes Simplex Virus type 1 (HSV-1) strain H129 has been observed to move almost exclusively in the anterograde-only direction,

while the HSV-1 strain MacIntyre moves only in the retrograde direction from the site of infection. The second tool is the Brainbow 1.0L genetic cassette; in transgenic animals, Brainbow-expressing cells express the red fluorescent protein tdTomato unless the cassette undergoes recombination to express either Cyan or Yellow fluorescent protein. This recombination occurs only in cells that are expressing the recombinase Cre, thereby selectively labelling Cre-expressing neurons. In genetic environments containing multiple copies of the Brainbow cassette, the diversity of color is sufficient for the visual distinction of individual cells within the target population. Recently, virologists have modified α Herpesvirus strains to express Brainbow. The construction of new Brainbow- or Cre-encoding recombinants of unidirectional HSV-1 strains is presented. These viral recombinants introduce visualization capabilities which address weaknesses in each of the above tools: 1. Infection with strains carrying only one labeling protein do not permit visual distinction between individual neurons in a circuit, particularly at the synaptic level; 2. As informational circuits are not genetically defined, transgenic Brainbow animals cannot be used to explore neural circuits.

Disclosures: **J.N. Brooks:** None. **E.A. Engel:** None. **L.W. Enquist:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

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Program#/Poster#: 850.04/UU67

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIA Grant AG025894

NINDS Grant NS045855

NINDS Grant NS057558

NINDS Grant NS086960

Title: Small numbers of neocortical neurons can be labeled with unique hues by helper virus-free HSV-1 vectors expressing Brainbow

Authors: *A. I. GELLER[1], G.-R. ZHANG[2], H. ZHAO[2], H. CAO[3], X. LI[2];
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Abstract: A critical problem in neuroscience is elucidating the connectome. As brains contain many neurons, labeling specific neurons with unique tags is desirable. Recently, a novel technology, Brainbow, was developed that labels neurons with hundreds of hues by combinatorial expression of multiple fluorescent proteins (FPs). But, in Brainbow mice, multiple neurons contain the same hue, as labeled neurons exceed the number of hues. We have labeled small numbers of neurons, and their axons, with unique hues, by expressing Brainbow from a HSV-1 vector. The vector that uses a glutamatergic-specific promoter, the vesicular glutamate transporter-1 promoter, to express a Brainbow cassette that contains four FPs. We used current monomeric FPs with readily separable spectra, and favorable protein stabilities and quantum properties, LSSmKate2 (red), mOrange2, emerald green FP (EmGFP), and enhanced blue FP-2. To target FPs to axons, the GAP-43 axon-targeting domain was fused to each FP. Brainbow recombination, and production of arrays of Brainbow cassettes, was achieved during vector packaging. We used standard helper-virus free HSV-1 vector packaging, and addied a plasmid that expresses Cre. Packaging produced a Brainbow array: Rolling circle DNA replication produces concatamers, and for HSV-1 vectors, a HSV-1 genome-sized array is packaged. HSV-1 is ~152 kb, and pVGLUT1brainbow is ~20 kb; an array of 7 or 8 Brainbow is packaged into each vector particle. A PCR assay showed that after packaging with Cre, each FP was in a position to be expressed from the VGLUT1 promoter, in different Brainbow cassettes. pVGLUT1brainbow labeled small number of neurons and axons with different, often unique, hues. pVGLUT1brainbow was injected into postrhinal (POR) cortex, and the rats were sacrificed 8 days later. POR cortex contained neurons labeled with different hues representing multiple FPs, including blue, green, yellow, and pink. We observed 100 to 200 hues, similar to Brainbow mice. Further, a POR cortex projection area, perirhinal (PER) cortex, contained axons with different hues. Specific axons in PER cortex were matched to specific cell bodies in POR cortex, based on hue. We drew a contour around a cell body or axon, and a macro calculated the average density in each channel. Specific cell bodies had unique hue profiles. Using these profiles, we matched 17 axons in PER cortex to specific cell bodies in POR cortex. Attractive properties of HSV-Brainbow include that single vector particles contain a sufficient number of Brainbow cassettes to represent a large number of hues, the recombination products are stabile, and experimental control of the number of labeled neurons.

Disclosures: **A.I. Geller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity in Alkermes Inc.. **G. Zhang:** None. **H. Zhao:** None. **H. Cao:** None. **X. Li:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.05/UU68

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Integral neuronal morphologies across the whole brain by fluorescence tomography

Authors: *S. ZENG[1,2], H. XIONG[1], F. XU[3,2], Q. LUO[1], H. GONG[1];
[1]Huazhong Univ. of Sci. & Technol., HB, China; [2]Britton Chance Ctr. for Biomed. Photonics,, Wuhan Natl. Lab. for Optoelectronics,, Wuhan, China; [3]Wuhan Inst. of Physics and Mathematics, Wuhan, China

Abstract: Resolving neuron morphology is always one of the fundamental themes in neuroscience. A hundred years ago, the hand-drawn artworks of Golgi stained neural processes has inspired Ramo´ n y Caja to propose those most fundamental laws lay behind the nervous system. During these past years, new results in exploring the neuron morphology continue to catalyze new breakthroughs in neuroscience. For example, Digital tracing of axons and dendrites shows projections, and helps to reveal functional connectivity or network organizations The 3D reconstruction of neurons leads to new neuron type discoveries, and neural harbors reconstruction provides the foundation for neural activity modeling. And the comparison of morphological characteristics has routinely been used to understand the gene function, diseases and neurodevelopment processes. Furthermore, the accumulation of the neuron morphology information directly supports large scale neuron network modeling and computation, which sheds light on the dream of understanding and utilizing the brain0. However, reconstruction the integral morphology of neurons in mammalian's brain is still a big challenge, as morphological characters of a single neuron may across 5 dimension orders: with pre- and post-synaptic structures locate at submicron size, but long neural fibers span centimeter range. As a result, there is always a tradeoffs between the reconstruction resolution and the field of view. Previous neuromorphological reconstructions based on election microscopy and super-resolution microscopy have revealed details of hundred-micron regions Although high resolution automatic laser scanning light microscope had been developed to enable whole brain neural tracing, successful imaging of the integral neurons with sufficient neural processes has never been reported. As a result, the inaccessible to analysis integral neuron morphology keeps impeding the neuroscience society. In this presentation, We show that by developing a new high throughput optical-imaging method based on chemical reactivation and sparse labeling, we finally enable routinely acquiring the integral morphology of GFP-labeled neurons at 0.3×0.3×0.2 μm3 3D resolution. The integral morphology enables comprehensive single cell projection revealing, connective statistics of projected regions. With the help of pre-/post- synaptic labeling, synapse is visible with this optical resolution with the help of 200nm sectioning-thickness. And since it is a GFP-based approach, the morphology of function-specified neurons has been acquired by applying those well-developed genetic and viral labeling techniques.

Disclosures: **S. Zeng:** None. **H. Xiong:** None. **F. Xu:** None. **Q. Luo:** None. **H. Gong:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.06/UU69

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: A simple and reliable technique for chronic non-invasive optical imaging of cortical activity in mice

Authors: ***M. TEICHERT**[1], A. DÖDING[2], K. LEHMANN[2], J. BOLZ[2];
[1]Inst. Für Allgemeine Zoologie Und Tierphysiologie, Jena, Germany; [2]Allgemeine Zoologie und Tierphysiologie, Jena, Germany

Abstract: An important goal of neuroscience is to characterize brain responses evoked by external stimuli. One standard tool allowing such investigations is optical imaging of intrinsic signals. It enables observations of neuronal activity over large cortical areas with a high spatial resolution (Grinvald et al., 1986; Kalatsky and Stryker, 2003). To follow cortical maps over time in individual animals, chronic imaging becomes more and more important. In previous studies, repeated invasive surgeries were required for chronic imaging in mice, including trepanations, and resectioning and re-suturing of the scalp (Hofer et al., 2006; Kaneko et al., 2008). We present here an improved technique which consists of fixing a permanent window (PW) on an animal's head allowing chronic imaging directly through the skull. After an initial surgery for the fixation of the PW, all following imaging sessions are completely non-invasive. During surgery a thin layer of 0.8 % agarose containing 1 % penicillin/streptomycin was applied to the skull over the region of interest and covered with a standard microscope cover slip. Subsequently, this assembly was fixed with cyanoacrylate and dental acrylic. We then used optical imaging of intrinsic signals to map the representations of the two eyes in the binocular visual cortex of adult mice. In order to test the reliability of this novel method, recordings were performed every other day for a period of up to two weeks, starting one day after the surgery. Our results show that the PW and the skull remain optically clear for the time tested. Imaging through the PW provides robust and stable data of neuronal activity in the visual cortex, such as response magnitude, retinotopy and ocular dominance index. An acceptable side effect of our technique is that the time required for data acquisition is reduced by half compared to conventional methods. In conclusion, the method presented here allows quasi non-invasive chronic imaging e.g. to study

mechanisms underlying the plasticity of cortical circuits. In addition, this approach might also provide new insights into alterations of cortical responses at different time points after drug administration in individual animals.

Disclosures: **M. Teichert:** None. **A. Döding:** None. **K. Lehmann:** None. **J. Bolz:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.07/UU70

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Grants-in-Aid for Japan Advanced Molecular Imaging Program

JST Grant, CREST to M.H. and H.I

Title: In-vivo imaging of neuronal differentiation and function of intracranially implanted induced pluripotent stem cells (ipscs) using a designer receptor exclusively activated by a designer drug (dreadd)

Authors: ***B. JI**[1], H. KANEKO[1], H. INOUE[2], H. TAKEUCHI[2], K. KUMATA[1], M.-R. ZHANG[1], I. AOKI[1], C. SEKI[1], M. ONO[1], M. TOKUNAGA[1], S. TSUKAMOTO[1], K. TANABE[2], K. TAKAHASHI[2], R.-M. SHIN[1], T. MINAMIHISAMATSU[1], T. SUHARA[1], M. HIGUCHI[1];
[1]Natl. Instit Radiolog, Chiba, Japan; [2]Kyoto Univ., Kyoto, Japan

Abstract: Induced pluripotent stem cells (iPSCs) provide a promising resource for cell replacement therapy in neurological diseases. In the present study, we have applied a designer receptor exclusively activated by a designer drug (DREADD) derived from human M4 muscarinic acetylcholine receptor (hM4D) and its synthetic ligand to in-vivo visualization of neuronal differentiation and function of iPSC-derived grafts implanted into the brain. We successfully captured expression of hM4D driven by neuron-specific Thy-1 promoter in newly-developed hM4D transgenic (hM4D Tg) mice with a 11C-labeled positron emission tomography (PET) ligand for hM4D. We also established iPSCs from a hM4D Tg mouse (hM4D-iPSC), and visualized time course of neuronal differentiation of grafts generated from these iPSCs in the living wild-type mouse brain by longitudinal PET imaging of hM4D with its specific radioligand. Quantitative assessment for cerebral blood flow using arterial spin labeling (ASL)

MRI indicated suppression of neuronal activity by clozapine-N-oxide (CNO), an exclusive activator of hM4D, in hM4D Tg but not wild-type mice, in consistency with attenuation of locomotion behaviors. Furthermore, we found CNO-induced reduction of cerebral blood flow in areas associated with implantation of hM4D-iPSC-derived grafts by ASL-MRI of recipient mice. Our results support the utility of hM4D in combination with PET and ASL-MRI for in-vivo longitudinal monitoring of neuronal differentiation and functional manipulation of iPSC-derived implants in the brain. Since this technology is potentially applicable to humans, it would accelerate translational research and development of cell replacement therapy towards clinical trials.

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Poster

850. Imaging Advances: In Vivo, Animals

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Program#/Poster#: 850.08/UU71

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Imaging deep-layer neuronal activity in mouse barrel cortex using 1040-nm excitation of a red fluorescent-protein calcium indicator

Authors: ***S. CARTA**[1], J. L. CHEN[1], F. F. VOIGT[1], B. SCHNEIDER[2], M. OHKURA[3], J. NAKAI[3], F. HELMCHEN[1];
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Abstract: Understanding the function of deep, infragranular layers of neocortex is essential because they communicate with subcortical and other cortical brain regions. Two-photon calcium imaging in infragranular layers 5 and 6 of living mouse neocortex has remained challenging, however, largely due to scattering loss of excitation light but also because of a lack of genetically-encoded calcium indicators suitable for fluorescence excitation at wavelengths above 1000 nm. Here, we demonstrate *in vivo* calcium imaging of cortical neurons in layers 5 to 6 using the red fluorescent indicator R-CaMP1.07 (Ohkura et al., PLoS One, 2012). R-CaMP1.07 expression was induced by viral infection with an adeno-associated viral (AAV)

vector. We excited R-CaMP1.07 fluorescence at 1040-nm wavelength with a compact, high-power (>2 W), low-cost femtosecond laser and imaged neurons in mouse barrel cortex using a standard two-photon microscope equipped with a 16x objective (Nikon). We readily could measure neuronal calcium transients in layer 5 at 550-700 micron depths and at depths greater than 800 micron, corresponding to layer 6. We imaged deep-layer neuronal population activity both in anesthetized mice as well as in awake, head-fixed mice during voluntary whisking. Using simultaneous juxtacellular recordings we are currently verifying the *in vivo* sensitivity of R-CaMP1.07 for reporting action potentials. The combination of new lasers operating above 1000 nm with red fluorescent proteins like R-CaMP1.07 offers new opportunities and an affordable approach to study the functional role of deep neocortical layers 5 and 6 for information processing in the mouse brain, especially in awake animals during specific behaviors.

Disclosures: S. Carta: None. **J.L. Chen:** None. **F.F. Voigt:** None. **B. Schneider:** None. **F. Helmchen:** None. **M. Ohkura:** None. **J. Nakai:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.09/UU72

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: HHMI

Title: Deep brain Ca2+ imaging of AGRP and POMC neuronal dynamics in the arcuate nucleus of freely moving mice

Authors: *S. XU, A.-K. EISELT, C. MAGNUS, S. STERNSON;
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Abstract: Agouti-related protein (AGRP)-expressing neurons and proopiomelanocortin (POMC)-expressing neurons in the hypothalamic arcuate nucleus (ARC) are two intermingled populations that positively and negatively regulate food intake, respectively. However, because of their depth and overlapping spatial distribution, AGRP and POMC neuronal activity dynamics have not yet been directly examined *in vivo* in freely moving and behaving mice. Here, we show the neuronal activity of AGRP and POMC neurons in mice in response to food intake and hormones by Ca^{2+} imaging. To image Ca^{2+} dynamics, we used Cre-dependent viral vectors and cell type specific Cre-recombinase expressing mouse lines to express the ultrasensitive Ca^{2+}-

indicator (GCaMP6) and implanted a long thin gradient refractive index lens into ARC. Time-lapse Ca^{2+} imaging was performed with an Inscopix miniature endoscope. This preparation enables us to investigate the neuronal dynamics of these two cell types with high resolution during energy deficit, in response to hormones, and during feeding behavior. The *in vivo* dynamics of AGRP and POMC neurons provide further understanding of how the brain encodes and decodes energy homeostasis.

Disclosures: **S. Xu:** None. **A. Eiselt:** None. **C. Magnus:** None. **S. Sternson:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

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Program#/Poster#: 850.10/UU73

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF 0954796

MURI W911NF-12-1-0594

R01 NS063226 (NINDS)

R01 NS076628 (NINDS)

Human Frontier Science Program (HFSP)

Title: Signal-based adaptive optics two-photon microscopy of in-vivo mouse brain

Authors: ***P. GALWADUGE**, S. H. KIM, L. E. GROSBERG, E. M. C. HILLMAN; Biomed. Engin., Columbia Univ., New York, NY

Abstract: Two-photon microscopy is widely used in neuroscience for in-vivo imaging due to its superior optical sectioning ability and depth penetration compared to confocal microscopy. While it is typically possible to image mouse brain down to depths exceeding 500 microns, the imaging depth, signal to noise ratio, contrast and resolution is limited by aberrations, scattering and photodamage. When performing adaptive optics, the aberrations and scattering induced by the sample are corrected by modulating the wavefront of the excitation light. The traditional approach to performing adaptive optics involves the use of direct wavefront sensing, yet this is impractical for in-vivo imaging. The other main approach is to acquire successive images of a

guide-star, a bright object embedded within the medium. Parameters of each image, such as its peak intensity or full width half maximum are assessed as a metric of image performance while iteratively improving wavefront corrections are applied. In-vivo, this technique has the disadvantage of needing an embedded guide-star, and can also be time-consuming and cause photodamage to the living brain. A method that permits very rapid calculation of wavefront corrections in the living brain would make performing adaptive optics corrections for in-vivo two-photon microscopy more accessible for routine use. Here we present a simple correction method that does not require sequential imaging of an object within the brain, permitting rapid optimization with minimal photodamage. The technique is based on measuring just the intensity of the two-photon intensity generated by a discrete fluorescent object. The basis of this approach is that the two photon emission signal varies as the square of the peak excitation intensity. This means that the detected signal is inherently sensitive to optical power, beam size and pulse width at the focal plane. This means that two-photon signal intensity alone can be used as the metric for image improvement during successive estimation of the optimized wavefront. This scheme is fast, does not require embedded guidestars and is implemented on a relatively cost effective liquid crystal modulator. We will present the results of in-vivo experiments using this signal based adaptive optics technique.

Disclosures: **P. Galwaduge:** None. **S.H. Kim:** None. **L.E. Grosberg:** None. **E.M.C. Hillman:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.11/UU74

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: DARPA

Title: Visualizing mammalian brain area interactions by dual-axis two-photon calcium-imaging

Authors: ***J. LECOQ**, J. SAVALL, D. VUCINIC, B. GREWE, J. LI, L. KITCH, M. J. SCHNITZER;
Biol., Stanford, Stanford, CA

Abstract: Calcium-imaging has become a vital tool for monitoring the dynamics of large numbers of neurons in behaving animals. However, a conventional microscope is usually limited

to fields of view <600 µm wide and individual regions of the mammalian brain. This approach has been fruitful for studies of local microcircuitry, but neuroscientists have generally lacked technology that can image interactions across pairs of mammalian brain areas in a way that simultaneously provides cellular resolution within each area. Creating such technology will be crucial for studying how inter-area network interactions shape behavior and cognition, and to understanding how cells in distinct regions coordinate their dynamics. Here we initiate imaging studies of multi-area interactions at cellular resolution by introducing a two-photon microscope with two movable imaging arms, for simultaneous imaging of two brain areas, nearby or distal, in head-restrained behaving mice. Each arm has a custom microendoscope providing satisfactory resolution and collection efficiency for neural calcium imaging, and sufficient mechanical freedom to concurrently image nearly any two brain areas in the mouse that can be accessed individually by microendoscopy. As illustration, concurrent calcium-imaging of ~100-300 neurons in each of visual areas V1 and LM in behaving mice revealed that the variability in LM neurons' visual responses was strongly dependent on that in V1, suggesting that fluctuations in sensory responses propagate through extended cortical networks. This observation regarding interactions between two visual areas is but an initial example of how dual-axis microscopy will help neuroscientists probe local and global information processing in behaving animals.

Disclosures: **J. Lecoq:** None. **J. Savall:** None. **D. Vucinic:** None. **B. Grewe:** None. **J. Li:** None. **L. Kitch:** None. **M.J. Schnitzer:** None.

Poster

850. Imaging Advances: In Vivo, Animals

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Topic: G.03. Staining, Tracing, and Imaging Techniques

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NSF Grant 1158914 to J.L.C.

Title: Multi-area imaging of large-scale cellular dynamics in anatomically defined neocortical networks

Authors: *F. VOIGT, J. L. CHEN, R. KRUEPPEL, F. HELMCHEN;
Brain Res. Inst., Zurich, Switzerland

Abstract: Within the past decades, two-photon microscopy has been established as a powerful method to study neuronal morphology and activity *in vivo*. Combined with synthetic or genetically encoded calcium indicators, two-photon microscopy serves as a key technique to measure population activity within neuronal circuits. The spatial scale of monitoring activity with cellular resolution is, however, still limited to field-of-views (FOV) typically less than 0.5 mm across. As information processing in the mammalian neocortex occurs in much larger distributed networks, improvements in the spatial coverage of *in vivo* imaging techniques with cellular resolution are highly desirable, especially since modern tracing techniques also allow the anatomical identification of specific cortico-cortical projections. To extend the spatial reach of two-photon microscopy beyond local activity patterns we have designed and built a modular two-photon microscope for imaging multiple cortical areas in awake, behaving mice. This multi-area microscope is based on a standard 16x NA 0.8 Nikon objective, which in combination with a large-area scanning system allows a maximum FOV of 1.8 mm at high resolution, a distance suitable for imaging across primary and higher sensory areas in the mouse cortex. Within this FOV, the user can select smaller sub-areas (two in the current configuration) for fast imaging which can be positioned independently in x and y. Additionally, each sub-area can be focused individually by an electrically tunable lens over a z-range of up to 550 µm. The scan system consists of a single pair of galvanometric mirrors and positioning systems, which send multiple laser beams onto the scan mirrors, each directed at one sub-area. To image two sub-areas simultaneously, we use a spatiotemporal multiplexing approach. Both microscope hard- and software are modular and can be readily extended to more sub-areas and other imaging or photostimulation strategies. Additionally, we established co-labeling methods to identify long-range projection neurons in mutually innervated areas by virus-mediated conditional expression of fluorescent reporters using Cre/loxP and Flpe/FRT recombinase systems. We demonstrate concurrent calcium imaging of layer 2/3 neurons in primary and secondary somatosensory cortex of the awake mouse, identifying subsets of ”feedforward” and “feedback” neurons. The ability to record activity with cellular resolution in two reciprocally connected cortical areas across much larger spatial scales than previously possible should help to further decipher the behavior-dependent flow of information within the neocortex.

Disclosures: F. Voigt: None. **J.L. Chen:** None. **R. Krueppel:** None. **F. Helmchen:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

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Support: Kavli Foundation

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Wallace H. Coulter Foundation

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Human Frontier Science Program

R01 NS063226 (NINDS)

R01 NS076628 (NINDS)

Title: High-speed, volumetric imaging of behaving organisms using swept oblique light sheet (solis) microscopy

Authors: *V. VOLETI[1], M. B. BOUCHARD[1], C. S. MENDES[2], C. LACEFIELD[3], W. B. GRUEBER[4], R. S. MANN[2], R. M. BRUNO[3], E. M. C. HILLMAN[5];
[1]Biomed. Engin., [2]Biochem. and Mol. Biophysics, [3]Neurosci., [4]Physiol. and Cell. Biophysics, Neurosci. and Col. of Physicians and Surgeons, [5]Biomed. Engin. and Radiology, Columbia Univ., New York, NY

Abstract: Genetically encoded GCaMP calcium sensors have yielded a wide range of living preparations in which the complex functional and structural relationships of the nervous system can be explored. However, capturing cellular-resolution 3D dynamic images of such samples, from intact rodent brain to freely moving zebrafish embryos or *Drosophila* larvae has presented a major challenge to conventional microscopy techniques. The data acquisition rates of conventional laser scanning techniques such as confocal and two-photon microscopy are inherently limited by their need to visit each voxel within the imaged volume. Light-sheet based imaging modalities such as selective plane illumination microscopy (SPIM) circumvent this limitation by illuminating and imaging each voxel in the focal plane simultaneously. However the need to translate the sample relative to the system in order to image an entire volume introduces a further constraint to imaging speed. Furthermore, the orthogonal configuration of the excitation and detection pathways of many light sheet imaging techniques limits sample selection to small, immobile organisms. Here, we present Swept Oblique Light Sheet Microscopy (SOLiS), a new translationless, single-objective light sheet microscopy technique capable of imaging upwards of 40 volumes/second over large fields of view. The system works by scanning an angled light-sheet through the sample using a high-NA objective lens. The

fluorescence generated by this sheet is then captured by the same objective and remapped via descanning optics onto a high-speed sCMOS camera. An image-splitter provides exactly simultaneous, co-registered dual color imaging. We have simultaneously captured high-speed, 3D activity in neuronal and non-neuronal structures of living animals. We demonstrate that the technique can image firing in apical dendrites and vascular dynamics within the brains of awake, behaving mice, as well as calcium activity and 3D motion in freely crawling *drosophila* larvae.

Disclosures: V. Voleti: None. **M.B. Bouchard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); We have an issued patent on this technology and are currently pursuing licensing.. **C.S. Mendes:** None. **C. Lacefield:** None. **W.B. Grueber:** None. **R.S. Mann:** None. **R.M. Bruno:** None. **E.M.C. Hillman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); We have an issued patent on this technology and are currently pursuing licensing..

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.14/UU77

Topic: G.03. Staining, Tracing, and Imaging Techniques

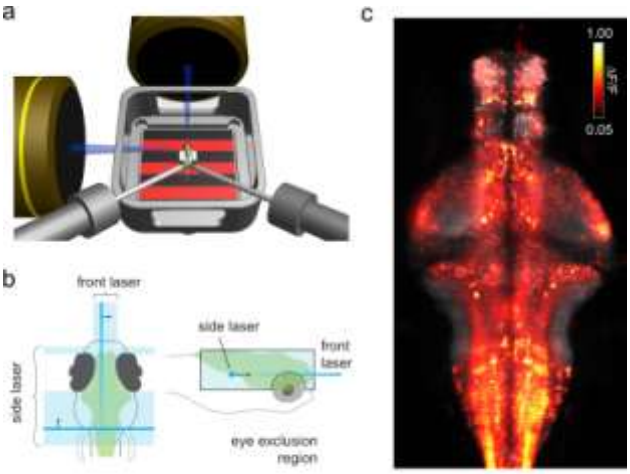
Support: HHMI funding

Title: Light-sheet imaging in zebrafish during visually driven virtual behavior

Authors: *N. VLADIMIROV, Y. MU, T. KAWASHIMA, D. V. BENNETT, C.-T. YANG, L. L. LOOGER, P. J. KELLER, J. FREEMAN, M. B. AHRENS;
Janelia Farm Res. Campus, HHMI, Ashburn, VA

Abstract: Brain function relies on communication within and between widely distributed networks of neurons. Imaging from large-scale, distributed networks during behavior will allow for new insights into sensorimotor transformations and other brain processes. We developed a light-sheet microscope capable of whole-brain imaging in paralyzed larval zebrafish, during simultaneous presentation of visual stimuli and monitoring of behavior. To overcome previous limitations of light-sheet microscopy and make this imaging modality compatible with visual stimulus presentation, we employ two orthogonal light sheets with temporal modulation to cover most of the brain while avoiding direct laser exposure of the retina. At the same time, visual

scenes are projected onto a screen below the fish. The intended behavior of the paralyzed animal is electrically recorded from the tail. In open-loop, visual stimulation is predetermined; in closed-loop 'virtual reality', the recorded motor output provides immediate visual feedback to the fish. With this system, activity in most neurons in the brain can be imaged at volumetric rates up to 3 Hz at single-neuron resolution during behavior. To assess whether whole-brain imaging affects behavior, we characterized in detail both the open-loop and closed-loop optomotor response, and verified that both behaviors are intact and robust in the presence of the light sheets. Whole-brain neuronal activity during the optomotor response in 5-7 dpf animals reveals wide-spread activity that, with the current method, can be correlated to behavior on a trial-by-trial basis. The system we describe promises new insights into how large neural networks implement behaviorally-relevant computations.



Disclosures: **N. Vladimirov:** None. **Y. Mu:** None. **T. Kawashima:** None. **D.V. Bennett:** None. **C. Yang:** None. **L.L. Looger:** None. **P.J. Keller:** None. **J. Freeman:** None. **M.B. Ahrens:** None.

Poster

850. Imaging Advances: In Vivo, Animals

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.15/UU78

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Comparison of the effect of sub-anaesthetic ketamine on brain activity and metabolism in conscious and anaesthetised rats by simultaneous electroencephalography (EEG) and 2-deoxyglucose autoradiography (2DG)

Authors: *C. SIMMONS[1], M. MESQUITA[1], A. KONING[1], T. C. WOOD[1], A. C. VERNON[2], S. C. R. WILLIAMS[1], D. CASH[1];
[1]Dept. of Neuroimaging, [2]Dept. of Neurosci., Inst. of Psychiatry, Kings Col. London, London, United Kingdom

Abstract: Ketamine (ket) is an NMDA antagonist, often used as a translatable assay for putative antipsychotics in both preclinical (animal) and clinical (human) studies. Non-invasive pharmacological MRI (phMRI) is frequently used to explore ket-induced effects in the awake humans[1]; however in preclinical MRI the subjects are typically anaesthetised rodents, and anaesthesia needs to be taken into account when conducting translational experiments. Our aim was to use EEG and 2DG to determine differences in the effect of ket on brain activity and metabolism in conscious and anaesthetised rats, and to corroborate phMRI conducted under anaesthesia. Two cortical (prefrontal and parietal) EEG electrodes were implanted 5-7 days before the recording. EEG (Pinnacle, USA) and 2DG[2] were conducted simultaneously, in anaesthetised (1.2% isoflurane in 1:9 O_2:air) and conscious restrained rats. After 2h stabilisation, ket (10mg/kg sc) was administered, followed by ^{14}C-2DG after 15 min. EEG data were processed by power spectral analysis per frequency (Hz: Delta 0.5-4, Theta 4-8, Alpha 8-12, Beta 12-30, Gamma 30-80). phMRI (BOLD-contrast sensitive gradient echo) was conducted in a separate cohort under 1.2% isoflurane. The results from the two EEG/2DG experiments showed several differences. In conscious rats ket increased EEG power in the delta, beta and gamma bands, peaking at 10-20 min, with no effect on alpha and theta. Glucose utilization (GU, from 2DG) was increased in the cortex, the hippocampus and the habenula, with no decreases. Under anaesthesia, EEG power was initially decreased in the lower frequencies (delta, alpha, beta, theta) but returned to baseline within 15-20 min, whereas gamma power increased in a delayed manner starting at 10 min and remaining increased by 60 min after ket. GU was significantly *decreased* in the striatum, nucleus accumbens and cingulate cortex, but increased in the hippocampus CA3 area. phMRI was analysed by correlating the signal timecourse with input functions derived from the anaesthetised EEG using a general linear model implemented in SPM8 (UCL, London). We detected early widespread BOLD signal decreases (during the first 10 min) matching decreases in the slow EEG frequencies, and later BOLD increases that correlated to the gamma power timecourse. These results confirm that ket-induced brain activity is influenced by anaesthesia. The data also show usefulness of EEG/2DG corroborative results for phMRI analysis. Careful delineation of the phMRI timecourse of ket establishes an assay in which to study the effects of antipsychotics in rodents under anaesthesia. 1. De Simoni et al (2013) NeuroImage 64(C) 75 2. Sokoloff L (1979) Brain 102(4) 653

Disclosures: **C. Simmons:** None. **M. Mesquita:** None. **A. Koning:** None. **T.C. Wood:** None. **A.C. Vernon:** None. **S.C.R. Williams:** None. **D. Cash:** None.

Poster

850. Imaging Advances: In Vivo, Animals

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.16/UU79

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: R01 NS44617

S10 RR15685

S10 RR019920

Title: Electrophysiological correlates of BOLD in whisker and visual cortices

Authors: *D. P. AKSENOV, L. LI, M. MILLER, G. IORDANESCU, A. WYRWICZ; NorthShore Univ. HealthSystem, Evanston, IL

Abstract: Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) relies upon the coupling between neuronal electrical activity and localized hemodynamic changes to map neuronal activation. A comprehensive understanding of this relationship is vital for the accurate interpretation of fMRI results and for the application of fMRI techniques to more sophisticated and detailed neurophysiological questions. However, this relationship may be altered by a variety of factors. One such potential factor that has not been well studied is difference in structure and physiology among brain regions. Local differences in properties including cortical thickness and organization, cell populations, or balance of excitatory vs. inhibitory activity could significantly influence the evoked and baseline responses measured in each brain region. In order to characterize regional differences in BOLD signal and neuronal activity, fMRI and electrophysiological data were obtained from the somatosensory and visual cortices of awake rabbits using either whisker or visual stimulation, and the BOLD and neuronal responses were compared. Images were acquired on a 9.4T Bruker BioSpec scanner from eight consecutive slices using a single-shot gradient-echo EPI pulse sequence (TR=2s and TE=20ms). For whisker experiments, the stimulus was a 20 s vibration (75 Hz) delivered to the whiskers on the left side by means of a nylon band coupled to an oscillating magnetic coil. For visual experiments, the stimulus (20 s) consisted of four LEDs flashing at 8 Hz. For each experiment, ten trials were averaged for each paradigm and analyzed by cross-correlation. Neuronal activity was recorded using Neuralynx system, the signals were amplified, band-pass filtered (300 Hz-3 kHz for single units (SU) and 1-150 Hz for local field potentials (LFP)), and digitized (32

kHz/channel). Data were analyzed after removal of blocks of gradient interference. Our results revealed a striking difference in BOLD time course adaptation (i.e., decrease to a plateau following an initial peak), between the visual and whisker cortices. In the visual cortex BOLD typically had an initial peak followed by a decrease to a plateau. In the whisker barrel cortex, a typical BOLD temporal response is mostly flat. In contrast, LFP and majority of SU responses showed adaptation in both cortices.

Disclosures: D.P. Aksenov: None. **L. Li:** None. **M. Miller:** None. **G. Iordanescu:** None. **A. Wyrwicz:** None.

Poster

850. Imaging Advances: In Vivo, Animals

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Program#/Poster#: 850.17/UU80

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Agence Nationale de la Recherche, Paris, France (ANR TECSAN VSD-IR)

Agence Nationale de la Recherche, Paris, France (ANR BRAINVASC)

Title: Chronic assessment of cerebral hemodynamics during rat forepaw electrical stimulation using functional ultrasound imaging

Authors: C. BRUNNER[1], E. MACE[1], H. MARC[1], J. ROSSIER[1], G. MONTALDO[2], *A. URBAN[1];
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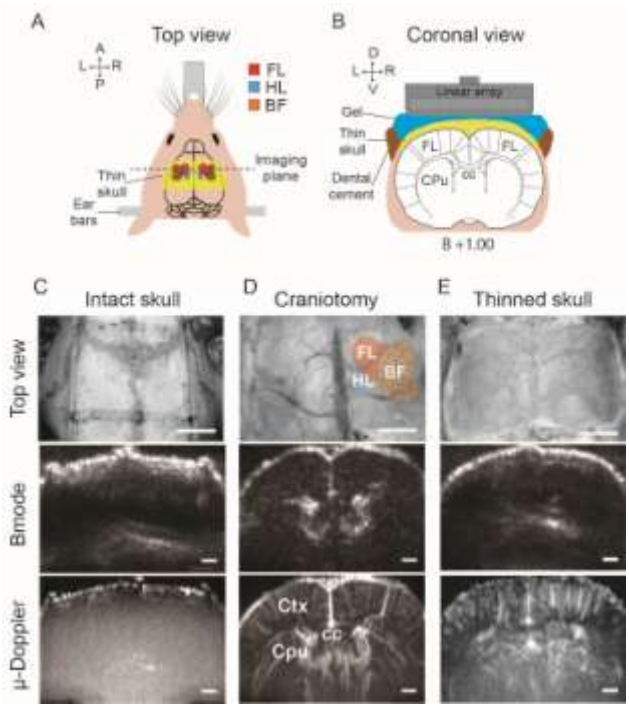
Abstract: Functional ultrasound imaging is a method recently developed to assess brain activity via hemodynamics in rodents. Doppler ultrasound signals allow the measurement of cerebral blood volume (CBV) and red blood cells (RBCs) velocity in small vessels. However, this technique originally requires performing a large craniotomy that limits its use to acute experiments only. Moreover, a detailed description of the hemodynamic changes that underlie functional ultrasound imaging have not been described but are essential for a better interpretation of neuroimaging data. To overcome the limitation of the craniotomy, we developed a dedicated thinned skull surgery for chronic imaging. This procedure did not induce brain inflammation nor

neuronal death as confirmed by immunostaining. We successfully acquired both high-resolution images of the microvasculature and functional movies of the brain hemodynamics on the same animal at 0, 2, and 7 days without loss of quality. Then, we investigated the spatiotemporal evolution of the CBV hemodynamic response function (HRF) in response to sensory-evoked electrical stimulus (1 mA) ranging from 1 (200 μs) to 25 pulses (5 s). Our results indicate that CBV HRF parameters such as the peak amplitude, the time to peak, the full width at half-maximum and the spatial extent of the activated area increase with stimulus duration. Functional ultrasound imaging was sensitive enough to detect hemodynamic responses evoked by only a single pulse stimulus. We also observed that the RBCs velocity during activation could be separated in two distinct speed ranges with the fastest velocities located in the upper part of the cortex and slower velocities in deeper layers. For the first time, functional ultrasound imaging demonstrates its potential to image brain activity chronically in small animals and offers new insights into the spatiotemporal evolution of cerebral hemodynamics.



Disclosures: **C. Brunner:** None. **E. Mace:** None. **A. Urban:** None. **H. Marc:** None. **J. Rossier:** None. **G. Montaldo:** None.

Poster

850. Imaging Advances: In Vivo, Animals

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.18/UU81

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIDA/NIAAA/NINDS-IRP, NIH

Title: Functional connectivity hubs in the conscious marmoset monkey

Authors: *A. M. BELCHER[1,2], D. TOMASI[3], C. C. C. YEN[4], L. NOTARDONATO[4], T. J. ROSS[2], Y. YANG[2], E. A. STEIN[2], N. D. VOLKOW[2], A. C. SILVA[4];
[1]Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Natl. Inst. on Drug Abuse, Bethesda, MD; [3]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; [4]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: The study of Resting-State Functional Connectivity (RSFC) has witnessed fast growth in recent years, and findings suggest its potential as a biomarker for abnormal brain function and for characterization of functional diversity among individuals. Animal models provide a method for mechanistic explorations of RSFC, but the need for anesthesia is a significant challenge to the interpretation of the data. We have developed a protocol to train marmoset monkeys (C*allithrix jacchus)* to tolerate light restraint during fMRI protocols, and have recently reported its successful employment for acquisition of RS data, finding that marmosets possess large-scale, functionally significant brain networks (Belcher et al., 2013). Here we extend these findings to utilize a data-driven method to investigate whether the marmoset brain exhibits local Functional Connectivity Density (*l*FCD) patterns that recapitulate those observed in the human brain. Six trained marmosets were scanned on a single day in a 7.0T scanner. A high-resolution anatomical scan as well as eight 10min-long EPI scans was collected for each monkey. Standard pre-processing steps were applied to functional datasets, and included skull-stripping, registration, and slice timing correction. Ultrafast data-driven FCD mapping with a stringent correlation threshold $R > 0.6$ was used to map the main functional connectivity hubs in the marmoset brain. Individual animals' EPI sessions were averaged, and voxelwise within-subjects ANOVA was used to assess the statistical significance of *l*FCD using a threshold $P_{FWE} < 0.05$, corrected for multiple comparisons at the voxel level. The *l*FCD in marmosets was highly significant in the whole brain (t-score > 5), and inter-subject variability was quite low. The strength of the *l*FCD hubs was maximal in the visual cortex and posterior cingulum, regions that also contain the strongest *l*FCD hubs in humans. The parietal and frontal cortices also included prominent *l*FCD hubs in the marmosets, with local maxima centered on somatosensory cortex and frontal pole, respectively. Species-specific differences emerged, with cortical visual hubs stronger in the marmoset, and temporal and posterior parietal cortical hubs stronger in the human. In conclusion, we present for the first time an approach to studying RSFC hubs in an

awake nonhuman primate, validating our previous findings that awake marmosets have strong patterns of brain connectivity that bear correspondence to those observed in awake humans.

Disclosures: **A.M. Belcher:** None. **D. Tomasi:** None. **C.C.C. Yen:** None. **L. Notardonato:** None. **T.J. Ross:** None. **Y. Yang:** None. **E.A. Stein:** None. **N.D. Volkow:** None. **A.C. Silva:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.19/UU82

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: The microanatomical origin of the high-field magnetic resonance imaging signal in the marmoset cerebral cortex: myelin sheaths or cell bodies?

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Abstract: High-field structural magnetic resonance (MR) imaging with field strengths of 7 Tesla and more maps the human brain with ever increasing spatial resolution but the microanatomical nature of the MR signal is still poorly understood. This project attempts to better understand the histological basis of this signal in a primate species with a less complex cortical anatomy compared to humans: the common marmoset monkey (Callithrix jacchus). In the cerebral cortex we compared regional differences in MR contrast with the distribution of myelin sheaths and cell bodies. We scanned two post-mortem marmoset brains (one male, 7.5 years, one female, 8 years, perfused and fixed with 4% formalin in phosphate-buffered saline) with a 7 Tesla whole-body MR scanner (MAGNETOM 7 T, Siemens) and generated quantitative maps of the longitudinal (T1) relaxation with a MP2RAGE sequence (Marques et al., NeuroImage 49 (2010), p. 1271 ff.) (voxel size (0.2 mm)3, scan medium: Fomblin (Solvay Solexis)). We sectioned the brains in the coronal plane (40 µm) with a freezing microtome (SM2000 R, Leica / Hyrax KS 34, Microm) and imaged the blockface with a digital camera (D90, Nikon) for section alignment and histological volume reconstruction. We stained every second section in an alternating sequence for myelin sheaths (Gallyas silver stain or avidin-

biotin-peroxidase immunohistochemistry with a monoclonal antibody (mAB) against myelin basic protein (Abcam)) or neuronal cell bodies (mAB against human neuronal protein HuC/HuD (Molecular Probes) or the pan-neuronal neurofilament marker SMI 311 (Covance)). We digitized the sections with a photomicroscope with a motorized stage (Axio Imager M1, Zeiss), analyzed the myelo- and cytoarchitectonic pattern in the cortex, and correlated histology- and T1-based contrast. The T1 distribution is anatomically very heterogeneous: T1 values are low in white matter tracts with high myelin content (e.g., corpus callosum, anterior commissure) and high in subcortical nuclei with low myelin content (e.g., basal ganglia, thalamus). In the cortex, differences in T1 values are smaller, but their spatial distribution shows a reproducible laminar and regional pattern. A comparison with histology reveals that this pattern reflects (i) cortical layers and areas and (ii) myeloarchitecture more than cytoarchitecture. In summary, the data show that quantitative T1 maps reflect predominantly myelin and are a powerful and emerging tool to map its distribution noninvasively in the non-human and human primate brain.

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Poster

850. Imaging Advances: In Vivo, Animals

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Intramural Research Program of the NIH, NIDA

Title: Amplitude of low-frequency fluctuations in the awake, truly resting marmoset monkey

Authors: *L. D. NOTARDONATO[1], D. G. TOMASI[2], A. M. BELCHER[3], C. C.-C. YEN[1], N. D. VOLKOW[2], A. C. SILVA[1];
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Abstract: The study of the spontaneous, low-frequency fluctuations captured during resting-state fMRI has received considerable attention in recent years. Animal models provide an ideal test-bed for applying resting-state fMRI to the study of human neuropsychiatric disease, yet relatively little work has been dedicated to exploring resting-state in animal models. The

common marmoset (Callithrix jacchus) is a small, New World nonhuman primate that is increasingly being used in preclinical behavioral, pharmacological and human disease studies. Recently, we have reported its successful use in an awake imaging protocol for acquisition of resting-state BOLD fMRI data, finding that these monkeys possess large-scale brain networks (RSNs) akin to those networks seen in humans. Previous work in humans has suggested that RSNs may be influenced by the amplitudes of the low-frequency fluctuations found in resting-state. Thus, in this study we subjected our data to a more fine-grained analysis to explore the influence of the amplitudes of low-frequency fluctuations (ALFF) on resting-state connectivity in the awake marmoset brain. We trained six male marmosets in a three-week period for behavioral acclimation to restraint in an MRI. Monkeys were then each scanned on a single day in a 7.0T Siemens scanner. A high-resolution anatomical RARE scan (FOV=4.5×4.5cm, mtx=160x160, slice thick=2mm, resol=0.281mm, 15 slices) as well as eight 10min-long single-shot gradient-echo EPI scans (TE/TR=24/1500msec, 400 time points, slice thick=2mm, 15 slices, mtx=80×80, resol=0.562mm) were collected in resting conditions for each monkey. Standard pre-processing steps were applied to functional datasets (skull-stripping, slice timing correction, and registration to a common template). Independent component analysis (ICA) was used to determine RSNs and a voxel-matched correlation analysis was performed to assess the relationship between network strengths and ALFFs. Here we report the relationship between several of these ICA-identified networks and ALFF in the marmoset brain, and compare and contrast those relationships to those reported to be observed in the human brain. These findings further support the use of this awake marmoset model as a platform for understanding normal human brain function, and for its applicability in existing disease models.

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Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

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Title: fMRI signal dropout in rhesus macaque monkey due to chronic contrast agent administration

Authors: *G. GAGIN[1], K. S. BOHON[1], J. CONNELLY[2], B. R. CONWAY[1,3];
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Abstract: A monocrystalline iron oxide nanoparticle (MION) solution has been found to enhance fMRI sensitivity compared to the blood oxygen level-dependent signal (BOLD) in macaque monkeys (Leite et al. 2001). MION boosts signal by increasing the amount of free iron circulating in the bloodstream. In humans and non-human primates, excess free iron in the bloodstream is managed in part by a class of iron storage proteins called ferritins, which render the iron essentially inert and prevent tissue damage. Ferritins carrying iron form intracellular complexes called hemosiderin, which allows for long-term iron storage within cells. While free iron circulation enhances fMRI signal, stationary intracellular iron in the form of hemosiderin does not vary with bloodflow and can contribute to signal dropout (Thickbroom et al. 2003). To quantify the effect of hemosiderin on fMRI signal quality across the visual cortex of macaque monkeys, we measured the temporal signal to noise ratio (tSNR) over the course of 44 (monkey 1) and 37 (monkey 2) scans spanning 15 months. Animals were injected with 10mg/kg of MION (Feraheme, AMAG) immediately prior to each scan. We investigated effects in retinotopic cortex (V1-V5) and the temporal lobe. To correct for variation in overall signal quality between sessions, tSNR values for regions of interest were normalized by whole brain tSNR. The effect of iron buildup on retinotopic cortex was minimal. But tSNR in the temporal lobe decreased with successive scans in both animals. We analyzed tSNR within subregions of the temporal lobe defined by functional criteria: patches selective for faces along the posterior-anterior axis of the temporal lobe (PL, ML, AL), and more ventrally, patches biased for color at corresponding locations along the posterior-anterior axis (PLc, CLc, ALc). tSNR decrease was minimal in the posterior regions (PL, PLc), but substantial within the anterior regions (ALc, AL). Effects on tSNR for the intermediate regions along the posterior-anterior axis were mixed: a significant decrease in the tSNR of the color-biased region (CLc), but no effect in the more dorsal face patch ML. In an attempt to recover fMRI signal, we treated both animals with two different iron chelators after the conclusion of the experiments described above. The monkeys received 21 treatments of 500mg of deferoxamine mesylate (Desferal, Novartis; IM injection) over 3 months and 104 treatments of 50mg/kg of deferiprone (Ferriprox, ApoPharma; PO) over 7 months. After

chelation, we found close to complete recovery of signal in CLc, and a slight recovery in ALc and AL.

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Poster

850. Imaging Advances: In Vivo, Animals

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Program#/Poster#: 850.22/UU85

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Use of MRI to non-invasively guide hydrogel treatment of stroke lesions in a rat model

Authors: *M. M. MODO[1], A. MASSENSINI[3], W. LING[2], C. MEDBERRY[2], F. NICHOLLS[2], S. BADYLAK[2];
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Abstract: Stroke remains the leading cause of adult disability worldwide, but there are currently no therapies that afford a replacement of lost brain tissue. Use of bioactive materials composed of extracellular matrix (ECM) has proved to be a promising strategy for the constructive remodeling of other tissue and organ systems (e.g. urinary bladder). For example ECM composed of porcine urinary bladder matrix (UBM) can offer both structural support and a mixture of bioactive molecules that promote tissue repair. Applications of this bioactive scaffold approach to treat stroke lesions requires that two technical challenges must be overcome: 1) delivery of an appropriate volume of material into the brain to fill the cavity without increasing intracranial pressure, and 2) retain a sufficiently high concentration of ECM material within the cavity. Herein, we show how MRI can be used to guide the site and volume of injection using a novel stereotactic surgical approach that injects ECM material through one burr hole, while draining extracellular fluid (ECF) from the cavity through another. Additionally, different concentrations of ECM are evaluated for their ability to gel inside the cavity whilst retaining structural integrity. In conclusion, we have developed a method that allows the complete coverage of a stroke lesion cavity with an ECM bioscaffold. These experiments will form the basis for a regenerative medicine approach for the stroke-damaged brain.

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Poster

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Support: NIH Grant 1R21NS081544-01A1

Title: Chemical exchange saturation transfer (CEST) MRI may aid in the detection of CNS graft death/rejection

Authors: ***S. SAJJA**[1,2], G. LIU[3,6], N. YADAV[3,6], J. XU[6], A. ARNOLD[1,2], A. JABLONSKA[1,2], M. MCMAHON[3,6], P. VAN ZIJL[3,6], J. BULTE[1,2,6,4,5], P. WALCZAK[1,2], M. JANOWSKI[1,2,7,8]; [1]Russell H. Morgan Dept. of Radiology and Radiological Sci. and Inst., [2]Inst. of Cell Engin., [3]Russell H. Morgan Dept. of Radiology and Radiological Sci., [4]Dept. of Biomed. Engin., [5]Dept. of Chem. & Biomolecular Engin., Johns Hopkins Sch. of Med., Baltimore, MD; [6]F. M. Kirby Res. Ctr. for Functional Brain Imaging, Kennedy Krieger Inst., Baltimore, MD; [7]Dept. of NeuroRepair, Mossakowski Med. Res. Ctr., [8]Dept. of Neurosurgery, Mossakowski Med. Res. Ctr., Polish Acad. of Sci., Warsaw, Poland

Abstract: Introduction: Cellular therapies for CNS disorders are of tremendous interest, but methods for the evaluation of graft survival are clinically inadequate (including T1 with/without contrast, T2 and T2* imaging modalities). Because stem cells require several months to differentiate and become functional, it is critical to know the fate of cells during that time-course to estimate therapeutic effect. The aim of this study was to develop a novel, clinically applicable approach for the non-invasive detection of cellular graft infiltration by immune cells, which could be used as a proxy for graft rejection. The current study used novel MR-based techniques, chemical exchange saturation transfer (CEST) and frequency-labeled exchange transfer (FLEX), to detect the exchange of protons between water and pools of mobile macromolecules and small metabolites. Methods: Luciferase+ (luc+) glial-restricted progenitor cells (GRPs) were transplanted into age-matched Balb/c and rag-/- mice (n=2/group) during stereotaxic surgery. Bioluminescence of transplanted GRPs was detected using an IVIS pre-clinical imaging system on alternative days, for up to two weeks. T1, T2, T2*, CEST, and FLEX MRI were obtained at days 1, 7, and 14 post-transplantation. At two weeks, immunohistochemistry was performed to

identify immune infiltration of the graft using CD4, CD8, CD68, and CD45 in addition to luc+ staining for graft cell viability. Results: Bioluminescence signal from the IVIS imaging system showed a ~92% drop for Luc+ cells two weeks after transplantation. T1, T2, and T2* showed no imaging contrast; however, CEST showed higher asymmetry at the transplantation site compared to the contralateral side of the brain. In addition, CEST magnetization transfer ratio (MTR) asymmetry changed from day 1 to day 14 at the transplantation site. Immunohistochemistry revealed higher immune cell infiltration with CD45+ and CD68+ cells at the transplantation site in Balb/c mice, but not in rag-/- mice. Discussion and conclusion: CEST could potentially be employed to understand the immune response following cell transplantation, as evidenced by changes in MTR asymmetry at the transplantation site (day 1 to day 14). This could be attributable to immune rejection of the graft, since the bioluminescence of luc+ showed a dramatic decline in transplanted cells (day 1 to day 14). The evidence of high immune cell infiltration (CD45+ and CD68+) at the transplantation site further supports graft rejection, which would also contribute to a change in the MTR asymmetry profile from day 1 to day 14.

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Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

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Carnot Institute

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Title: High resolution MR-thermometry is a proper method to characterize thermal safety of chronic implantable medical device

Authors: F. REINHART[1], V. AUBOIROUX[1], C. CHABROL[1], F. DARLOT[1], N. TORRES-MARTINEZ[1], *D. JOHNSTONE[2], A.-L. BENABID[1], J. MITROFANIS[2], C. MORO[1];

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Abstract: Context: A growing number of medical devices are designed for chronic implantation. They produce heat which increases tissue temperature and may eventually create tissue damage. Characterization of these devices requires a precise evaluation of the temperature at the site of implantation. High resolution MR-thermometry allows noninvasive quantification of thermal variations in rat brain, without any bias such as induced by a thermocouple. Similarly to the follow-up of thermal elevation already used during laser induced thermotherapy (LITT), we present here a high spatial resolution, high precision characterization of the thermal effects of active, implanted optical fiber device in order to assess its safety. Methods: We tested a device specifically designed for low-light intra-cerebral laser therapy at 670 nm, with an optical fiber for deep delivery of light in the rat brain at several light powers at the tip of the fiber. Using proton resonance frequency shift (PRFS)-based MR-thermometry (resonance frequency dependence to biological tissues temperature), high resolution (0.3 x 0.3 x 0.1 mm3) thermal maps were acquired during *in vivo* illumination in rat. Multi-slice MR acquisitions were performed with an actual optical power ranging from 5 to 115 mW, during 5 and 30 minutes. Results: Here we show that a 115 mW optical power at the end of the fiber causes a very fast temperature increase, which stabilizes at 7 °C within less than 2 minutes, then decreases in less than 2 minutes after turning off the light. A 15 mW optical power does not cause a temperature increase above 2 °C. Moreover, the heat increase pattern is linearly correlated with the light power injected (R^2=0.996). The analysis of heat diffusion shows that it is light power dependent and it has a spherical pattern. Conclusion: MR-thermometry is an adequate method to precisely measure *in vivo* deep brain thermal variations and allows thermal characterization of chronic implantable medical devices.

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Poster

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Title: In-utero diffusion magnetic resonance imaging of the embryonic mouse brain

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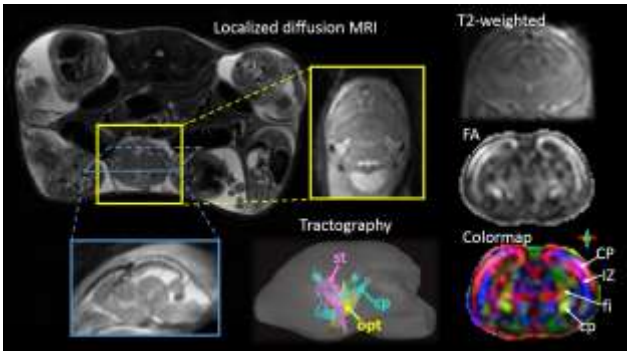
[1]Biomed. Engin., [2]Gynecology and Obstetrics, [3]Radiology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: In-utero imaging of the embryonic mouse brain is useful for longitudinal monitoring of the normal/abnormal brain development. Diffusion magnetic resonance imaging (dMRI) provides superb tissue contrasts in the embryonic brain compared to conventional T1/T2 MRI. However, *in vivo* dMRI of the embryonic mouse brain was not yet shown to be feasible due the motion and poor spatial resolution In this study, we developed a localized imaging technique (the yellow box in the figure indicates a selected embryo for localization) in combination with a 3D fast imaging sequence to accelerate image acquisition, and thereby reduce the exposure to motion. The residual motion artifacts were amended using navigator-based motion correction. With these techniques we were able to achieve high-resolution in-utero dMRI of the embryonic day 17 mouse brains (from five pregnant dams with average litter size of 11 pups). Imaging was performed on an 11.7 Tesla scanner at 0.2 x 0.2 x 0.2 mm resolution with 30 diffusion directions in 72 minutes. The dMRI data clearly delineated the major gray matter and white matter structures, e.g., the cortical plate (CP), intermediate zone (IZ), cerebral peduncle (cp), and fimbria (fi). DMRI-based tractography revealed early white matter tracts in the E17 mouse brain. Quantitative measures obtained *in vivo*, such as the fractional anisotropy (FA) and apparent diffusion coefficients (ADC), were significantly different from *ex vivo* measurements and reflected tissue properties under the normal physiological condition. In addition, high-throughput T2-weighted images can be obtained at 0.13 mm isotropic resolution in 10 minutes, from which the overall brain morphology can be visualized in 3D. This in-utero dMRI technique can be used for detection of abnormality in mouse models of fetal injury and phenotype screening in mutant mouse models.



Disclosures: **D. Wu:** None. **I. Burd:** None. **J. Zhang:** None. **T. Borbiev:** None.

Poster

850. Imaging Advances: In Vivo, Animals

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Support: LABEX WIFI

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ANR-10-EQPX-15

Title: Highly-resolved ultrasound imaging of functional connectivity in the living rat brain

Authors: ***S. PEZET**[1], A. RICOBARAZA[1], B. OSMANSKI[2], M. TANTER[2], Z. LENKEI[1]; [1]Neurorestoration, Lab. of Brain Plasticity, Paris, France; [2]Inst. Langevin, ESPCI, Paris, France

Abstract: Long-range coherences in spontaneous brain activity reflect functional connectivity. We developed a novel, highly resolved connectivity mapping approach, using ultrafast functional ultrasound (fUS) imaging of microvascular hemodynamics deep in the anesthetized rodent brain, through a large thinned-skull cranial window. Both seed-based and singular value decomposition analysis of spatial coherences in the low frequency (<0.1 Hz) spontaneous fUS signal fluctuations reproducibly found, at different coronal planes, high-contrast intrinsic functional connectivity patterns. These patterns corresponded to known major functional networks, such as the task-dependent lateral sensorimotor network, which was temporally anticorrelated with prominent midline hubs of the default-mode network. Pixel dimension was 100 µm x 100 µm in-plane and the millisecond-range temporal resolution allowed unambiguous cancellation of low-frequency artifacts resulting from cardio-respiratory motion. These results demonstrate that fUS is a powerful novel neuroimaging method, which could be extended to portable systems for three-dimensional functional connectivity imaging in awake and freely moving rodents.

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Poster

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NIH Grant R01 EB009041

Kinetics Foundation

Title: Enhanced localized delivery and therapeutic effects of the Neurturin neurotrophic factor through the focused ultrasound-mediated blood-brain barrier opening in wild-type and Parkinson's disease mouse model

Authors: *G. SAMIOTAKI, C. ACOSTA, S. WANG, E. KONOFAGOU;
Columbia Univ., New York, NY

Abstract: The blood-brain barrier (BBB) constitutes a major obstacle in drug delivery to the brain. Focused Ultrasound (FUS) in conjunction with microbubbles has been shown to open the BBB non-invasively, locally and transiently to allow the delivery of molecules. Neurturin (NTN), a member of the GDNF family, has been demonstrated to have neuroprotective and regenerative effects on dopaminergic neurons, suggesting its therapeutic potential for Parkinson's disease (PD). The ascending nigrostriatal pathway, i.e. neurons in the substantia nigra (SN) projecting to the caudate putamen (CP), is the most severely damaged brain system in PD and was therefore selected as the target area in this study. First, the acoustic parameters and sonication locations were optimized in CB57/bl wild-type mice (n=20) for efficient and safe drug delivery in both CP and SN using FUS (center frequency: 1.5 MHz, PRF: 10 Hz, acoustic pressure: 0.45 MPa) covering the entire areas of interest. BBB openings were monitored longitudinally and reversibility timeline was found to 4-5 days for both the CP and SN. No damage was detected upon histological examination of the brain tissue. For the second part of this study, NTN (20 mg/ kg, Invitrogen, CA, USA) diluted in saline was injected intravenously to wild type mice after BBB opening. The diffusion and the downstream signaling bioactivity were detected using immunostaining. Increased phosphorylation of the receptor RET, the kinase ERK1/2 and the CREB transcription factor in the sonicated areas was detected and quantified. No bioeffects were detected due to FUS alone. Immunostaining of brain horizontal sections revealed NTN bioavailability, i.e. diffusion in the brain tissue, in the entire area of CP (9.1 mm2 ± 1.1 mm2) and SN (4.6 ± 0.7 mm2) using FUS, compared to an average area of 0.23 ± 0.04

μm2 following the conventional method of direct injection . Finally and most importantly, in the third part of the study, NTN effects in the PD disease MPTP-model were studied. 15 mice received a sub-acute MPTP regimen, and the lesions were let stabilize for 4 weeks. Mice then received sonication (FUS+) or not (FUS-), and NTN (NTN+) or saline (NTN-) iv injection, they were divided into 3 groups (FUS+NTN+, FUS+NTN-, FUS-NTN-), and they were let survive for 4 weeks. Brains were harvested and stained for TH+ neurons. Preliminary results showed increased numbers of TH+ neurons, in the FUS+NTN+ group compared to the other groups, suggesting neuroregeneration following NTN iv administration after FUS. These findings confirm the effective delivery of NTN using FUS to brain parenchyma and indicate the potential of the technique for reversing the PD phenotype in animal models.

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Poster

850. Imaging Advances: In Vivo, Animals

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Program#/Poster#: 850.28/UU91

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: SPECT/CT imaging of parkinson's disease in preclinical models

Authors: *T. HUHTALA[1], V. JANKOVIC[2], U. HERZBERG[2], R. PUSSINEN[1], P. SWEENEY[1], A. NURMI[1];
[1]Charles River Discovery Res. Services Finland Ltd, Kuopio, Finland; [2]Celgene Cell. Therapeut., Warren, NJ

Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder, which includes, among other pathologies, death of dopaminergic cells in the substantia nigra (SNc). Cocaine analogues such as ^{123}I-β-CIT can be used to image dopamine transporters (DAT) by SPECT. With small animal imaging scanners, the striatum can be visualized and ligand binding quantified in rodents. In this study, DAT density in three preclinical rodent models of PD was studied using SPECT/CT. Unilaterally induced PD was generated using intrastriatal 6-hydroxydopamine (6-OHDA) injection or AAV-α-synuclein gene transfer into SNc in rats. Also, a PD model using MPTP neurotoxin (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to cause Parkinson-like symptoms in mouse was studied. DAT imaging was performed 4 and 8 weeks after 6-OHDA lesion, and 3 and 8 weeks after AAV-α-synuclein gene transfer as well as 4 and 11 days post MPTP dosing. For the quantification of DAT ligand binding, ^{123}I-β-CIT was used. From the

MPTP-induced mice, neuroendocrine transmitter dopamine (DA) and its metabolites [3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] concentrations were also analyzed from striatum using HPLC. SPECT/CT imaging of the 6-OHDA-induced PD model in rat 4 and 8 weeks after lesion showed 20% decrease in DAT density on the ipsilateral side at both time points. For the unilateral AAV-α-syn gene transfer model, overall DAT density of striatum imaged 3 weeks after transfection was decreased. When ipsi- and contralateral sides were compared in this model, DAT concentration on the ipsilateral side had decreased ca. 20%. In the MPTP model, a significant decrease ($p<0.05$) in DAT binding was seen 11 days post induction. Concentrations of DA, DOPAC and HVA in striatum were also decreased in diseased mice compared to healthy controls. Modern imaging modalities offer a translational method to study functional changes of PD in rodents. Fully quantitative results can be obtained *in vivo*, and data can be obtained quickly and cost-efficiently over several time points, enabling longitudinal follow-up of disease progression within individual animals, and offering a valuable tool for preclinical research and efficacy studies.

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Poster

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Title: Label-free optically quantified brain metabolic rate of oxygen consumption

Authors: ***V. J. SRINIVASAN**, H. RADHAKRISHNAN, C. LEAHY, S. CHONG, C. W. MERKLE;
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Abstract: Cerebral metabolic rate of oxygen (CMRO2) is an important pre-preclinical endpoint for experimental model studies of cerebrovascular disease that involve ischemia. Moreover,

CMRO2 quantification at the microscopic level is important to better interpret macroscopic neuroimaging techniques. While traditionally difficult to measure optically, oxygen metabolism can be inferred by quantitatively measuring the flux of vascular oxygen to and from a region of tissue (Fick's principle). Recently-introduced phosphorescent probes enable precise quantification of oxygenation, but are costly and generate singlet oxygen that may perturb physiology, while calibration of probe lifetime depends on the local probe environment. Here, we introduce a novel method that quantifies oxygenation with label-free coherence-gated spectroscopy, and measures nutritive blood flow with Doppler Optical Coherence Tomography. These techniques are label-free and robust, and importantly, can be applied dynamically. Taken together, these methods promise to enable investigation of metabolic rate of oxygen consumption at the microscopic level, under a wide range of conditions in health and disease. Cortical cerebral blood flow (CBF), arterial oxygen saturation (Ya), and venous oxygen saturation (Yv) were measured on two different optical systems in the cortices of mices. A Spectral / Fourier domain OCT system, used to quantify blood flow, achieved a depth resolution of approximately 5 microns, a lateral resolution of approximately 8 microns, and a penetration depth of approximately 1 mm with a 1310 nm dual superluminescent diode light source. The second visible light OCT system, used to spectroscopically determine oxygenation, achieved a depth resolution of approximately 22 microns, a lateral resolution of approximately 20 microns, a spectral resolution of approximately 6 nm, and a penetration depth of ~300 microns with a 585 nm supercontinuum light source. Assuming that the cortex is predominantly supplied and drained at the cortical surface, these measurements can be combined to determine CMRO2 using the following expressions: CMRO2 = OEFxCBFxCa = OEFxCBFx[Hb]xYa, where Ca is the arterial oxygen content, [Hb] is the total hemoglobin concentration of blood, and OEF =(Ya-Yv)/Ya is the oxygen extraction fraction. Using these measurements, a CBF of ~120 mL/100g/min was estimated, while an OEF of 30% and an arterial oxygen saturation (Ya) of 95% were estimated. Thus, a CMRO2 value of 316 umol/100g/min was determined. This measurement is in agreement with literature values.

Disclosures: V.J. Srinivasan: None. **H. Radhakrishnan:** None. **C. Leahy:** None. **S. Chong:** None. **C.W. Merkle:** None.

Poster

850. Imaging Advances: In Vivo, Animals

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 850.30/VV1

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Dana Foundation

American Heart Association National Scientist Development award (09SDG2260983)

Western States Affiliate Beginning Grant in-Aid (0665051Y)

University of Missouri Department of Pathology and Anatomical Sciences research fund (ZG)

Title: Histological quantitation of brain injury using whole slide imaging: A pilot validation study in mice

Authors: Z. CHEN[1], D. SHIN[2], S. CHEN[1], K. MIKHAIL[1], O. HADASS[1], B. TOMLISON[1], D. KORKIN[2], C.-R. SHYU[2], J. CUI[1], D. ANTHONY[1], *Z. GU[1];
[1]Pathol & Anat Sci., Univ. Missouri Sch. Med., Columbia, MO; [2]Univ. of Missouri, Columbia, MO

Abstract: Quantitative assessment of serial brain sections provides an objective measure of neurological events at cellular and molecular levels, but is difficult to implement in experimental neuroscience laboratories because of variation from person-to-person and the time required for analysis. Whole slide imaging (WSI) technology, recently introduced for pathological diagnoses, offers an electronic environment and a variety of computational tools for performing high-throughput histological analysis and managing the associated information. In our study, we applied various algorithms to quantify histologic changes associated with brain injury and compared the results to manual assessment. WSI showed a high degree of concordance with manual quantitation by Pearson correlation and strong agreement using Bland-Altman plots in: (i) cortical necrosis in cresyl-violet-stained brain sections of mice after focal cerebral ischemia; (ii) intracerebral hemorrhage in ischemic mouse brains for automated annotation of the small regions, rather whole hemisphere of the tissue sections; (iii) Iba1-immunoreactive cell density in the adjacent and remote brain regions of mice subject to controlled cortical impact (CCI); and (iv) neuronal degeneration by silver staining after CCI. These results show that WSI, when appropriately applied and carefully validated, is a highly efficient and unbiased tool to locate and identify neuropathological features, delineate affected regions and histologically quantify these events.

Disclosures: **Z. Chen:** None. **D. Shin:** None. **S. Chen:** None. **K. Mikhail:** None. **O. Hadass:** None. **B. Tomlison:** None. **D. Korkin:** None. **Z. Gu:** None. **C. Shyu:** None. **J. Cui:** None. **D. Anthony:** None.

Poster

851. Optogenetics: Experimental Uses

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 851.01/VV2

Topic: G.04. Physiological Methods

Title: Optogenetic and calcium-sensing mouse models available from The Jackson Laboratory

Authors: *J. BECKWITH, S. F. ROCKWOOD, M. SASNER;
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Abstract: The Jackson Laboratory (JAX) Mouse Repository distributes mouse lines that incorporate recently developed optogenetic and calcium-sensing technologies. Opsins are light-activated proteins that alter membrane potential in neurons, so that stimulation with light allows rapid control of neuronal activity. Several mouse models are engineered to express next generation improved/optimized opsins fused to fluorescent proteins. These include mice with channelrhodopsin expression directed to defined cell populations by the *Thy1*, parvalbumin (*Pvalb*), *ChAT*, Vgat (*Slc32a1*), *Tph2*, Vglut2 (*Slc17a6*) or *Olfr160* promoters. Additional control is available in mice with Cre-dependent expression of archaerhodopsin, channelrhodopsin, or halorhodopsin variants. Variants of GCaMP exhibit fluorescence in response to calcium binding, thus serving as an indication of neuronal activation. Included are the *Thy1*-promoter driven GCaMP2.2c, GCaMP3, GCaMP6 fast (GCaMP6f) or GCaMP6 slow (GCaMP6s) transgenic lines, and the Tet-dependent GCaMP6s transgenic line. Mice with Cre-dependent GCaMP3, GCaMP6f or GCaMP6s expression provide additional flexibility. Several strains utilize both Cre-lox and Tet-On/-Off functionality. After removal of an upstream floxed-STOP, such mice allow Tet-dependent expression of the newest generation channelrhodopsin (Chronos/EGFP), halorhodopsin (Jaws/EGFP) or GCaMP6f. Similarly designed mice have Cre- and Tet-dependent expression of a voltage-sensitive FRET chromophore pair upon membrane depolarization. This set features recent models from Allen Institute for Brain Science, GENIE project (Janelia Farm/HHMI), Duke/MIT and others. It also includes mice with pharmacogenetic receptors and photoactivatable GFP. The JAX Mouse Repository is a centralized facility for the development, rederivation, distribution and cryopreservation of mouse models. Hundreds of new strains are added annually to one of the largest collections of characterized mouse strains available to the international biomedical research community. Repository holdings may be searched online (JAXMice database: jaxmice.jax.org/query). Researchers wishing to donate their mouse strains may submit them using our online submission form (jax.org/donate-a-mouse). The JAX Mouse Repository is supported by NIH, The Howard Hughes Medical Institute and several

private charitable foundations. *Please stop by for a detailed list of recently added optogenetic mouse lines. Please also visit our resources for optogenetics strains (research.jax.org/grs/optogenetics.html) and Cre-dependent optogenetics tool strains (jaxmice.jax.org/list/ra2600.html).*

Disclosures: J. Beckwith: None. **S.F. Rockwood:** None. **M. Sasner:** None.

Poster

851. Optogenetics: Experimental Uses

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 851.02/VV3

Topic: G.04. Physiological Methods

Support: NIMH Grant R01MH099231

Neuroscience Training Program Grant T32-GM007507

Advanced opportunity fellowship through SciMed Graduate Research Scholars at UW-Madison

Title: Sleep homeostasis in optogenetically fatigued neurons

Authors: *A. V. RODRIGUEZ, C. M. FUNK, G. TONONI, C. CIRELLI;
Univ. of Wisconsin Madison, Madison, WI

Abstract: In the mammalian cortex, neuronal activity during non-rapid eye movement (NREM) sleep is characterized by periods of activity (ON periods) alternating with periods of silence (OFF periods). After sleep deprivation (SD), the number and duration of OFF periods increases when compared to baseline sleep, but it is unknown whether these changes are due to neuronal "tiredness" triggered by synaptic plasticity and/or to neuronal "fatigue" caused by sustained firing during prolonged wake. To address this question, we combined *in vivo* electrophysiology with optogenetic stimulation using a mutant of Channelrhodopsin-2 (Chr2-C128S/D156A) known as the stable step function opsin (SSFO) to chronically stimulate neurons during sleep. We injected a Cre-dependent adeno-associated virus (rAAV5) coding for SSFO into the right frontal cortex of male transgenic CamKIIα-Cre mice (n=8). We then implanted 16-channel electrode arrays bilaterally to record unit activity, along with fiber optics and electrodes to

measure EEG and EMG. At least one week after surgery, each mouse was subjected to two experimental conditions (random order, at least 2 days apart) both starting at light onset (10am). In both experiments, the goal was to maintain firing for 6 hours at levels comparable to or higher than during wake. In the SD experiment, we used exposure to novel objects to keep mice awake. In the second experiment, we delivered light pulses (473 nm) of 1-2 seconds every 5-10 minutes during NREM sleep starting with the first sleep episode and continuing until 6 hours after light onset. During SD, mice slept less than 1% of the time, and the average firing rate in all channels (n=40) was 132.6%+-33.3% of baseline wake. The mean duration of OFF periods increased (+5.8% p=.003) in the first hour of recovery sleep compared to the first hour of baseline sleep. Number and duration of OFF periods increased (+16% p=.01; +10.2% p=.0001) compared to the same circadian time in baseline. During laser stimulation, sleep was unaffected, but the overall (all behavioral states) firing rate of activated channels (n=13) increased to 153.7%+-27.9% of baseline wake. Other channels were minimally or not affected. In the first hour following laser stimulation, number and duration of OFF periods did not change compared to the same circadian time in baseline (p=.9; p=.5). Thus, though firing rates during laser stimulation were comparable to those observed during SD, OFF period measures only increased when neuronal activity was high during wake, but not during sleep. These data suggest that sustained firing alone does not account for the increase in OFF periods following SD.

Disclosures: **A.V. Rodriguez:** None. **C.M. Funk:** None. **G. Tononi:** None. **C. Cirelli:** None.

Poster

851. Optogenetics: Experimental Uses

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 851.03/VV4

Topic: G.04. Physiological Methods

Support: NICHD R15HD075207

Title: Optogenetic studies of synaptic function in spinal motor neurons

Authors: ***A. THARANEETHARAN**, M. A. HARRINGTON;
Biol., Delaware State Univ., Dover, DE

Abstract: Motor neurons have traditionally been thought of as strictly cholinergic in phenotype. However, recent work has shown that motor neurons release glutamate or another excitatory amino acid transmitter at central synapses during the early post-natal period. Earlier work from

our lab and others has shown that in cultures of motor neurons dissociated from embryos, synaptic transmission is almost entirely glutamatergic. We have used transgenic mice expressing a yellow fluorescent protein (YFP) channelrhodopsin2 (ChR2) fusion protein (mhChR2::YFP) under the control of the choline acetyl transferase (ChAT) promoter to assess synaptic function of motor neurons in culture. We investigated cultures established on embryonic day 13.5, before neuromuscular junction formation, and the early postnatal period, after the development of the neuromuscular junction. The ChR2-YFP fusion protein is expressed in cholinergic neuronal populations due to the mouse ChAT promoter/enhancer regions on the bacterial artificial chromosome (BAC) transgene. Like cultures of other types of neurons, cultured motor neurons organize themselves into spontaneously active networks with frequent "network bursts" of synchronized activity that are recorded by all active electrodes. We used the 64-electrode, MED64 multielectrode array to characterize network activity resulting from photostimulation of ChR2 expressing motor neurons. Using immunofluorescence we found changes in ChAT expression from early postnatal cultures as compared to embryonic cultures, while MED64 recordings also show changed bursting activity in postnatal as compared to embryonic. Our results highlight interesting variations between the synaptic activation and network properties of motor neurons established in culture during embryonic development as compared to cultures of motor neurons that have matured in the spinal cord. These findings pose interesting implications upon the use of primary motor neuron cultures and differentiated stem cells as model systems and potential therapeutics for motor related neuropathologies.

Disclosures: A. Tharaneetharan: None. **M.A. Harrington:** None.

Poster

851. Optogenetics: Experimental Uses

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 851.04/VV5

Topic: G.04. Physiological Methods

Title: An optogenetic model to study motor and psychiatric symptoms of parkinson's disease

Authors: *D. ZWILLING, W. ZHU, H. LEE, I. GALLAGER, S. P. BRAITHWAITE, K. R. THOMPSON;
Circuit Therapeut., Menlo Park, CA

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder with an array of symptoms including motor, cognitive and psychiatric components. Pathological hallmarks of

parkinsonian disorders include loss of dopaminergic neurons in the substantia nigra pars compacta that innervate the dorsomedial and dorsolateral striatum. While motor symptoms can effectively be treated with agents such as L-DOPA, there is an unmet need to effectively treat non-motor symptoms such as cognitive impairments and depression. However, the traditional *in vivo* models used in the field only reliably recapitulate the motor component, thus disease-relevant models are needed to address other symptoms. We have generated a mouse model in which the activity of the indirect pathway neurons (D2/A2A) in the dorsomedial striatum is modulated. Using optogenetics we initiated motor symptoms by activating the indirect pathway and demonstrated that the severity of the motor impairment can be titrated by varying the illumination intensity. This establishes a model in which submaximal pathway activation produces a partial motor phenotype that can be reflective of early stages of disease. The titratable motor phenotype can be reversed with an A2A adenosine receptor antagonist. Furthermore, in this optogenetic model psychiatric effects can be dissociated from motor effects. Stimulation of the indirect pathway neurons results in real-time place aversion with stimulation parameters that limit motor dysfunction. Activation of the indirect pathway also reduced sucrose preference, a widely used behavioral paradigm for depression, that is not confounded by motor phenotype. Together these studies demonstrate that an optogenetic model of indirect pathway activation in the dorsomedial striatum poses many advantages in modeling both the progressive motor and psychiatric symptoms of Parkinson's disease. This model can be used to test drugs and address multiple symptoms relevant in disease progression.

Disclosures: D. Zwilling: None. **W. Zhu:** None. **H. Lee:** None. **I. Gallager:** None. **S.P. Braithwaite:** None. **K.R. Thompson:** None.

Poster

851. Optogenetics: Experimental Uses

Location: Halls A-C

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Program#/Poster#: 851.05/VV6

Topic: G.04. Physiological Methods

Support: Boerhinger Ingelheim Award

TILL Photonics Technology Prize

DFG , SPP1665

Title: Optogenetic manipulation of oscillatory activity in the visual system of the cat

Authors: *T. WUNDERLE[1], C. LEWIS[1], J. NI[1,2], I. DIESTER[1], P. FRIES[1,3];
[1]Ernst Strüngmann Inst. (ESI) for Neurosci., Frankfurt, Germany; [2]2 Intl. Max Planck Res. Sch. for Neural Circuits, Frankfurt, Germany; [3]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Optogenetics has become an important tool in the study of brain circuits and their functions. In order to apply this technique to the study of visual processing, we established optogenetics in the cat visual cortex. Cats provide a well-developed visual system with a known hierarchy and high degree of functional specificity. We used an adeno-associated viral vector (AAV2.1 or 2.9) to deliver channelrhodopsin-2 (hChR2-H134R) under the control of a CamKIIα promoter, in order to primarily target excitatory neurons. Neurons were activated through surface illumination and recorded with metal electrodes or with laminar probes during anesthesia. Injections were made either in the primary visual cortex (area 17) or in a higher visual area (21a). With this setup, we tested three different stimulation and recording protocols: (1) Direct stimulation of the infected neurons by placing the optical fiber and recording electrode in the same area where we injected the virus. (2) Feedforward stimulation by placing the optical fiber and electrode in area 21a, while the virus was injected in area 17 (stimulating feedforward axons). (3) Feedback stimulation by placing the optical fiber and electrode in area 17, while the virus was injected into area 21a (stimulating feedback axons). In each scenario, we calculated time-frequency plots to obtain the spectral power of the local field potential (LFP) during light stimulation. Interestingly, for both areas, constant (DC) illumination at the site of injection led to the induction of narrowband oscillations in the gamma frequency range (around 60Hz). The frequency of this oscillation matched the one obtained by visual stimulation with a high contrast moving grating. The amplitude of the optogenetically-induced gamma oscillation increased with increasing light intensity but remained stable at a fixed frequency. A similar narrowband oscillation was induced by DC stimulation of the feedforward axons of area 17 in area 21a, albeit at a lower frequency (~35Hz). On the other hand, stimulation of the feedback axons of area 21a by DC illumination in area 17 did not lead to any noticeable oscillatory activity. However, DC stimulation of these feedback axons, paired with visual stimulation, could selectively enhance the visually induced narrowband oscillations in the gamma range. In summary, we established an optogenetic model system in a highly visual mammal to study the impact of inter-areal interactions on the rhythmic activity of neuronal populations in the visual system. Our results indicate that feedforward projections alone can induce narrowband oscillations, whereas feedback projections selectively amplify visually induced activity.

Disclosures: T. Wunderle: None. **C. Lewis:** None. **J. Ni:** None. **I. Diester:** None. **P. Fries:** None.

Poster

851. Optogenetics: Experimental Uses

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Program#/Poster#: 851.06/VV7

Topic: G.04. Physiological Methods

Support: Boerhinger Ingelheim Award

TILL Photonics Technology Prize

DFG SPP1665

Title: Optogenetic entrainment of neuronal activity in the cat visual cortex

Authors: *J. NI[1,2], T. WUNDERLE[1], C. M. LEWIS[1], I. DIESTER[1], P. FRIES[1,3];
[1]Ernst Strüngmann Inst. (ESI) for Neurosci., Frankfurt, Germany; [2]Intl. Max Planck Res. Sch. for Neural Circuits, Frankfurt, Germany; [3]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: For decades, the cat has been a prime model system to study the neuronal mechanisms of vision. Yet, manipulation of its neuronal circuits with cell-type specificity and high spatiotemporal precision has not been established. Here we report that the injection of genetically engineered viral vectors in areas 17 and 21a can provide expression of channelrhodopsin and corresponding optogenetic control of neuronal activity. We used recombinant adeno-associated viral vectors (AAV2.1, 2.5 and 2.9) to transduce neurons in the visual cortex of adult cats. The vector contained the channelrhodopsin-2 (hChR2) gene under the control of a CamKIIα promoter in order to target excitatory neurons (AAV1/5/9.CamKIIα.hChR2(H134R).eYFP.WPRE.hGH), and was injected either in the primary visual cortex (area 17) or a higher area (area 21a) in three cats. An injected area received 8 µl of virus solution distributed over four 2 µl injections targeted at 1 mm below the cortical surface (virus titer was on the order of 1E13/ml). After 6-7 weeks, neuronal recordings were performed under isoflurane/sufentanil anesthesia using metal electrodes or laminar probes. Optical stimulation with blue light (473 nm) was done by a 200 µm optical fiber glued close to the electrode's tip or by surface illumination via a 2 mm optical fiber. Yellow light (593 nm) was used as a control. Surface and optrode stimulation both caused pronounced firing of the recorded multi-units in area 17 and 21a using the AAV2.1 and 2.9 vector. In area 17, spike latency in response to a 2 ms light pulse was 4.5 ms at a power density of 30 mW/mm2 and decreased with higher light intensities to 2.5 ms (60 mW/mm2). At the same time, firing rates increased with increasing light power, saturating around 70 mW/mm2. Spikes could be entrained to the

stimulation frequency by both rectangular light pulses (at least up to 80Hz) and continuous wave stimulation. However, we could not evoke any spiking or LFP activity using the AAV2.5 viral vector. Transduction quality was assessed by post mortem histology using confocal microscopy of the eYFP fluorescence. We identified individual infected cells for both, the AAV2.1 and AAV2.9 vectors several millimeters away from the injection sites. Interestingly, labeling of somata and their processes was strongest in layer 2+3 and 5+6. For AAV2.5 we could not identify a clear fluorescence, and no labeled cell bodies were observed. This is in agreement with the physiological measurements. In summary, these results demonstrate that hChR2 expression in cat visual cortex mediated by rAAV2.1 or rAAV2.9 provides a promising tool for investigating neuronal processing and in particularly rhythmic activity in visual cortex.

Disclosures: J. Ni: None. **T. Wunderle:** None. **C.M. Lewis:** None. **I. Diester:** None. **P. Fries:** None.

Poster

851. Optogenetics: Experimental Uses

Location: Halls A-C

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Topic: G.04. Physiological Methods

Support: NIH grants (NS041083-10, NS073947)

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Title: Striatopallidal neurons control motor activity through striatal collaterals

Authors: *M. P. SURPRIS[1], J.-H. YOO[1], X. HOU[1], P. LI[1], Y. WANG[1], X. ZHOU[2], J. QU[2], S. DUAN[3], J. LU[4], H. YIN[5], J.-F. CHEN[1];
[1]Neurol., Boston Univ., Boston, MA; [2]Wenzhou Med. Univ., Wenzhou, China; [3]Zhejiang Univ. Sch. of Med., Hangzhou, China; [4]Harvard Med. Sch. and Beth Israel Deaconess Med. Ctr., Boston, MA; [5]Duke Univ., Durham, NC

Abstract: The indirect pathway is considered as the main modulatory locus for basal ganglia control of motor and learning activity and is responsible for inhibiting undesired motor and learning programs, potentially through motor learning/information processes. To further define the critical role of the indirect pathway in the control of motor activity, we have developed two

new transgenic mouse lines expressing ChR2 or Arch-GFP selectively in the medium spiny neurons (MSNs) of the indirect pathway under the control of adenosine A2A receptor (Adora2a) gene promoter. We verified the specific expression pattern of ChR2/Arch in the indirect pathway: i) Green fluorescence protein (GFP, fused with ChR2 or Arch) express in the striatum was largely detected in cell bodies, and was specifically co-localized with the Adora2a but not with substance P+ cells. Consistent with a previous optogenetic study, we found that light activation of ChR2 in the striatopallidal neurons suppress motor activity while light activation of Arch in the striatopallidal neurons in the DLS produced the predicted motor stimulant effect. We further hypothesized that the presence of profuse projections and collaterization within the striatum may contribute to striatopallidal pathway control of motor activity. To test this hypothesis, we optogenetically stimulated and silenced the striatopallidal neurons in the dorsal striatum and examined immediate early gene expression in the striatum. We found that ChR2 activation in the striatopallidal neurons in the DLS induced c-Fos expression in the GFP-positive MSN while Arch activation in the striatopallidal neurons in the DLS induced c-Fos expression in the GFP-negative MSN surrounding the GFP-positive MSN immediately underneath the optogenetic cannula. This is consistent with the suppression of GABA release in GFP-positive cells in DLS, resulting in the induction of c-Fos in the GFP-negative cells with likely the collateral connections with the GFP-positive cells. Our findings raise new questions regarding the complexity of the role of the indirect pathway and the net effect of the collaterals in control of motor activity under normal physiological conditions.

Disclosures: **M.P. Surpris:** None. **J. Chen:** None. **J. Yoo:** None. **X. Hou:** None. **P. Li:** None. **Y. Wang:** None. **X. Zhou:** None. **J. Qu:** None. **S. Duan:** None. **J. Lu:** None. **H. Yin:** None.

Poster

851. Optogenetics: Experimental Uses

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Program#/Poster#: 851.08/VV9

Topic: G.04. Physiological Methods

Support: NIH/NIBIB R00 Award (4R00EB008738)

Okawa Foundation Research Grant Award

NIH Director’s New Innovator Award (1DP2OD007265)

NSF CAREER Award (1056008)

Alfred P. Sloan Research Fellowship

Title: Distinct global dynamics of direct and indirect pathway striatal neurons

Authors: *H. LEE[1], M. CHOY[1], A. WEITZ[2], A. KRAVITZ[3], A. KREITZER[4], J. LEE[2]; [1]Neurol. and Neurolog. Sci., [2]Bioengineering, Stanford Univ., Stanford, CA; [3]NIH, Bethesda, MD; [4]Univ. of California, San Francisco, San Francisco, CA

Abstract: The basal ganglia are functionally heterogeneous structures that play a key role in motor function and many neurological conditions such as Parkinson's and Huntington's disease. The two key pathways of the basal ganglia, known as the direct and indirect pathway, are classically thought to provide opposing effects on downstream targets. In both pathways, the caudate putamen represents the first synapse in the system and distinctively involves the striatal D1- or D2-receptor medium spiny neurons (MSNs). Until recently, the networks driven by D1 and D2 MSNs were difficult to isolate and interrogate separately, since both cell types are highly intermingled within the striatum. With the recent introduction of optogenetics and special transgenic mouse lines, however, they can now be separately accessed and have been shown to contribute to distinct behavioral and electrophysiological outputs. Nevertheless, the large-scale brain networks driven by these pathways remain unknown. We sought to dissect these circuits through direct, *in vivo* visualization of whole-brain activity driven by D1 and D2 MSNs using the novel imaging modality of optogenetic functional magnetic resonance imaging (ofMRI). To enable selective stimulation of the direct and indirect pathway, we targeted D1 and D2 MSNs in the dorsomedial striatum of D1- and D2-cre BAC transgenic mouse lines, respectively. We performed whole-brain fMRI during repeated periods of optogenetic D1 or D2 stimulation. Changes in the blood-oxygen-level dependent (BOLD) signal were observed throughout basal ganglia, cortical, and subcortical regions with varying dynamics and polarity. Importantly, the local signal at the site of stimulation was positive for both D1 and D2 MSN stimulations, confirming a widely debated issue that activity of inhibitory neurons can evoke a positive BOLD response. Surprisingly, we also found that D1 stimulation resulted in positive BOLD signal activity in the substantia nigra and the subthalamic nucleus, which is seemingly contradictory to the classical model of the basal ganglia circuit. However, since D1 stimulation led to increases in thalamic and cortical activity, activation of the corticosubthalamic, or hyperdirect, pathway may in turn have led to activation of the subthalamic nucleus and substantia nigra. Collectively, these results demonstrate that the ofMRI technology can reveal highly distinct, multi-synaptic network function driven by two neuronal populations at the same anatomical location, and provide important insight into the global influence of the direct and indirect pathways.

Disclosures: H. Lee: None. **M. Choy:** None. **A. Weitz:** None. **A. Kravitz:** None. **A. Kreitzer:** None. **J. Lee:** None.

Poster

851. Optogenetics: Experimental Uses

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Program#/Poster#: 851.09/VV10

Topic: G.04. Physiological Methods

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Okawa Foundation Research Grant Award

NIH Director's New Innovator Award (1DP2OD007265)

NSF CAREER Award (1056008)

Alfred P. Sloan Research Fellowship

California Institute for Regenerative Medicine (CL-00518)

Title: Direct *in vivo* assessment of human stem cell graft-host neural circuits

Authors: *A. WEITZ[1], B. BYERS[1], H. J. LEE[2], J. LIU[5], P. LIN[2], P. ZHANG[3], A. SHCHEGLOVITOV[4], R. DOLMETSCH[4], R. REIJO PERA[3], J. H. LEE[1];
[1]Bioengineering, [2]Neurolog. Sci., [3]Inst. for Stem Cell Biol. and Regenerative Med., [4]Neurobio., Stanford Univ., Stanford, CA; [5]UCLA, Los Angeles, CA

Abstract: Stem cell mediated therapies aim to directly restore the neural circuitry lost during disease progression of the central nervous system (CNS). Yet despite the potential of stem cell based CNS repair, successful therapy development has been hindered by the lack of methods that can assess the functional connections and causal interactions between a neural transplant and host circuitry in an intact animal. Although many techniques exist to characterize transplant integration (e.g. *in vivo* tracking of labeled stem cells, visualization of synapse formation, and behavioral monitoring of host organisms), they are all limited by their inability to directly assess the global, functional impact of the graft's electrical activity on host neural networks *in vivo.* Here, we overcome this problem and report a novel method that uses selective stimulation of stem cell-derived human neurons, combined with whole-brain functional magnetic resonance imaging (fMRI), to directly evaluate the causal influence of transplanted neurons as they integrate into the nervous system of a living subject. To achieve selective control of neural graft cells, we first engineered human induced pluripotent stem cells to express the light-sensitive membrane channel channelrhodopsin-2 (ChR2). After differentiating cells to neurons and transplanting them to striatum of rats, we found that transplanted cells successfully engrafted,

expanded, grew projections to distant regions, and maintained ChR2 expression. To identify the global circuitry causally driven by the neural grafts, we performed high-field fMRI during simultaneous optical stimulation of transplanted cells. Stimulation resulted in significant increases in the local, striatal fMRI signal, which was found to coincide with electrophysiologically measured increases in the local neuronal firing rate. Remarkably, optically evoked changes in the fMRI signal were identified in remote regions such as the hippocampus, where increases in neuronal firing rate were also observed. Our findings demonstrate that selective stimulation of neural grafts *in vivo* combined with high-resolution fMRI readouts can be used to non-invasively measure the spatiotemporal dynamics of graft-host neural circuitry at both the local transplantation site and at downstream targets. By having a means to directly compare the neural circuit function that results from different differentiation or transplantation protocols, future studies in regenerative medicine may utilize this technique for the development and optimization of stem cell based CNS therapies.

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Poster

851. Optogenetics: Experimental Uses

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

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Topic: G.04. Physiological Methods

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Okawa Foundation Research Grant Award

NIH Director's New Innovator Award (1DP2OD007265)

NSF CAREER Award (1056008)

Alfred P. Sloan Research Fellowship

Title: Whole brain dissection of central thalamic circuit function with optogenetic fMRI

Authors: ***J. LEE**[1], J. LIU[2], H. LEE[1], P. LIN[1], Z. FANG[1], A. WEITZ[1], R. FISHER[1], V. PINSKIY[3], P. MITRA[3], N. SCHIFF[4];

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Abstract: Neurons within the central thalamus (CT) have a well-established role in forebrain arousal and awareness. As a result, they have been explored as a potential target of deep brain stimulation (DBS) for remediating acquired cognitive disabilities. Until now, the mechanisms of thalamic DBS have largely been studied using electrophysiology techniques that provide limited information on whole-brain activity. In order for such therapies to be effectively designed, however, the causal influence that CT neurons exert on large-scale brain networks must be properly understood. To address this problem and investigate the global functional properties of central thalamic circuits, we combined selective stimulation of distinct CT nuclei at different frequencies with large-scale readouts of brain activity using optogenetic functional magnetic resonance imaging (ofMRI). The behavioral correlates of selective CT stimulations were also studied in separate video-EEG experiments. To achieve selective stimulation, channelrhodopsin-encoding virus was injected unilaterally into the central thalamus region of adult rats and targeted toward specific nuclei, such as the intralaminar group. *In vivo* optical stimulations at 10, 40, and 100 Hz all resulted in widespread cortical recruitment measured with fMRI, including prefrontal, motor, sensory, and limbic regions. Subcortical structures such as the striatum were also recruited. Importantly, distinct patterns of activation were observed across brain regions and frequencies, including delayed responses and both positive and negative changes in the blood-oxygen-level dependent signal. To identify the behavioral correlates of these networks as they relate to arousal, video-EEG experiments were performed during equivalent stimulations in sleeping and awake animals. We found that the likelihood of stimulation leading to arousal could be predicted by specific patterns of circuit function as revealed through ofMRI. Collectively, our results offer insight into the relationship between specific central thalamic circuits, frequency-dependent responses of neuromodulation, and arousal regulation. In addition, they add to a growing body of evidence that ofMRI can dissect complex, causally driven neuronal dynamics across the brain.

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Poster

851. Optogenetics: Experimental Uses

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NIH Director's New Innovator Award (1DP2OD007265)

NSF CAREER Award (1056008)

Alfred P. Sloan Research Fellowship

Title: Optogenetic fMRI reveals distinct, frequency-dependent networks recruited by dorsal and intermediate hippocampus stimulations

Authors: *M. CHOY[1], A. J. WEITZ[2], Z. FANG[2], H. LEE[2], R. S. FISHER[2], W. C. SMITH[3], J. LIU[3], P. LIN[2], M. ROSENBERG[3], J. LEE[2];
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Abstract: Based on a large volume of anatomical, behavioral, and genetic data, it has been argued that the hippocampus is made up of functionally distinct regions along its dorso-ventral axis. However, the way in which these regions contribute to different large-scale networks in the brain remains unknown. To address this issue, we dissected the circuitry causally driven by two anatomically distinct subregions of the hippocampus using optogenetic functional magnetic resonance imaging (ofMRI). Using this novel imaging approach, we selectively stimulated excitatory neurons within the dorsal and intermediate hippocampus while simultaneously visualizing the spatial and temporal dynamics of resulting activity across the brain. To achieve selective control of these two regions, channelrhodopsin-encoding virus was injected into the dorsal (DH) or intermediate (IH) region of adult rat hippocampi. Five frequencies of stimulation, ranging from 6 to 60 Hz, were evaluated to investigate the specific frequency response of recruited networks. IH stimulation resulted in widespread cortical and subcortical recruitment at frequencies between 10 and 40 Hz, whereas DH stimulation led to activity primarily restricted to the hippocampus at all tested frequencies. Within the hippocampus, selective recruitment of the intermediate region was observed during IH stimulation only, and a negative signal in the dentate gyrus was observed with DH stimulation only. These signals may reflect mechanisms that control the spread of synchronized hippocampal activity to other regions of the brain. Within the visualized 4D networks, diverse hemodynamic response shapes were also observed across the brain, including sustained hippocampal responses during high frequency stimulation. Importantly, these high frequency stimulations produced seizure-like afterdischarges in separate video-EEG experiments, with only IH stimulations leading to both electrographic and behavioral seizures. These results suggest that ofMRI-measured activities such as sustained hemodynamic responses and specific patterns of BOLD activation are associated with different seizure phenotypes, and provide insight into the frequency-dependent function of hippocampal networks.

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Poster

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Topic: G.04. Physiological Methods

Support: DFG Grant SPP1665

EMBO Long-Term Fellowship

Marie Curie Actions

Title: Projection-specific optogenetic identification of midbrain dopamine neurons *in vivo*

Authors: ***S. DUVARCI**[1], L.-M. SELESNEW[2], B. KERN[1], G. SCHNEIDER[3], T. SIGURDSSON[1], M. CHILLON[4], J. ROEPER[1];
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Abstract: Midbrain dopamine (DA) neurons are located in the substantia nigra (SN) and ventral tegmental area (VTA) and play essential roles in a variety of brain functions including motor control, reward processing and cognition. In accordance, recent studies suggested that the midbrain DA system is composed of functionally distinct subpopulations of DA neurons with largely non-overlapping projection areas. This projection-specific functional specification exists in both SN and VTA. For instance, in SN, while the lateral SN DA neurons projecting to dorsolateral striatum mediate initiation of habitual actions, the medial SN DA neurons projecting to dorsomedial striatum mediate initiation of exploratory actions. In VTA, DA neurons projecting to the various nucleus accumbens regions mediate different aspects of reward and aversion processing, whereas the prefrontal cortex-projecting DA neurons are involved in cognitive functioning. It is therefore important to target, identify and manipulate DA neurons in a projection-specific manner in the adult mammalian brain. In this study, we aimed to identify DA neurons depending on their projection targets *in vivo*. To this end, we combined *in vivo*

electrophysiological recordings with projection-specific optogenetic identification. We utilized a dual viral strategy by injecting a retrogradely-transported canine adenovirus type 2 (CAV2) carrying Cre-recombinase in one of the projection targets of DA neurons and an adeno-associated virus (AAV) expressing channelrhodopsin-2 (ChR2) in a Cre-dependent manner in the SN or VTA. After assessing the specificity of our targeting approach for striatal target regions, we performed simultaneous single-unit recordings and optical stimulation using a chronically implanted optrode consisting of a bundle of stereotrodes and an integrated optic fiber. Currently, we are using this strategy for *in vivo* identification of distinct populations in the rostro-medial SN, where DA neurons either project into dorsomedial striatum or the lateral shell of the nucleus accumbens. Our next step will be to test the specificity and feasibility of this approach for the projection-specific identification of nucleus accumbens medial shell and core projecting as well as prefrontal projecting VTA DA neurons.

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Poster

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Topic: G.04. Physiological Methods

Support: NIDA (DA025279)

NIDA (DA031900)

Title: Hypocretin regulation of catecholaminergic responses to cocaine

Authors: ***D. L. BERNSTEIN**[1], C. BASS[2], R. ESPAÑA[1];
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Abstract: The mesolimbic dopamine (DA) projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is crucial in transducing the effects of drugs of abuse, such as cocaine. Dopaminergic signaling in this pathway is subject to heavy modulation by a variety of afferent inputs and neurotransmitters. The hypocretins are hypothalamic neuropeptides that promote reward-seeking behavior and exert a strong excitatory effect on DA signaling . For

instance, infusions of the hypocretin-1 peptide into the VTA enhance DA responses to cocaine in the NAc and promotes cocaine self-administration. Conversely, intra-VTA delivery of the hypocretin 1 receptor antagonist, SB-334867, attenuates DA signaling and reduces cocaine self-administration. Hypocretin neurons also send strong projections to the Locus Coeruleus (LC), a major source of norepinephrine (NE) in the brain. While this pathway is important for the regulation of wakefulness and stress, it is also likely to influence reinforcement processes associated with cocaine. Cocaine increases signaling of both DA and NE, largely through actions on the DA and NE transporters. Therefore, it is possible that hypocretin-induced alterations in NE signaling could contribute to cocaine seeking. To examine the extent to which the hypocretin system modulates phasic DA or NE signaling, we used optogenetics in combination with fast scan cyclic voltammetry. In initial studies, we injected the VTA with 0.5 µl of a virus that expresses ChR2-EYFP, with expression driven by a tyrosine hydroxylase promoter. Following a 4-week period, animals were placed into a stereotaxic apparatus and implanted with a carbon fiber electrode in the NAc and an optrode in the VTA. Pulses of 473nm light were used to evoke release of DA, and electrode positions were adjusted until a stable baseline of release was obtained. Preliminary results suggest that optogenetic stimulation of DA neurons reliably produced DA release in the NAc, which was capable of being modeled for concentration and uptake kinetics. Further, these studies also suggest that blockade of hypocretin 1 receptors reduced evoked DA release and attenuated DA responses to cocaine. Our continuing studies focus on targeting of NE cells of the LC and examining the extent to which hypocretins regulate NE signaling under baseline conditions and following cocaine. Together, these studies offer additional evidence for hypocretin's involvement in the regulation of DA and NE signaling and its involvement in the regulation of reward and reinforcement processes underlying cocaine abuse.

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Poster

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Topic: G.04. Physiological Methods

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The State of Sachsen-Anhalt and the “European Regional Development Fund” (Center for Behavioral Brain Sciences, CBBS)

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Title: Imaging the functional networks activated by optogenetic VTA stimulation in rats with fMRI and SPECT

Authors: M. J. BROCKA[1], D. VINCENZ[1], J. TEGTMEIER[1], T. LÜCKNER[1], K. TAKAGAKI[1,2,3], T. WANGER[1], J. GOLDSCHMIDT[1,2], F. W. OHL[1,2,3], F. ANGENSTEIN[1,2,4], *M. T. LIPPERT[1];
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Abstract: The ventral tegmental area (VTA) is a major source of dopaminergic projections in the brain. As such, it plays a central role in learning and reward-associated behavior. Here we present cell-type specific optogenetic stimulation, behavioral training in an intracranial self-stimulation task and functional imaging of the resultant brain-wide activity patterns with fMRI and 99mTc-HMPAO SPECT imaging of regional cerebral blood flow. To combine these different techniques, we developed an fMRI-compatible fiber-optic implant as well as an optical coupler to administer light stimulation inside an MRI scanner. The implant enables rapid and stress-free connection of the animal to the light source during training, and does not interfere with either fMRI or SPECT imaging. In addition, our micro-prism based coupler allows for low light-loss stimulation even in spatially confined RF-coils, necessary to achieve high SNR. Our results show a partial dissociation of functional activity evoked by optogenetic VTA stimulation and anatomical projection regions of the VTA. Evoked activity in the tectal region and hippocampus is significantly stronger than those in mPFC, NAcc and striatum. Similar to previous reports, we also find only comparatively small signals at the immediate stimulation site, despite vigorous dopamine release throughout the brain, which confirms effective stimulation. A comparison of functional networks activated by this stimulation during wakefulness and anesthesia supports the hypothesis that the influence of dopamine on cerebral blood flow unfolds through modulation of underlying activity instead of through direct action. This could explain the local dissociation of blood flow changes and dopamine release.

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Poster

851. Optogenetics: Experimental Uses

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Title: Optogenetically-induced spatiotemporal gamma oscillations and neuronal spiking activity in primate motor cortex

Authors: *Y. LU[1], W. TRUCCOLO[2,3,5], C. E. VARGAS-IRWIN[2,3], I. OZDEN[4], J. B. ZIMMERMANN[2,3], T. MAY[4], F. WAGNER[2], A. V. NURMIKKO[4,3];
[2]Neurosci., [3]Inst. for Brain Sci., [4]Engin., [1]Brown Univ., Providence, RI; [5]DVA, Ctr. for Neurorestoration and Neurotechnology, Providence, RI

Abstract: There is considerable interest in cortical gamma oscillations (40-80 Hz) which may play multiple important roles in cortical function, both in the healthy and diseased brain. Gamma oscillations are thought to provide a spatial and temporal structure for information processing in the neocortex. Here, combining local optogenetic stimulation with multichannel microelectrode array recording, we were able to study how gamma local field potential (LFP) oscillations can be induced in primate neocortex, and how their various transient spatiotemporal patterns within a sampled 4 by 4 mm neocortical patch evolve in time. A novel monolithic intracortical device with 95 recording electrodes and a polymer optical fiber positioned at the center of the array was used, allowing simultaneous electrophysiological recording and optogenetic stimulation. The optoelectronic array was chronically implanted in primary motor cortex (M1) of one monkey and ventral premotor cortex (PMv) in another monkey. Targeted brain tissue was transduced with the red-shifted opsin C1V1 (from K. Deisseroth). Constant light stimulation (1s square pulses) induced very clear narrowband (40-80 Hz) gamma oscillations during wake resting states. Increasing 'ramp' stimulation also showed that gamma oscillations appeared only after a critical stimulation level. The oscillations were induced not only within the direct light stimulation volume (few hundred microns from the fiber tip) but also, via network interactions, at more distant sites on the microelectrode array. Transient gamma spatiotemporal patterns including

concentric expansion patterns and spiral waves were mapped. Despite observed modulation of neuronal spiking rates, point process models showed that coupling between single-neuron spiking and the phase of gamma oscillations was weak, with spiking remaining highly asynchronous and irregular during induced gamma LFP oscillations. In one monkey, where it was also possible to track optogenetically induced gamma oscillations during movement preparation and execution, gamma oscillations were largely attenuated at movement onset. Overall, our findings demonstrate that beyond previously reported gamma resonance mechanisms, strong enough constant inputs may carry network dynamics into gamma oscillations likely via a Hopf bifurcation. Furthermore, our framework combining microelectrode array recordings and optogenetic stimulation provides an important tool for probing spatiotemporal dynamics in primate cortical networks during various physiological and behavioral conditions.

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Poster

851. Optogenetics: Experimental Uses

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Title: Optogenetic and 3-D anatomical mapping of intact neural circuits of the dorsal raphe nucleus

Authors: *J. B. TREWEEK[1], J. CHO[2], C. XIAO[3], L. R. BREMNER[3], B. YANG[3], C.-K. CHEN[3], V. GRADINARU[3];
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Abstract: A hallmark of chronic stress disorders in both humans and rodent models alike is the diminished capacity to cope during traumatic stress. In addition to regulating emotional status, the dorsal raphe nucleus (DRN) plays a role in patterning the psychobehavioral response to stressors, a feat accomplished in part through its numerous points of contact with the corticotropin releasing factor (CRF) system. Notably, the DRN is among only a few brain regions to express both major CRF receptor subtypes: CRF_1 and CRF_2. And, innervating these CRF-responsive DRN neurons are CRF-immunoreactive (ir) afferents from hypothalamic and extrahypothalamic sources, including the amygdala and bed nucleus stria terminalis. That a subset of DRN 5-HT neurons also co-express CRF hints at the potential for CRF system feedback between the DRN and the extended amygdala, or for DRN neurons to transmit stress-related signals to its limbic forebrain targets. Despite these provocative initial reports of CRF-5HT crosstalk at the neuronal level, the topography of DRN circuits and the mechanism through which they modulate anxiety have remained poorly characterized. Hindering their further study was the neuronal heterogeneity of the DRN and the inherent neuroplasticity of the CRF system, wherein CRF_1 and CRF_2 exhibit activity-dependent shifts in their membrane localization. Herein, optogenetic tools, which grant local or retrograde opsin expression in Cre recombinase-expressing neurons, and a whole-brain clearing protocol, which we recently developed, now enable the isolation, activity modulation, and fiber-tracing of DRN circuits, including both their sources of innervation and their projections. Thus, using rodent crosses between Cre driver lines for CRF_2, tyrosine hydroxylase (TH), and tdTOMATO, we endeavored to map the sources of CRF input to the DRN (1), to determine the neurochemical identity and projection pathways of DRN CRF-responsive cells (2), to trace DRN CRF-ir cells to their post-synaptic targets (3), and to study the functional relevance of CRF activity in the DRN (4). With respect to the latter, subjects were exposed to rounds of behavioral stress during the simultaneous optical excitation or inhibition of CRF_2-gated circuits or of DRN TH neurons, respectively, followed by EEG-EMG sleep recording. It follows that our 3D visualization of DRN CRF-responsive circuits sheds light onto the neuroanatomical connectivity between the DRN and brain areas involved in the neuroendocrine stress response. Our future work will continue to explore this hypothesized interaction between wakefulness-promoting DRN TH cells and the CRF stress system.

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Poster

851. Optogenetics: Experimental Uses

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Support: National Natural Science Foundation of China NSFC 91132306

Strategic Priority Research Program (B) XDB02050003

Title: A subpopulation of neurons in the medial intermediate layer of the superior colliculus mediates visually guided innate defensive responses

Authors: *P. WEI[1], N. LIU[1], X. LIU[1], Z. ZHOU[1], L. WANG[1,2];
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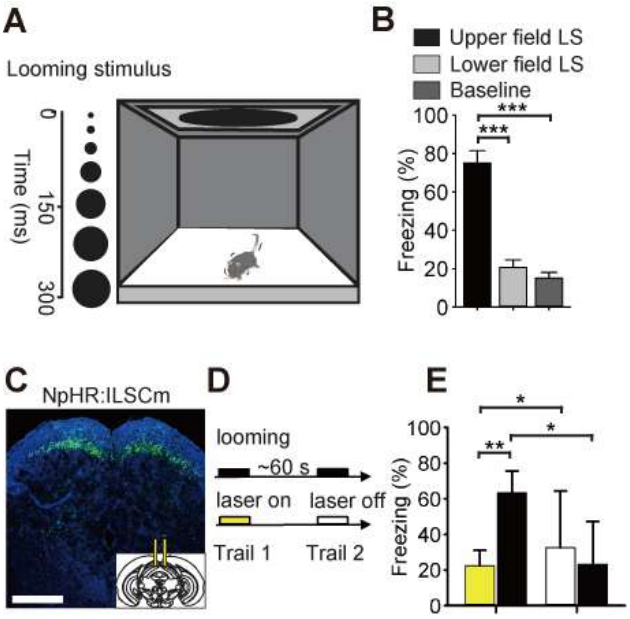
Abstract: Innate fear reaction to threat stimuli is a basic survival capability across species, but the underlying neural circuit remains to be elucidated. A recent study reported laboratory mice innately respond to an above looming visual stimulus, which mimics appearance of an aerial predator, by freezing or flight behavior. Here, we found that this visually guided innate defensive response was associated with activation of a population of glutamatergic neurons in the medial intermediate layers of superior colliculus (ILSCm), and optogenetics inhibition of these cells blocked the expression of the behavioral response. Reversibly, optogenetics activation of these ILSCm excitatory neurons partially imitate the visually salient stimulus which initiated an exaggerated long-lasting freezing followed by sustained anxiety-like responses. The mammalian superior colliculus is an important structure for rapid responding to a biological salience by receiving and transforming sensory inputs to behavior reflection. Our current results suggest that the subpopulation of neurons in ILSCm could detect and signal the overhead motion information as a potential threat and initiate the innate defensive responses without conditioning.



Disclosures: **P. Wei:** None. **N. Liu:** None. **X. Liu:** None. **Z. Zhou:** None. **L. Wang:** None.

Poster

852. Modeling Neurons, Circuits, and Behavior

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant R01 MH086638

Title: Calcium regulation of HCN supports persistent activity associated with working memory: A multiscale model of prefrontal cortex

Authors: *S. A. NEYMOTIN[1,2], R. A. MCDOUGAL[2], M. L. HINES[2], W. W. LYTTON[1,3]; [1]Physiol. & Pharmacol., State Univ. of New York, Downstate Med. Ctr., Brooklyn, NY; [2]Neurobio., Yale Med. Sch., New Haven, CT; [3]Neurol., Kings County Hosp. Ctr., Brooklyn, NY

Abstract: "Bump attractors" are hypothesized to enable short-term memory via persistent activation in prefrontal cortex (PFC). They have been primarily assessed in terms of electrical mechanisms, without attention to molecular events. To assess this contribution, we developed a multiscale model in NEURON going from molecular to network levels, assessing contribution of

calcium (Ca) release from endoplasmic reticulum (ER) to alteration in hyperpolarization-activated cyclic-nucleotide gated channels (HCN) thought to provide continued activity via rebound. The network had 800 neurons arranged in 6 cortical layers. Neurons included pyramidal (E) and 2 interneuron (I) types, with Na, K, Ca, and HCN channels. Cells connected with AMPA/NMDA/GABAA synapses using data from M1. Metabotropic glutamate receptors (mGLUR) produced inositol triphosphate (IP3). Intracellular components included: Ca, Ca buffers, ER Ca stores, IP3, ER IP3 receptors (IP3Rs; release ER Ca), sarco/ER Ca-ATP-ase pumps (SERCA; pump Ca into ER), Ca extrusion pumps, E cell HCN regulated by Ca-bound protein kinase. 18 s of reaction-diffusion simulation ran on 24 Intel XEON CPUs in 7 minutes. Stimulus-induced depolarization led to Ca influx via NMDA and L-type channels. After a delay, mGLUR activation led to ER Ca release via IP3Rs. These factors increased HCN conductance and firing (0.5-8 Hz), lasting 5-10 s. During this time, alpha oscillations decreased, and beta/gamma increased. Non-stimulated cells were suppressed from more inhibition via extra drive from activated to I cells. The network encoded stimulus strength in the ratio of firing rates of stimulated vs non-stimulated neurons (firing-rate distinction; FRD). The network supported stimulus-induced switching between 2 populations (P1, P2) with sensitivity to inter-stimulus delays. Short delays suppressed P2's response, due to P1's dominance. Free Ca regulated FRD and was manipulated via parameter changes, e.g.: 1. Ca extrusion pump time constant (tau) had an inverted-U relationship with FRD: slow tau caused Ca to saturate all neurons; fast tau prevented Ca from having time to regulate neurons. 2. Lowering concentration or binding rate of Ca buffers caused Ca to saturate all neurons, reducing FRD. 3. ER Ca stores modulated network excitability: both SERCA rate and priming time of ER stores correlated positively with FRD, since both provided ER with more Ca (released after mGLUR stimulation). The network therefore supported nonsynaptic plasticity, a Ca-dependent memory trace of neuronal excitability modulation. Our model demonstrates nanoscale electrochemical interactions may lead to persistent activity associated with working memory.

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Poster

852. Modeling Neurons, Circuits, and Behavior

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Topic: G.06. Computation, Modeling, and Simulation

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NIH

Title: Stochastic diffusion simulation in NEURON

Authors: *C. TROPPER[1], M. PATAORY[2,3,4], R. MCDOUGAL[3], M. HINES[3], W. LYTTON[4]; [1]Dept. of Computer Sci., McGill Univ., Montreal, Canada; [2]Dept. of Computer Sci., McGill Univ., Montreal, QC, Canada; [3]Neurobio., Yale Univ., New Haven, CT; [4]Physiol. and Pharmacol., SUNY Downstate, New York City, NY

Abstract: Neuronal simulation with reaction-diffusion presents a variety of computational demands. Among these is the need to couple simulation of the large volumes of major dendrites and soma with simulation in small structures such as the dendritic spine. The former situation requires deterministic (Fick's law) simulation for reason of efficiency, whereas the latter requires stochastic simulation due to the variable effects of small *n* which requires that individual molecules or ions be handled independently. We are developing a subsidiary parallel discrete event simulator interfaced with 1D and 3D deterministic diffusion within the NEURON simulator. We are developing Neuron Time Warp (NTW, a stochastic method based on the next sub-volume method (NSM) an outgrowth of the Gillespie algorithm. We compared a shared memory version of our simulator to a MPI based version because of the continuous increase in the number of cores in multi-core machines. We have discovered that the shared memory version consistently out-performed the MPI based version in spite of the increased synchronization necessary due to the shared memory. Because of the relatively small number of cores (8) in our platform, the priority queue did not experience as much contention as it would have if the machine had a larger number of cores. Further work on priority queue algorithms will be necessary. We have also implemented threads in NTW in order to better take advantage of multi-core machines. While our results indicated that execution time scaled well with the number of processors, the number of rollbacks also increased and caused the decrease in execution time to flatten. Both dynamic window management and dynamic load balancing are necessary in order to contain the number of rollbacks. (The window size controls the optimism of Time Warp, preventing an excessive number of rollbacks). We have previously developed AI based algorithms for dynamic load balancing and window management-we are going to implement them in NTW (simulated annealing, multi-state Q-learning and genetic algorithms) shortly. We note that threads complicate matters for load balancing, as both the thread level and the processor level have to be accounted for differently when developing algorithms to deal with load balancing.

Disclosures: C. Tropper: None. **M. Pataory:** None. **R. McDougal:** None. **M. Hines:** None. **W. Lytton:** None.

Poster

852. Modeling Neurons, Circuits, and Behavior

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Title: Calcium 'impedance mismatch' -- the role of geometry on diffusion dynamics

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Abstract: Calcium (Ca) is a major intracellular second messenger. As such, it is heavily regulated within a neuron and is thought of as forming a partnership with membrane voltage in providing the major state variables across the dendritic tree of a pyramidal cell. In many pyramidal cells, regenerative calcium waves propagate through a portion of the morphology in response to a multitude of factors, including inputs to metabotropic glutamate receptors providing IP3 that releases Ca from intracellular stores, and contributions from plasma membrane Ca fluxes from both NMDA receptors and voltage-sensitive calcium channels. We have begun to explore these complex interactions using a new version of the NEURON simulator, a widely used tool for the computational study of neuronal electrophysiology, now being extended to provide a specialized reaction-diffusion syntax. In NEURON version 7.3, diffusion was restricted to one-dimension. It has now been extended to also support 3D simulation in the developmental version available online. Prior experimental observations demonstrated that Ca waves often approach the soma but rarely enter it, even though the soma can sustain wave propagation. We investigated the specific role of geometry in wave blocking by varying apical dendrite broadening as well as the specifics of the shape of the dendrite-soma connection in a deterministic simulation of diffusion augmented by calcium-induced calcium release. Abrupt expansions in geometry slowed or blocked wave propagation in a parameter-dependent fashion in a manner reminiscent of the membrane depolarization failures seen due to electrical impedance mismatch, which occurs where small low-current-sourcing dendrites attempt to drive the much larger somatic membrane. We therefore characterize this is a [Ca]-

impedance mismatch and compare and contrast with the voltage impedance mismatch at transition points, using identical geometry.

Disclosures: **R.A. McDougal:** None. **M.L. Hines:** None. **W.W. Lytton:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH grant R01MH086638

Title: Integrating Systems Biology Markup Language (SBML) with NEURON

Authors: ***A. S. BULANOVA**[1], R. A. MCDOUGAL[2], S. A. NEYMOTIN[3], V. K. MUTAI[2], W. W. LYTTON[3], M. L. HINES[2];
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Abstract: The NEURON simulator software is widely used by the computational neuroscience community for electrophysiology modeling. We have recently extended it to provide support for reaction-diffusion dynamics (RxD class). Many reaction simulations, written for a variety of different cell types in various organs, have been developed in the Systems Biology Markup Language (SBML), a standard XML-based format supported by over 200 software packages. We therefore have developed routines to import SBML simulations into NEURON, enabling NEURON users to import a large number of previously developed cell biology models and use them in computational neuroscience research. These can then combine with NEURON's electrophysiological simulation capabilities, and with other intracellular reaction models written in the new RxD syntax. This allows combined use of 2 extensive collections: the neuroscience ModelDB (http://senselab.med.yale.edu/modeldb/) and the SBML BioModels (http://www.ebi.ac.uk/biomodels-main/) databases. Import and export of SBML models is handled using the libSBML library (http://sbml.org/Software/libSBML). A critical feature is a method to match state variables across the different models and different modeling levels. We also give the user facilities to make sure that semantically identical parameters are identified across models and to manage parameters, regardless of their origin. The importation procedure is as follows: 1) an electrophysiology model is loaded from ModelDB or constructed de novo; 2)

NEURON loads SBML model descriptions (and creates corresponding SBMLModelComponent objects) 3) the user identifies semantically identical names across the component model name spaces; 4) Diffusion constants are associated with appropriate SBML states; 5) appropriate RxD objects (rxd.Region, rxd.Species, rxd.Reaction) are instantiated; 6) the user adjusts parameters and makes simulation runs of the model.

Disclosures: **A.S. Bulanova:** None. **R.A. McDougal:** None. **S.A. Neymotin:** None. **V.K. Mutai:** None. **W.W. Lytton:** None. **M.L. Hines:** None.

Poster

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NHARP 003652-0136-2009

Title: Detection and morphological quantification of dendritic spines from *in vivo* multi-photon images

Authors: ***D. LABATE**[1], P. K. SINGH[1], P. HERNANDEZ-HERRERA[2], T. KECK[3], M. PAPADAKIS[1];
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Abstract: Despite the significant advances in the development of automated image analysis algorithms devoted to the study of neuronal structures, current software tools offer only rudimentary capabilities for the analysis of dendritic spines; for example, they cannot reliably estimate spine volume and shape and typically require time-consuming and labor-intensive expert intervention. The problem is especially challenging in *in vivo* imaging, where the difficulty of extracting morphometric properties of spines is compounded by lower image

resolution, higher noise levels due to numerous labeled processes, and tissue motion due to blood circulation and respiration. To address this challenge, we introduce a new computational framework for the automated extraction and quantitative analysis of dendritic spines from *in vivo* multi-photon imaging. This framework includes: (i) a new algorithm for 3D image segmentation tailored to robustly extract dendritic tree features even under very low signal-to-noise ratio; (ii) a centerline algorithm that computes the central axis of dendritic branches; (iii) an algorithm that detects spine location with respect to the centerline trace and extracts the complete 3D structure of dendritic spines. This framework enables the computation of a wide range of geometric features such as spine length, spatial distribution and spine volume in a high-throughput fashion. We illustrate our approach for the automated extraction of dendritic spine features in time-series multi-photon images of layer 5 cortical excitatory neurons from the mouse visual cortex. Our computational framework will facilitate the development of a scalable software platform that can rapidly and objectively process large-scale multi-photon images from complex neuronal structures.

Disclosures: **D. Labate:** None. **M. Papadakis:** None. **P.K. Singh:** None. **P. Hernandez-Herrera:** None. **T. Keck:** None.

Poster

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Support: Project for Developing Innovation Systems and Grants-in-Aid for Scientific Research in a Priority Area and Targeted Proteins Research Program from the Ministry of Education, Science, Sports and Culture, Japan

Title: Computational analysis of the effects of anti-neoplastic agents on intraneuronal transport of human iPSC derived neurons

Authors: ***H. NAKAMURA**[1,2], N. YAMASHITA[1], Y. KANAMARU[3], Y. SEKINO[4], T. GOTOH[3], F. TANAKA[2], Y. GOSHIMA[1];
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Abstract: Intraneuronal transport is a fundamental mechanism for survival, morphogenesis, and function of neurons. This transport system is affected in several neurodegenerative disorders. We have developed an automated monitoring system for axonal transport in primary cultured chick dorsal root ganglion (DRG) neurons, in which chloromethylbenzamide dialkycarbocyanine (CM-DiI) was utilized to visualize membranous organelles transported along the axons. We here applied this system to assess the intraneuronal transport in human neurons differentiated from induced pluripotent stem cells (iCell Neurons, Cellular Dynamics International). iCell Neurons were generated through a proprietary forebrain differentiation protocol resulting in a population of neurons without glial or oligodendrocyte cells (Cellular Dynamics). The histograms of instantaneous velocity of anterograde and retrograde intraneuronal transport of iCell Neurons showed a bimodal distribution pattern. We further assessed the effects of anti-neoplastic drugs on intraneuronal transport of iCell Neurons. Vincristine and paclitaxel suppressed anterograde and retrograde intraneuronal transport significantly. Cisplatin and oxaliplatin did not produce the effects on intraneuronal transport of iCell Neurons. Our system may be useful for evaluation of intraneuronal transport in human neurons. For future research, this system will be applied for studying neurodegenerative disorders.

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Poster

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Support: Hunkele Dreaded Disease Award

Samuel and Emma Winters Foundation Award

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Pittsburgh Tissue Engineering Initiative Interface Seed Grant

Title: In-vivo and in-silico studies implicate interleukin (IL)-18 as a central mediator in chronic pain

Authors: *K. VASUDEVA[1,2], Y. VODOVOTZ[4], N. AZHAR[4], D. BARCLAY[4], J. M. JANJIC[3,2], J. A. POLLOCK[1,2];
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Abstract: Neuropathic pain affects more than 6 million Americans and is caused by injury to the nervous system. Following nerve injury, Wallerian degeneration or anterograde degeneration occurs as part of the neurodegeneration of the injured axons in the peripheral nervous system, leading to inflammation and neuropathic pain. Both innate and adaptive immune responses contribute to pain sensitization by producing various inflammatory mediators. However, the interplay among these cytokines and chemokines following peripheral nerve injury is unclear. We hypothesized that key inflammatory interactions can be defined by computational modeling based on the dynamics of protein-level expression of inflammatory mediators in the sciatic nerve of rats subjected to chronic constriction injury (CCI). Here we show that elevated pain sensitivity in CCI rats was correlated with changes in the tissue expression of 14 inflammatory mediators. Dynamic Bayesian Network (DBN) inference strongly implicated the cytokines interleukin (IL)-1β and IL-18 as central nodes in CCI-induced inflammation and neuropathic pain in this animal model. Immunofluorescence confirmed the presence of IL-1β and IL-18 in infiltrating macrophages. Though IL-18 has been implicated previously in inflammation in both the central and peripheral nervous system, this is the first study to utilize data-driven computational modeling to define an inflammatory target in the setting of chronic pain.

Disclosures: K. Vasudeva: None. **Y. Vodovotz:** None. **N. Azhar:** None. **D. Barclay:** None. **J.M. Janjic:** None. **J.A. Pollock:** None.

Poster

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Support: 5R90DA033460-02

NSF-DMS-1042134

Title: Dynamic Neural Simulator - a simple tool for rapidly building and sharing large neural models

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Abstract: The Dynamic Neural Simulator (DNS) is a tool for rapid prototyping of large neural models, batch simulation management, and efficient model sharing. It is designed to speed up and simplify the process of generating, sharing, and exploring network models of neurons with a few compartments. It requires no user programming and interfaces a crowd-sourced model database (DNS DB) to facilitate model sharing and modular model building. DNS DB includes cellular mechanisms, spiking and rate neurons, and networks of them; users can arbitrarily combine existing models stored in DNS DB or on their local machine, create new ones and add them to DNS DB for others to use. The graphical interface (DNS GUI) has interactive and batch modes. In interactive mode, a running simulation with real-time display is automatically updated as users adjust parameters, add ionic mechanisms to compartments, compartments to cells, new populations, and connections between them. Auxiliary functions and network connectivity can be interactively tuned during simulation. In batch mode, users indicate variations on the active model and parameters to simulate and have the option of running simulations serially on a local machine or submitting them to a compute cluster for parallel simulation; results are automatically analyzed, plotted, and stored according to user specifications. DNS includes tools for visualizing results of past simulations, performing across-batch meta-analysis, and visualizing performance metrics over a batch's model and parameter search space. DNS GUI has two views for specifying models: an equation view for computationalists and a semantic view for experimentalists to construct models by combining meaningful labels that point to existing models stored locally or in DNS DB. The design of DNS incorporates a simulator-independent model specification that facilitates interoperability with other specifications (e.g., NeuroML, SBML), simulators (e.g., NEURON, GENESIS), and web-based applications (e.g., Geppetto). The software is presently implemented in Matlab to leverage its efficiency of vectorized computation and may be ported to Python in the future for easier integration with web applications. Present limitations of DNS include a lack of spatial representation and minimal support for discrete-event simulation. The hope is that this tool will reduce barriers to exploring dynamics in complicated neural models, facilitate collaborative modeling, and complement other tools being developed in the neuroinformatics community.

Disclosures: J.S. Sherfey: None. **N. Kopell:** None.

Poster

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Title: Multiscale modeling of the Pacinian corpuscle shows that receptor depth and structure contribute to unique response of receptor

Authors: *J. QUINDLEN[1], V. K. LAI[2], V. H. BAROCAS[1];
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Abstract: Stretch receptors within the skin respond to different mechanical stimuli and have distinct geometries, but it is unknown precisely how structure determines sensitivity. The Pacinian corpuscle (PC) is a tactile mechanoreceptor located in the dermis of the skin. The PC is comprised of lamellae layers, which serve as mechanical filters to the nerve fiber at its core. It responds to high frequency vibrations with 10 nm sensitivity and allows the perception of distal events in its large receptive field. The goal of this study was to determine the effect of structure and receptor depth on PC sensitivity through a multiscale finite-element model (FEM). We hypothesized that a layered, anisotropic structure, embedded deep within the skin, would produce low spatial sensitivity and nonlinear strain transmission. To observe the effect of structure, a layered, ellipsoidal model of the PC was utilized. Previous studies that used standard FEM under-predicted PC deformation to small indentations (Güçlü et al., 2006). A multiscale FEM was used, containing pre-aligned Delaunay networks rotated to form ellipsoidal shells around the PC core. Unlike a homogeneous model, the structural model was able to predict the decreased displacement of nodes closer to the center and therefore simulate the nonlinear transmission of displacement through the receptor. The reduction in displacement of lamellae located closer to the core is in agreement with the hypothesis that the layers act as filters that suppress low-frequency vibrations and large compressions that could damage the nerve fiber. To measure the effect of receptor depth, an ellipsoid model was embedded either 125 μm or 2.125 mm below the surface of a Neo-Hookean mesh containing networks to mimic the epidermis or dermis, respectively. In both models, a node located on the surface of the mesh was indented by 10 μm and strain at the nerve fiber was calculated. This study uses the working model that stretch along the axes of the nerve fiber causes stretch-gated Na+ channels to open and initiates a neural response. The epidermis model resulted in more concentrated strain around the PC that drops off as the indenter moves away from the receptor. This results in more sensitivity and a smaller receptive field. The dermis model resulted in a more uniform strain distribution and

larger receptive field. This study suggests that the nonlinear sensitivity and large receptive field of the PC can be attributed to its structure and location. Thus, these physiological properties are necessary to capture the mechanical response of the PC. The accurate simulation of PC mechanics is a major step in creating a mechano-to-neural transduction model of the receptor.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Title: A novel approach to modeling cortical activation of forearm muscles using genetic programming

Authors: *J. BAARBÉ[1,2,3], H. SALEHINEJAD[2], D. FORMAN[3], M. W. R. HOLMES[1]; [1]Fac. of Hlth. Sci., [2]Dept. of Electrical, Computer, and Software Engin., Univ. of Ontario Inst. of Technol., Oshawa, ON, Canada; [3]Sch. of Human Kinetics and Recreation, Mem. Univ. of Newfoundland, St, John's, NL, Canada

Abstract: The Boltzmann sigmoidal function has been used traditionally to model activity of neurons with wide applications including H-reflexes, cervicomedullary evoked potentials (CMEPs) and motor evoked potentials (MEPs). Although a notable function, applications of the Boltzmann sigmoidal function may be improved by utilizing an evolutionary genetic programming (GP) technique that applies advanced varieties of mathematical operators and constants to fit the data. The aim of this work was to conduct a comparative study on methods for modeling cortical activation of forearm muscles that occur with changes in angle and plane during static shoulder positions. Ten participants (8 male, 2 female, mean age: 22.4 ± 2.5 years) positioned their arm at three elevation angles (45º, 90º and 120º) in two planes (flexion and abduction) for a total of six postures per participant. Surface electromyography (Cambridge Electronic Design, Cambridge, UK) monitored muscle activity from four muscles including extensor carpi ulnaris (ECU), extensor carpi radialis (ECR), flexor carpi ulnaris (FCU) and flexor carpi radialis (FCR). Single pulse transcranial magnetic stimulation (TMS) was delivered sequentially to vertex between 85% and 205% of participants' resting threshold. To analyze the data, peak-to-peak MEP amplitude was fitted to an advanced function using GP (Eureqa, Nutonian, Somerville, MA), and data was also fitted to the Boltzmann sigmoidal function using

the Levenberg-Marquardt method. Mean squared error was assessed separately for each function and results were compared to each other with independent student t tests. Significantly less mean squared error was found when using the GP technique. Preliminary results from one participant demonstrate that during shoulder abduction at 45º, muscle activity of ECR with the GP technique produces a mean squared error of 1.81×10^(-5) and with the Boltzmann sigmoidal function, a mean squared error of 0.85. Further characteristics including slope of the steepest section on the curve and plateau levels were also assessed using the GP technique and Boltzmann sigmoidal function. Results were compared with independent t tests in order to assess differences between the two techniques and to model characteristics of the four muscles across the three elevation angles (45º, 90º and 120º) and two planes (flexion and abduction). Findings suggest that the GP technique may be used to model characteristics of stimulus-response curves and is comparable to traditionally-used techniques for comparing patterns of muscle activity.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH NS40412

NIH NS54894

NSF CRCNS IIS 0827684

Title: A computationally efficient 3D muscle simulation

Authors: *A. RAMAKRISHNAN[1], S. GISZTER[2];
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Abstract: Conventionally, biomechanical models describe muscles as massless Hill-type line actuators acting through mono-anchored tendons on a skeletal frame which carries the lumped mass of that limb segment. These approximations fail to capture the inertial and viscoelastic properties of muscles and the muscle-muscle and muscle-bone interactions. These interactions are crucial in determining the lines of action of the muscle force and the inertial properties of the

segment. Tendons, in real life, attach over an extended area of the bone and have a richer moment application which is also not captured in a line actuator model. Further muscle recruitment and proprioception could be better modeled in a 3D environment. Hence the construction of a detailed realistic biomechanical model coupled to a compatible computational model is essential. We have developed a novel technique for biomechanical simulation by separating the 3D muscle wrapping and the physical plant simulation. A very high resolution (13micron voxel size) 3D mesh model of an adult bullfrog was constructed in Autodesk Maya by segmenting block-face images. Muscles were modeled as constant volume objects. We build a corresponding physical model in Opensim with paired forces at the muscle origin and insertion locations and the muscle mass represented at its mass center. In a simulation, Opensim, which supplies kinematic information, and Maya, which provides muscle lines of action and mass centers, work in a frame-by-frame loop. Preliminary static analysis of a simple 2 link-2 muscle model reveals a shift in center of gravity of individual members up to 16% of muscle length, but a shift of the entire segment center of mass approximated as a rigid body of only 2.4% of the link length between full flexion and full extension. This means rigid body approximations which have been used for decades may have represented controlled movements pretty well, possibly within acceptable tolerance for most tasks. We speculate that rotational effects of the muscle mass motions may also be folded into rigid body moment of inertia parameter approximations. However with the high demand for additional detail in today's biomechanical simulations, we believe that our extensible and therefore 'future-proof' strategy of separating the volumetric simulation and physical plant simulations will enable the transition into building still more realistic future musculoskeletal biomechanical models to occur more seamlessly. Development supported by NIH NS40412, NS54894 and NSF CRCNS IIS 0827684.

Disclosures: A. ramakrishnan: None. **S. Giszter:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Title: Bayesian estimation of traction force generated by neuronal growth cone

Authors: *S. KOZAWA[1,2], Y. SAKUMURA[1,3,4], M. TORIYAMA[5], N. INAGAKI[4], K. IKEDA[1,2,6];
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Abstract: Neuronal morphogenesis is essential for precise wiring of intricate neuronal circuitry. Recent studies have begun to analyze mechanical forces generated by neurons, as its morphogenesis depends on their ability to produce mechanical forces. Traction force microscopy uses fluorescent nano beads that are embedded on the surface layer of an elastic gel substrate. When neurons cultured on the substrate generate traction force, the beads change their locations due to the deformation of the gel substrate. In our previous work, we proposed a force estimation algorithm in which Bayesian framework is applied to dataset of bead displacements. The proposed algorithm performs accurate estimation by using prior of force direction which is corresponding to actin retrograde flow. However, the previous algorithm is sensitive to hyper-parameters of the prior and is applied only to synthetic data. In this study, we analyzed dependency of the algorithm on the hyper-parameters and proposed an efficient procedure to determine values of the hyper-parameters. Using the new algorithm with experimental dataset, we estimate mechanical force generated by neurons.

Disclosures: **S. Kozawa:** A. Employment/Salary (full or part-time):; Advanced Telecommunications Research Institute International. **Y. Sakumura:** None. **M. Toriyama:** None. **N. Inagaki:** None. **K. Ikeda:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: Sandia LDRD 151345

Title: How to model the whole brain?

Authors: ***F. ROTHGANGER**, D. TRUMBO, C. WARRENDER, J. AIMONE;
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Abstract: A premise behind Neuroinformatics is that sharing models and data is necessary to make progress toward a full understanding of brain function. The Neuroscience Information Framework (NIF) has largely accomplished this for many forms of descriptive data. Ongoing work on standardization (such as NeuroML/LEMS) promises to facilitate model interchange. Repositories exist (such as ModelDB) for holding models in various formats. Excellent graphical tools (such as NeuroConstruct) support intuitive model building. However, simply sharing models and data is not sufficient. It is also necessary to assemble those shared models into larger functional units, ultimately reaching the level of an entire nervous system. Beyond that, it is necessary to abstract out algorithmic structures hiding within the details, and to validate models against data. We propose an approach to this integration that represents both structural relationships and the explicit quantitative behavior of each part. It packages neural components as bundles of simple declarative equations that describe the system dynamics. Parts are loosely coupled to each other via additional equations that relate their variables. Such models are capable of both computation (via a simulator) and structural analysis (via, for example, graph algorithms). This approach is embodied in a new modeling language called "Neurons to Algorithms" (N2A). While similar in many ways to standardized interchange languages like NeuroML and NineML, the focus of N2A is on assembling models into larger structures. The N2A language is partially implemented in an open source tool of the same name. While many necessary features (integration with NIF, model exchange via NeuroML) are still missing, the software is already capable of equation-set editing and generating simulations on supercomputers using Xyce, a parallel version of the electrical circuit modeling language SPICE. In addition, the tool can generate C++ code for single-threaded simulations which support the full range of N2A language features (that is, a reference implementation).

Disclosures: **F. Rothganger:** A. Employment/Salary (full or part-time):; Sandia National Laboratories. Other; University of Illinois, Urbana-Champaign. **D. Trumbo:** None. **C. Warrender:** None. **J. Aimone:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NSERC/CIHR CHRP

Title: Simulation of the effect of TMS on a single neuron

Authors: A. SABOUNI[1], D. SIEVENPIPER[2], *A. SHMUEL[3];
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Abstract: TMS involves the application of time-pulsed magnetic fields to cortical tissue. By using a coil located close to the head, it is possible to induce currents in neuronal tissues. By localizing the magnetic field according to prior anatomical MRI information, it is possible to modulate cortical function by exciting or inhibiting neuronal activity in a local area. This modification of neuronal activity has several applications in medical and clinical research including brain mapping, psychiatry, treatment of epilepsy and treatment of chronic pain. To date, using TMS is still empirical and the application of TMS has been hindered by a lack of understanding of its mechanism of action. Understanding TMS and its mechanisms of action has been the subject of very active research. The effect of induced current has been evaluated in phantoms, in animal models, and in humans with intracranial electrodes. These results can be complemented by modeling studies of electromagnetic field propagation in tissues. Numerical solvers have been developed for calculating the induced current in brain tissue during TMS. Relatively few models investigated the effect of TMS at the cellular level. In this work, the stimulation of a single neuron is simulated using CST (Computer Simulation Technology) software. We used a 3D brain neuron model including the cell membrane, nucleus, axon, and synapses. A small magnetic coil is used to induce current in the neuron. This creates voltage across the synaptic cleft (between the axon terminus and the adjacent neuron) and therefore a neurotransmitter molecule is released. Since today's TMS systems use repetitive pulses, the lower end of the required operating band may be in the kHz range. The upper end of the operating band is based on the fact that the current density generated in the brain depends on the rate of change of the magnetic field, governed by the higher frequency components. We will demonstrate the effect of changing the frequency on the current induced in neurons and the dependency of the synaptic voltage for different synaptic cleft width.

Disclosures: A. Sabouni: None. **D. Sievenpiper:** None. **A. Shmuel:** None.

Poster

852. Modeling Neurons, Circuits, and Behavior

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 852.15/VV33

Topic: G.06. Computation, Modeling, and Simulation

Support: Hertz Foundation

Department of Energy Computational Sciences Graduate Fellowship

NIH Grant K25 GM098875-02

Title: The deterministic information bottleneck

Authors: ***D. J. STROUSE**, D. SCHWAB;
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Abstract: A fundamental and ubiquitous task that all organisms face is prediction of the future; accurate predictions of, for example, the locations of food or predators are matters of life and death. The basis of those predictions are, of course, past sensory experience. Since an individual's memory resources are limited and costly, however, there is a tradeoff between memory cost and predictive payoff. The information bottleneck (IB) method (Tishby, Pereira, & Bialek 2000) formulates this tradeoff as a mathematical optimization problem using an information theoretic cost function. IB encourages storing as few bits of past sensory input as possible while selectively preserving the bits that are most predictive of the future. The optimization is done over the stochastic encoding distribution q(m|p), where p and m are random variables representing past observations and encoded memory, respectively. Algorithmically, the optimization is done via an iterative algorithm, except in a few simple cases where an analytic solution is available. Here we introduce an alternative formulation of the IB method, which we call the deterministic information bottleneck (DIB). First, we argue for an alternative cost function, which better represents the biologically-motivated goal of minimizing required memory resources. Then, we show that this seemingly minor change has the dramatic effect of converting the stochastic encoding distribution q(m|p) into a deterministic encoding function m(p). Next, we propose an iterative algorithm for solving the DIB problem. Additionally, we compare the IB and DIB methods on a variety of synthetic datasets, and examine the performance of retinal ganglion cell populations relative to the optimal encoding strategy for each problem. We conclude by discussing the relative advantages and appropriate uses of each method.

Disclosures: **D.J. Strouse:** None. **D. Schwab:** None.

Poster

852. Modeling Neurons, Circuits, and Behavior

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant R01NS063360

Title: 3D computational model of infusion into rat brain hippocampus that accounts for fissures and fiber tracks

Authors: *W. DAI, M. SARNTINORANONT, G. ASTARY, A. K. KASINADHUNI, P. R. CARNEY, T. H. MARECI;
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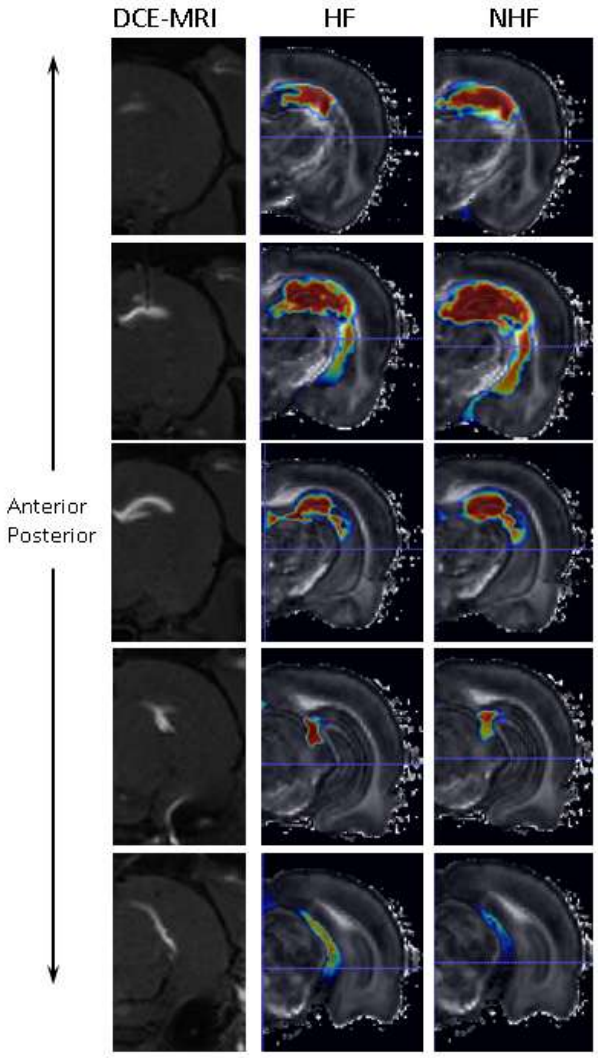
Abstract: Convection-enhanced delivery (CED) is a promising drug delivery method to treat neural diseases, as it is a technique that can transport macromolecular therapeutic agents over large volumes through direct infusion. Distribution of drug significantly depends on infusion protocol parameters as well as tissue properties. To predict CED distributions, our group has developed 3D computational transport models based on magnetic resonance diffusion tensor imaging (MR-DTI) that simulate infusion flow fields and tracer distributions within the brain. In previous studies by our group as well as others, brain tissue segmentation was based on diffusion tensor measures such as fraction anisotropy and average diffusivity. This method was able to segment major regions of white matter, gray matter, and cerebrospinal fluid (CSF). In this study, generalized anisotropy, another scalar measure of anisotropy obtained from high angular resolution diffusion imaging (HARDI), was adopted to improve tissue segmentation within complex tissue regions of the hippocampus. This allowed for better segmentation of white matter regions with crossing fibers. Improved assignment of hippocampal fissures was also achieved (increased volume and improved connectivity of ventricle regions). CED simulations predicted preferential transport into fissures whenever they are present, resulting in lower tissue spread and greater CSF accumulation. Predicted tissue distribution volumes decreased by 17% and 13% for dorsal and ventral hippocampus infusion sites, respectively. Predicted tracer distributions were also compared with experimental CED studies previously collected by our group. Overall, better spatial representation of fissures improved the simulation of infusate leakage into ventricles. The model accounted for this major source of non-specific targeting with local drug delivery. Figure 1. Comparison of experimentally measured MR distributions of albumin tracer (Column 1) with computational models that include a hippocampal fissure (Column 2) and no hippocampal fissure (Column 3).



Disclosures: **W. Dai:** None. **M. Sarntinoranont:** None. **G. Astary:** None. **A.K. Kasinadhuni:** None. **P.R. Carney:** None. **T.H. Mareci:** None.

Poster

852. Modeling Neurons, Circuits, and Behavior

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Program#/Poster#: 852.17/VV35

Topic: G.06. Computation, Modeling, and Simulation

Title: Self-organized critical phenomena in neural coding linked to synchronized high and low frequency oscillations

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Abstract: Objective Brain systems are expected to have not only the low frequency below 100 Hz caused by neuronal electrical activities but also the high frequency of 10^{11}-10^{12} Hz caused by the dipolar oscillation of cell membranes or giant molecules. Focusing on transition phenomena of cognitive biases, our objective is to investigate how the evaluation system of uncertainties is coded in neural circuits using a simulation model through the switching of synchronization phenomena in this frequency range, and discuss the properties of brain's information processing systems. Methods We think that cognitive biases are cognitive distortions that are the macroscopic reflection of the microscopic limitation in neural circuits. When this macroscopic phenotype is called a phase, the change of phenotypes can be called a phase transition. Based on the assumption that the switching of information processing circuits between the cerebral cortex (such as the prefrontal area) and the subcortex (such as the basal nucleus and amygdala) through the synchronized high or low frequency oscillations in the brain, emerges as the phase transition phenomena of cognitive biases, we present a neural-circuit model on the risk estimation under control of the normalcy bias or the prospect theory, and examine the evaluation mechanism in the brain. Results Our neural coding model using the synchronization phenomena indicates that the rational evaluation can be led from the phase transition of cognitive biases. Possibilities of the existence of the self-organized criticality, which induces a self-invited phase transition independent of external parameters, are suggested. Conclusion A high frequency of 10^{11}-10^{12} Hz corresponds to the natural frequency of hydrogen bonds in biological molecules. We think that biological systems can realize various functions using this feature. Neural coding linked to the synchronization phenomena can simulate phenotypes of cognitive biases. It can be thought that some cognitive biases are entrenched in the methods of perception, memory formation and determination of the brain. It is expected that the investigation of properties of phenotypes of cognitive biases will reveal the relationship between microscopic phenomena of neurotransmitters and macroscopic actions to lead understandings of decision making algorithms and implementation approaches.

Disclosures: **M. Hirabayashi:** None. **H. Kojima:** None. **H. Ohashi:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: NSF PoLS 1058034

NIH DP2MH104119

Young Investigator Award, Brain and Behavior Research Foundation

Title: Quantifying dynamics in neuronal networks

Authors: *D. MARUYAMA[1], N. OGNJANOVSKI[2], S. ATON[2], M. ZOCHOWSKI[1,3]; [1]Physics, [2]Molecular, Cellular, and Developmental Biol., [3]Biophysics, Univ. of Michigan, Ann Arbor, MI

Abstract: The steady development of MEAs and other modern measurement devices is opening up access to data streams composed of large numbers of neuronal signals. The ability to effectively analyze these signals is already bottlenecking device use and threatens to fall far further behind the projected advances in data collection. Here we investigate two methods capable of handling large network throughput to assess patterns in neuronal datasets. Specifically, by employing a fast analytic approach to capture neuronal correlations over short periods of time we can quantify both slower structural changes, by looking at large scale functional connectivity shifts, and faster dynamical changes, by tracking the evolution of the neuronal network. These methods are applied to simulated datasets and to in-vivo data of contextual fear conditioning in mice. The techniques reveal signs of network wide structural changes and measure significant changes in the network stability during mouse sleep states. Namely we observe that increased stabilization of network wide functional structure with mice improved performance on the task.

Disclosures: D. Maruyama: None. **N. Ognjanovski:** None. **S. Aton:** None. **M. Zochowski:** None.

Poster

853. Data Analysis and Statistics III

Location: Halls A-C

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Program#/Poster#: 853.02/VV37

Topic: G.07. Data Analysis and Statistics

Support: Alfred P. Sloan Foundation

Title: Node dynamics in time-dependent brain networks

Authors: *Q. K. TELESFORD, D. BASSETT;
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Abstract: Network science methodologies represent a powerful approach for understanding the complex organization of the brain. Traditional approaches to functional brain network analysis often utilize the correlation or coherence between signals in brain regions to build a network. However, this approach often ignores changes in network organization that may occur on shorter time scales. In place of a single adjacency matrix to represent a network, a time-dependent network is represented as an adjacency tensor linking nodes across epochs. In this work, we describe community detection in time-dependent networks that are able to capture dynamic changes in network topology. To understand changes in the network, new algorithms have been developed to describe how often a node changes community assignment, its level of community change across time, and how diverse is its community participation over time. We explore these metrics using an fMRI data set acquired from 109 healthy subjects that performed a memory paradigm with words and faces. Node flexibility was measured between two runs for each task. Flexibility was found to be significantly higher in the first run compared to the second run for the word ($p<0.01$) and face ($p<0.01$) tasks. Understanding changes in a network over time can provide more robust classifiers for understanding differences in populations.

Disclosures: Q.K. Telesford: None. **D. Bassett:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: Swiss National Science Foundation Grant 135736

Alamaya Foundation

Title: *In vivo* whole brain dti analysis in cohort studies in mice, an analysis of statistical power

Authors: *A. CORCOBA[1,2], J. M. N. DUARTE[1], Y. VAN DE LOOIJ[1,3], N. KUNZ[1], R. GRUETTER[1,4], K. Q. DO CUENOD[2,5];
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Abstract: Diffusion tensor imaging (DTI) is a non-invasive imaging modality sensitive to alterations in white matter (WM) structure and myelination. The available software for whole-brain analysis makes DTI an useful tool for WM phenotyping in mice. Nevertheless, most tools have been developed for human clinical data and studies in rodents remain scarce. Here we compare the statistical power of two methods of whole-brain MRI analysis in a cohort study at the state-of-the-art in-vivo resolution, a voxel- and ROI-based approaches. We acquired mouse DTI data, from which we generated a second group of images with localized differences in DTI-derived fractional anisotropy (FA) for comparison with a known ground truth. Spin-echo diffusion-weighted images of 15 C57Bl/6 mice were acquired in a 14T Varian/Magnex scanner with a transmitting-receiving quadrature surface coil (TR=2s TE=31.35ms Vxl=0.156x0.312x0.6mm3, Av=4, 6 directions and b-value=1000 s/mm2, SNR=155±21 on b0 images). We fitted a tensor voxel-wise to model water diffusion and calculated FA maps. Anatomical images with 0.078x0.078x0.6mm3 resolution were acquired for image registration to a target atlas using linear (FLIRT) and non-linear (FNIRT) tools (FMRIB Oxford, UK). The atlas was obtained by registering and averaging images from an independent group of 11 mice and manually segmenting it into 27 ROIs. We reduced the FA value of all voxels within two WM tracts, the fimbria/ fornix (FF) and anterior commissure (AC), in all images of the second group. Subsequently, we compared the power of a voxel-based approach using SPM8 (The Wellcome Trust Centre for Neuroimaging, London) and a ROI-based method (extracting the average FA values from the 27 atlas ROIs) to detect between-group differences (with family-wise and Holm's correction for multiple testing respectively). We started with 1% FA difference and increased until both methods reported significant between-group p-values. In the FF, voxel-wise slightly outperformed ROI-based analysis (12 vs 15% effect size reported significant) probably due to the higher localizing power of voxel-based method, not constrained by a-priory localization and the overlap of the ROIs with the affected area. In the AC, both methods required bigger FA differences (28 and 26% respectively), probably due to the higher distance from the coil (lower signal-to-noise ratio). Our study provides guidance on the application of whole brain analysis tools to mouse and more generally to rodent MRI and points out the different spatial sensitivity due to hardware settings.

Disclosures: **A. Corcoba:** None. **J.M.N. Duarte:** None. **Y. van de Looij:** None. **N. Kunz:** None. **R. Gruetter:** None. **K.Q. Do Cuenod:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: NIH RO1MH064537 Analysis of Nonstationary Neural Data

Title: Inferring oscillatory modulation in neural spike trains

Authors: *K. ARAI[1], R. E. KASS[2], J. SCOTT[3];
[1]Statistics, [2]Dept. of Statistics, Carnegie Mellon Univ., Pittsburgh, PA; [3]McCombs Sch. of Business, The Univ. of Texas at Austin, Austin, PA

Abstract: Oscillations of local and global scope entrain large populations of neurons in the brain. Extracellular neural recordings reveal only the action potentials from each neuron, which are point-like events ("spikes") that convey very little information about the underlying oscillation. Approaches to study the oscillatory structure of the action potentials often compare the spiking to continuous signals that more obviously convey the oscillations, such as the local field potential (LFP) [Fries et al, 2008]. However, the LFP may not reflect the oscillatory activity that actually drives the recorded neuron. It is possible to apply spectral analysis to spike trains [Jarvis and Mitra, 2002] or methods based on auto- and cross-correlation histograms [Muresan et al, 2008], but these approaches assume stationarity of the spike train, and do not estimate the instantaneous phases or amplitudes of oscillation. Instead, we have investigated a point-process modeling approach [Smith and Brown, 2003; Sarma et al, 2010], in which a latent oscillatory autoregressive (AR) process is inferred from the observed spike trains. A state-space model is fit by taking advantage of a recently-developed method for Gibbs sampling in logistic regression models [Pillow and Scott, 2012]. We find this approach to be effective in inferring oscillations in spiking data from V1 of monkey viewing oriented bars (300ms per orientation).

Disclosures: K. Arai: None. **R.E. Kass:** None. **J. Scott:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: NIH Grant R90DA023426

NIH Grant RO1MH064537

Title: Statistical association of oscillatory lfp and neural synchrony

Authors: *P. ZHOU[1], S. D. BURTON[1], R. C. KELLY[2], M. A. SMITH[3], N. N. URBAN[1], R. E. KASS[1];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Google Inc., New York City, NY; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Pairs of active neurons recorded simultaneously will frequently fire spikes nearly synchronously (e.g., within 5 ms of each other). Such spike synchronization may occur by chance, or it may occur too frequently to be explicable by chance alone, based solely on the individual neurons' fluctuating firing rates. When excess synchrony, above chance, is present, it may subserve computation for specific cognitive process (Riehle et. al, 1997), or it could be an irrelevant byproduct of such computation. Either way, it is a feature of the data that should be explained. A point process regression framework has been developed for this purpose, using generalized linear models (GLMs; Kelly & Kass, 2012). In this method the observed number of synchronous spikes is compared with the number predicted by chance under varying assumptions about the factors that affect each of the individual neurons' firing-rate functions. An important possible source of synchrony is neuronal oscillations, because they could provide an essential mechanism of neural network information flow (Colgin et. al, 2009). To establish the statistical link between synchronous spiking and oscillatory field potential we have used point process regression. We first extended the spike-field association models of Lepage et al. and showed that we could recover phase relationships between input current and spiking of hippocampus CA1 pyramidal neurons. In simulation studies this approach also performed well. We then demonstrated the method using both CA1 pyramidal neurons and *in vivo* V1 data.

Disclosures: P. Zhou: None. **S.D. Burton:** None. **R.C. Kelly:** None. **M.A. Smith:** None. **N.N. Urban:** None. **R.E. Kass:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: Barrow Neurological Foundation

NIH 1R21DC009871-0

Title: Recording human intracranial single neuron activity in electrically noisy clinical environments

Authors: *P. N. STEINMETZ[1], C. K. THORP[2];
[1]Neurol. and Neurosurg., Barrow Neurolog. Inst., PHOENIX, AZ; [2]Devicix, LLC, Eden Prarie, MN

Abstract: Recording single neuron activity in the brains of human patients provides an extraordinary opportunity to study the single neuron correlates of human cognition, but also presents significant engineering challenges. These challenges are primarily due to the low amplitude of signals - on the order of 10 μV. Additionally, the recordings are made in an electrically noisy environment, which often contain 5 μV of noise in the passband for single neuron recording. In this work, we examine the input referred power spectrum (system background noise vs frequency) of 4 systems that have been used to record single neuron activity in human epilepsy patients. We also contrast their spectral characteristics in terms of their ability to recover single neuron activity. Surprisingly, due to the high input impedance of the pre-amplifiers which are typically used, these systems can introduce inadvertent hardware filtering which reshapes the actual system passband for recording. Since this filtering is introduced prior to digitization, it cannot be removed by signal processing because the signal is so close to the noise floor. This frequency-domain reshaping has a significant impact on spike sorting since all impulses passed through a narrow bandpass filter resemble the impulse response of the filter. In summary, a careful examination of the power spectrum of neurophysiological recordings, particularly in electrically noisy clinical environments, is a key tool in ensuring the quality of single neuron recordings.

Disclosures: P.N. Steinmetz: None. **C.K. Thorp:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: NSF-DMS Grant 1042134

Title: Rate correction for spike-field coherence: Detection and estimation properties

Authors: *M. C. AOI, M. KRAMER, K. Q. LEPAGE, U. T. EDEN;
Dept. of Math. & Stats., Boston Univ., Boston, MA

Abstract: Spike field coherence (SFC) has found been found to be a useful metric of rhythmic synchrony between spike trains and local field potentials (LFP's). Recently however, it has been demonstrated that SFC is a function of the mean spike rate, confounding tests of cross-condition difference in synchrony when spike rates differ between experimental conditions [1]. The confound is illustrated in the form of a decomposition of the SFC that characterizes two sources of variability, one associated with the stochastic process determining the probability of spiking and one associated with the probability of spiking itself. One approach to correcting for this confound is the "thinning" method, which corrects the number of spikes via a Monte Carlo method [2]. In the present work, we propose a simple adjustment to the spike-field coherence estimator, inspired by the thinning procedure, that corrects for variability associated with changes in spike rate, while leaving the variability associated with variation in the probability spiking unchanged. We develop estimation procedures for the parameters of the rate correction, derive sampling properties of the new estimator, and provide simulation experiments demonstrating the validity of the theoretical results. We also provide a detailed analysis of the receiver operating characteristics of the test for cross-condition differences in coherence using the corrected estimator and compare these properties to the test using the uncorrected estimator. While the ROC properties of the uncorrected estimator are dependent upon the unknown experimental outcome, the proposed estimator is not. Furthermore, we show that the proposed estimator improves detection of cross-condition differences compared to the thinning method. Since the rate-adjusted SFC retains many of the optimality properties associated with the coherence, we suggest that the rate-adjusted SFC is an attractive first-order descriptive statistic of spike - LFP coupling. [1] Lepage, K., Kramer, M., Eden, U., 2011. The dependence of spike field co- herence on expected intensity. Neural Computation 23, 2209-2241. [2] Gregoriou, G., Gotts, S., Zhou, H., Desimone, R., 2009. High frequency, long-range coupling between prefrontal and visual cortex during attention. Science 324, 1207.

Disclosures: **M.C. Aoi:** None. **M. Kramer:** None. **K.Q. Lepage:** None. **U.T. Eden:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Support: Swiss National Fund

Mathers Foundation

Title: Quantification and analysis of kernel smoothed peristimulus time histograms

Authors: *M. R. HILL[1], I. FRIED[2], C. KOCH[3];
[1]Behavioral Biol., Caltech, Pasadena, CA; [2]Dept. of Neurosurg., UCLA, Los Angeles, CA;
[3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: In neurophysiological experiments single-unit recordings are often presented as peristimulus time histograms (PSTH). In a PSTH, the modulation of a neuron's mean firing rate over time is plotted across multiple trials, aligned to the onset of a stimulus (t = 0). A better estimate of the actual shape of this response envelope can be achieved by smoothing the PSTH with a kernel convolution. In the current study, we developed an algorithm to classify such a kernel convoluted PSTH as containing either a real response or only random fluctuations having occurred by chance. This algorithm, called the h-coefficient, quantizes the response shape in a smooth PSTH and measures the probability of such a response shape to occur by chance for the respective unit. We tested the performance of the h-coefficient in a large dataset of 14'700 Monte Carlo simulated smooth PSTH with varying response amplitudes, response durations, trial numbers and baseline firing rates. Across all these conditions, the h-coefficient outperformed more traditional classifiers significantly, with a mean false alarm rate of 0.004 and a mean hit rate of 0.494 within this dataset. We also applied the h-coefficient's improved performance in a dataset of real neuronal responses recorded in patients. The h-coefficient is a PSTH classifier that systematically quantifies and classifies the actual shape of a neuronal response envelope. Our findings demonstrate that the h-coefficient can provide a conservative and powerful PSTH analysis method with great potential for further development and adaptation.

Disclosures: M.R. Hill: None. **C. Koch:** None. **I. Fried:** None.

Poster

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Topic: G.07. Data Analysis and Statistics

Support: Travis Roy Foundation

Title: Comparing anatomy and physiology of the corticospinal tract across subjects: Microstimulation motor mapping and retrograde tracing of motor cortex neurons co-registered in 3-dimensional space

Authors: *D. GUPTA[1], J. B. CARMEL[1,2];
[1]Motor Recovery Lab., Burke-Cornell Med. Res. Inst., White Plains, NY; [2]Brain and Mind Res. Inst. and Departments of Neurol. and Pediatrics, Weill Cornell Med. Col. of Cornell Univ., New York, NY

Abstract: In systems neuroscience, comparing anatomy and physiology often leads to insights about functional connectivity. Two commonly used methods in animals are retrograde labeling of corticospinal neurons and motor mapping with intracortical microstimulation (ICMS). There are limited tools available to compare these methods within and across animals. The main gaps are in tools, especially for i) co-registeration of the ICMS and labeling results within subject, ii) combination of these results across subjects, and iii) quantifying the group-level changes. Here we describe a method that we achieved for co-registering, combining and quantifying the ICMS and labeling results based on spatial normalization to a common 3-dimensional rat brain derived from the Paxinos atlas. This required 3 steps. In the first step, we combine the anatomical results across animals. We label corticospinal neurons by injecting the retrograde tracer fast blue into the spinal cord. After 2 weeks, we perfuse the rats and identify labeled neurons in coronal sections. We then extract and separate the structural contours and labeled neurons and import them in the Atlas3D software. We match the data slice contour to the slice from the Atlas by appropriately translating, scaling and rotating the data. The resulting transformation matrix is then applied to the labeled neurons from the corresponding slice. Repeating this for each slice from multiple animals, allows us to overlay all labeling results in a common brain volume. In step 2, we co-register the ICMS and labeled neurons. Using stereotaxic coordinates, we add the ICMS results onto the anatomical results. These are projected in the form of spheres with a diameter that corresponds to the stimulation threshold response. In step 3, we quantify the labeled neurons. For quantification of the irregular volumetric spread of the labeled neurons, we use a Gaussian Mixture Model_a parametric probabilistic density estimation method_to estimate

the 2D and 3D probability density maps. These maps allow quantification of spread, density and center of gravity of the labeled neurons in the 3D brain volume. We demonstrate this method as applied to rats with both ICMS and anatomical tracing. Our method allows us to quantify the motor maps in control rats and to compare these results with maps in rats with injury.

Disclosures: D. Gupta: None. **J.B. Carmel:** None.

Poster

853. Data Analysis and Statistics III

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Topic: G.07. Data Analysis and Statistics

Title: EEG evoked-responses during viewing of natural stimuli are strongly driven by overt orienting of attention

Authors: *J. J. KI, A. ALAM, L. PARRA, S. HAUFE;
Neural Engin. Lab., The City Col. of the City Univ. of New Yor, New York, NY

Abstract: When subjects watch an engaging movie their brains are reliably driven by the stimulus [1,2]. This results in similar EEG responses across individuals, which can be measured as inter-subject correlation of the raw EEG [2]. We wondered whether these reliable responses are modulated by the level of attention to the stimulus. To test this, we manipulated viewer attention while subjects (N=15) watched short (6 min.) sections of two feature films: Bang you're dead!, Alfred Hitchcock (BYD); and Good Bad and the Ugly, Sergio Leone (GBU). In a first viewing, subjects watched the clip normally without any distraction (attend condition). In the second viewing, subjects were asked to count backwards in their mind in decrements of 7 starting with 1000 (disattend condition). To determine the effect of overt orienting of attention, i.e. natural eye movements driven by exogenous attention, we repeated the experiment in a new cohort (N=15), but now asking subjects to focus their gaze at the center of the screen. We measured how similar the EEG signals are between subjects using correlated components [2]. In this manner we obtain for each viewer and each condition a correlation value that captures how similar their EEG evoked response is to that of normally attending individuals. As expected, correlations to the normally attending group differed between “attend” and “disattend” conditions. The difference was remarkably strong for BYD, allowing us to perfectly determine the attentional state of the subject from the EEG alone (Az=1.0) and somewhat less strong for GBU (Az=0.77). During analysis, we noticed that eye movements were a major contribution to

correlation. Indeed, correlations computed from the electro-oculogram yielded even better discrimination (Az=0.96 and 0.82). However, when fixations were limited to a central area of the screen, all correlations dropped significantly. From these data, we conclude that natural stimuli drive eye movements and EEG evoked responses similarly across individuals. However, the level of similarity or reliability of these driven responses is strongly affected by the viewer's attentional state as well as the ability of the stimulus to draw overt attention. [1] Hasson et al. Science, 2004 [2] Dmochowski et al, Front. Hum. Neurosci., 2012

Disclosures: J.J. Ki: None. **A. Alam:** None. **L. Parra:** None. **S. Haufe:** None.

Poster

853. Data Analysis and Statistics III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 853.11/VV46

Topic: G.07. Data Analysis and Statistics

Title: Thalamotomy using MRI-guided focused ultrasound alters spontaneous brain networks in the essential tremor patients

Authors: *M. YOON[1,2], H.-J. PARK[2,3], W. CHANG[4], M.-K. OH[3], J.-I. KIM[2], C. DO[2], J. LEE[2,3], J. CHANG[4];
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Abstract: Introduction: One of effective treatments of essential tremor is to remove part of the thalamus, called thalamotomy. Recently, thalamotomy can be conducted using a minimal-invasive ablation method, Magnetic Resonance-guided Focused Ultrasound Surgery (MRgFUS). Despite its successful treatment effects on essential tremor, it is not yet clear how this thalamotomy alters whole brain network and leads to effective outcomes. In this study, we investigated brain network changes in the essential tremor patients using graph theoretical measures after MRgFUS. **Methods**: We obtained the resting state fMRI from 8 patients both before MRgFUS and after surgery (mean age=66 years; female 1) and 16 normal subjects (mean age=66.31 years female 8). After all fMRI data were preprocessed using SPM8, nuisance signals were regressed out and high-pass filtered (0.009~ Hz). To construct individual functional network, we used FSL's Harvard-Oxford atlas regions (http://www.fmrib.ox.ac.uk/fsl/) with 25%

threshold. We split 110 regions to functionally more homogeneous 315 regions using an Anatomical-constrained Hierarchical Modularity Optimization (Park et al., 2013). After calculating functional correlation among 315 regions, we converted them into z-map with Fisher's r-to-z transformation. Graph properties were calculated with respect to weighted graph. And we decomposed networks into edge-sharing independent subnetworks using Graph ICA (Park et al., 2014). Finally, we conducted two-sample t-test between the weights of each component between patients and normal group. **Results**: We found significantly lower characteristic path length in pre-surgery data than control data from healthy subjects ($p<0.02$ for all subjects). On the contrary, no significant difference was found between post-MRgFUS data and control data. In addition, there were subnetworks, which had significantly high weights before the surgery but had no difference after treatment (e.g. fronto-parietal network, fronto-temporal-parietal network) compared to control data. **Conclusions**: According to the current study, lower characteristic path length in essential tremor patients reduced to the level of normal group after thalamotomy. The hyper strengthend involvements of some independent subnetworks were also decreased after the surgery. From these results, we revealed the cause of essential tremor as an excessive compact form in the brain network globally, also with immoderately high involvement of fronto-parietal attention network. Thalamotomy seems to lead the abnormal status of network to normal degree.

Disclosures: **M. Yoon:** None. **H. Park:** None. **W. Chang:** None. **M. Oh:** None. **J. Kim:** None. **C. Do:** None. **J. Lee:** None. **J. Chang:** None.

Poster

853. Data Analysis and Statistics III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 853.12/VV47

Topic: A.01. Brain Patterning

Support: NIH / NICHD grant HD062499

Title: The Gene Expression Database (GXD): integrated access to mouse developmental data

Authors: ***J. H. FINGER**, T. F. HAYAMIZU, I. J. MCCRIGHT, C. M. SMITH, J. XU, J. T. EPPIG, J. A. KADIN, J. E. RICHARDSON, M. RINGWALD;
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Abstract: The Gene Expression Database (GXD) provides the neuroscience and developmental biology research community with an extensive, integrated, and curated resource of mouse expression information. GXD contains more than 260,000 expression images and more than 1.4 million annotated expression results for over 13,000 genes covering all stages of mouse development. Expression data from multiple assay types, including RNA *in situ* hybridization, immunohistochemistry, reporter knock-in, northern, western and RT-PCR experiments are integrated via the use of standard genetic nomenclature, controlled vocabularies, and an extensive anatomical ontology. Curators collect expression data from the literature as well as through collaboration with large-scale data providers. GXD is part of the Mouse Genome Informatics (MGI) resource (http://www.informatics.jax.org), and integration with genetic and phenotypic information provides neuroscientists with the access to multi-faceted information related to their research perspective. Expression data from wild-type mice and from more than 1,900 mouse mutants can be accessed in GXD. The best starting point for exploring GXD's utilities is the GXD Home Page at http://www.informatics.jax.org/expression.shtml. The Gene Expression Data Query Form allows researchers to search for expression data using many parameters. One can search for expression based on gene nomenclature or on functional, phenotypic, or disease classification; for expression in specific anatomical structures and/or at developmental stages; and for expression in wild-type mice or in specific mouse mutants. By combining parameters very specific queries can be built. Search results are represented at different levels of detail via tabbed data summaries for genes, assays, assay results, and images. These summaries can be sorted in different ways and interactively refined using newly implemented data filtering functions. The images tab permits researchers to quickly find expression images that match specific search criteria. Following links from summaries to assay details, users can see the expression images together with their annotations. Individual image panes are linked to full figures to preserve the original context. The recently improved Mouse Developmental Anatomy Browser allows users to search and navigate the developmental anatomy ontology and to look up expression data associated with specific anatomical structures. The Gene Expression Literature Query Form lets researchers quickly find publications that report developmental expression data for specific genes, ages, and assay types.

Disclosures: J.H. Finger: None. **T.F. Hayamizu:** None. **I.J. McCright:** None. **C.M. Smith:** None. **J. Xu:** None. **J.T. Eppig:** None. **J.A. Kadin:** None. **J.E. Richardson:** None. **M. Ringwald:** None.

Poster

853. Data Analysis and Statistics III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 853.13/VV48

Topic: G.07. Data Analysis and Statistics

Title: Mapping genetic variation to morphology and behavior in free-ranging rhesus macaques

Authors: *S. MADLON-KAY[1], K. WATSON[2], L. BRENT[2,4], M. PLATT[2,3];
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Abstract: Though much behavioral and morphological variation in phenotypes are thought to be related to genetic variation in neurological pathways, determining the relationship between specific sets of genes and variation in normal populations is a difficult task. The difficulty stems in part because effects of individual loci on phenotypes are often small and confounded with environmental factors or population stratification. One way forward is to study genotype-phenotype relationships in wild animal populations where environmental, social, and life-history effects that may confound or interact with genetic effects can be measured and factored out using statistical tools. As a first step to seeing if the genetic basis of important human phenotypes could be studied in wild populations, we examined the genetics of obesity in a population of free-ranging rhesus macaques on the island of Cayo Santiago. In order to establish relationships between variation in different genomic pathways and typical variation in morphology, we developed modeling tools that allow us to partial out the influences of environmental, life-history, and shared genetic backgrounds from the effects of genetic variation in neurobiological pathways. Specifically, we adopted the general linear mixed-model framework, combining multiple random and fixed effects components to capture correlations between organisms due to genetic relatedness and shared environment, along with a sparse regression component to model the effects of genetic variation in pathways of interest. The data set contained 251 single nucleotide polymorphisms (SNPs) from 70 genes related to neurological development and function across 397 animals. The body mass index (BMI), a proxy for percentage body fat, was calculated for 501 animals between the years 2011 and 2013. We found that BMI had a large genetic component in rhesus macaques, with additive genetic variance explaining approximately one third of the total genetic variability in the sample. Decomposing genetic variance into the amount explained by kinship versus the variance explained by the known SNPs in the neurobiological pathways revealed that as much as half of the genetic component of the variance was accounted for by the SNPs. The gene contributing the largest proportion of variance per identified SNP was MC3R, a gene coding for G-coupled protein receptor that has been linked to obesity in rodents and humans. These results suggest that the genetic basis of morphological and behavioral traits in humans can be fruitfully examined in wild animal populations.

Disclosures: **S. Madlon-Kay:** None. **K. Watson:** None. **L. Brent:** None. **M. Platt:** None.

Poster

853. Data Analysis and Statistics III

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Title: Application of advanced feature recognition and statistical physics methods to quantify age-related changes in myelinated nerve fibers in monkey brain

Authors: *J. R. SANTOS[1,2], C. H. COMIN[4], D. CORRADINI[2], W. MORRISON[2], C. CURME[2], D. L. ROSENE[3], A. GABRIELLI[5], L. F. COSTA[4], H. STANLEY[2];
[2]Dept. of Physics, [3]Sch. of Medicine, Dept. of Anat. & Neurobio., [1]Boston Univ., Boston, MA; [4]Univ. of Sao Paulo, Inst. of Physics at Sao Carlos, Sao Carlos, Brazil; [5]Dept. di Fisica, Inst. dei Sistemi Complessi, Rome, Italy

Abstract: We present a new method to identify and quantify differences in myelinated axons and their surrounding myelin sheath. Our method uses statistical physics tools to characterize properties ranging from morphologic characteristics of individual fibers to macroscopic and structural properties of collections of fibers and their spatial relationships. This allows quantification of their differences and improves on traditional measures, such as packing density and fiber size. Understanding axons at the ultrastructural level is a critical issue for understanding the human connectome, as the action potential conduction and hence communication among neurons depends upon both axon integrity and the integrity of the myelin sheath. Changes in the myelin sheath alone alter action potential conduction during development, across the adult life and into old age. Only electron microscopy can provide a detailed ultrastructural analysis of the axon and myelin sheath but previous studies have been limited by

either the tedious task of quantifying features from these samples in sufficient numbers to make solid inferences or by using conventional stereological techniques to quantify simple macroscopic features (e.g. number of axons, axon density). While these studies, relying on expert observation and measurement, have shown age-related changes in axons and myelin, they have not been able to assess enough features of enough axons to fully characterize higher dimensional properties like the spatial or angular regularity of axons. In this study, we analyze cross-sectional electron micrographs from the fornix of young and old rhesus monkeys using a semi-automatic feature recognition algorithm to identify and characterize myelinated axons. We then use a feature selection approach to identify the features that best discriminate between young and aged monkeys. Initial results show that there is a change in the spatial relationship between myelinated axons in the fornix, best described by the effective local density, a modified calculation of axon density which reflects how closely axons are packed. We have further extended our method to separately capture the myelin sheath and the axon, applying it to samples of over 40,000 axons from 25 rhesus monkeys. This will allow us to fully characterize significant features like the ratio of axon diameter to myelin sheath thickness. Moreover this feature analysis approach can be applied to characterize differences resulting from biological processes such as aging or damage from trauma or disease, as well as differences between anatomical regions such as the fornix and the cingulum bundle or corpus callosum.

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Poster

853. Data Analysis and Statistics III

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 853.15/VV50

Topic: G.07. Data Analysis and Statistics

Support: KAKENHI

Title: Cohort removal induces hyperthermia, increased pain sensitivity, and decreased anxiety-like behavior

Authors: *K. TAKAO[1,2], S. HATTORI[3,2], H. SHOJI[3,2], T. MIYAKAWA[3,1,2];
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Abstract: Various factors affect mouse behavior and must be considered in behavioral analyses. It was previously reported that sequential removal of animals from a common cage (cohort removal) induces hyperthermia in group-housed mice. The effects of cohort removal on mouse behavior, however, are not well characterized. To investigate the effects of cohort removal on rectal temperature, pain sensitivity, and anxiety-like behavior, we performed a large-scale analysis of behavioral data from more than 1000 mice that we collected using a comprehensive behavioral test battery. Mice were housed in groups of 4-5/cage. Rectal temperature increased in a stepwise manner according to the position of sequential removal, suggesting that cohort removal induces hyperthermia in mice. In the hot plate test, mice removed first from the cage had a significantly longer latency to the first paw response than mice removed later, suggesting that cohort removal increases pain sensitivity. Mice removed first from the cage also spent significantly less time on the open arms of the elevated plus maze test compared to mice removed later. This finding suggests that anxiety-like behavior is decreased by cohort removal. The results of the present study demonstrated that cohort removal induces hyperthermia, increased pain sensitivity, and decreased anxiety-like behavior in mice. Thus, the position of sequential removal from the cage should be carefully counter-balanced when characterizing mouse behavior.

Disclosures: K. Takao: None. **T. Miyakawa:** None. **S. Hattori:** None. **H. Shoji:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.01/VV51

Topic: G.07. Data Analysis and Statistics

Support: R01 DC006938

R01 DC00407

Title: Image J software designed to quantify multiple labels in sectioned tissue from confocal stacks in large experimental datasets

Authors: A. DAYAL[1], *D. L. HILL[2];
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Abstract: The ability to image sectioned neuronal tissue containing multiple labeled structures with a confocal laser scanning microscope has become standard in recent years. While the increased access to this important tool has led to many discoveries involving qualitative descriptions of the neuroanatomical and functional organization within neural structures, a precise and unbiased quantification of multiple stacks of optical sections in tissue containing three or more labeled channels has been challenging. Commercial software has been extremely useful in providing some quantification of labeled images within neural tissue; however, applications often are expensive and cumbersome for multiple uses. Here, we describe an ImageJ-based (NIH), open source program that we use to analyze terminal fields of three nerves that carry gustatory information to the nucleus of the solitary tract in mice. We use the program to first rotate image stacks so that the orientation of user-defined regions of interests is normalized among physical sections. Using a thresholder algorithm, the user then applies an unbiased pixel intensity frequency histogram for the image stack to generate an 8-bit binary image stack of pixels above threshold. A particle analysis is then used to quantify pixel areas above threshold, ultimately leading to a calculation of volumes of single and colocalized pixels for all optical sections in a stack. The program then converts data for all channels to a single data string that can be uploaded with the region of interest information and metadata to a server that organizes data defined by the user (e.g., animal name, group, experiment). These data can then be quickly and efficiently retrieved and analyzed by another ImageJ routine, which is designed to aggregate user-defined parameters (e.g., density of label, spread of label within the region of interest) for group-related analysis. We find that this open source software can be readily modified for multiple users and applications, and allows analyses of large datasets efficiently with the use of laptop computers.

Disclosures: A. Dayal: None. **D.L. Hill:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.02/VV52

Topic: G.07. Data Analysis and Statistics

Title: Acquiring practical population estimates of neurons through design-based stereology: Dissecting the disector

Authors: D. ZADORY, E. BURTON, *J. C. WOLF;
Exptl. Pathology Laboratories, Inc., Sterling, VA

Abstract: A design-based stereological probe known as the optical disector is employed frequently to acquire unbiased neuronal population estimates from thick histologic sections. This methodology includes customizable parameters for systematic sampling through the X, Y, and Z axes of the region of interest (ROI). For the purpose of this study, cryosections of 40 μm nominal thickness were immunostained for tyrosine hydroxylase to detect dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) of C57BL/6J mice. Stereological estimates of DA neuron populations were acquired using the Optical Fractionator Workflow module of the Stereo Investigator software system (MBF Bioscience). Because the histologic processing and immunostaining of thick sections may result in asymmetric distribution and density of DA neurons throughout the Z axis of the SNpc, various disector heights were analyzed systematically to determine the degree to which disector height selection contributed substantially to the total population estimates. We concluded that the choice of disector height had a major influence on total population estimates of DA neurons in the SNpc. For studies that utilize the optical disector method to quantify neuronal population estimates, adequate preliminary sampling should be performed initially through the entire Z axis with extrapolation of the data in order to achieve accurate population estimates.

Disclosures: D. Zadory: None. **E. Burton:** None. **J.C. Wolf:** None.

Poster

854. Data Analysis and Statistics: Software Tools

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Topic: G.07. Data Analysis and Statistics

Support: DARPA Contract No. N66001-11-C-4171

Title: Managing experimental data, metadata and automatic data importing in a neurophysiology lab

Authors: M. NOVELLI[1], *L. E. FISHER[2], R. GAUNT[1], D. J. WEBER[1];
[1]Univ. of PIttsburgh, Pittsburgh, PA; [2]Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Managing and organizing large data sets can be a daunting challenge, often including difficult tasks like format conversion and data importing into custom analysis systems. Our lab focuses on multichannel microelectrode recording and stimulation in the peripheral and central nervous systems to restore function after injury or disease and to investigate the role of the sensory feedback in normal motor control. Experiments may include hundreds of individual trials that generate multiple files totaling gigabytes. Further, each trial may involve a unique hardware setup that impacts structure and interpretation of the data. In order to manage these data sets, experimental data are converted to a standardized intermediate format and imported into our analysis framework. However, this process is time-intensive, error prone and difficult to debug, and, to date, must be performed manually. These overall challenges are common to many labs, yet common supported tools to solve these problems remain elusive. In order to standardize, organize and automate these procedures, we have developed a metadata database (MDDB) to improve the capture and organization of metadata related to each experiment (hardware setup, experimental goals, etc.) and a Matlab Database Importing Framework (MDIF) to facilitate automated format conversion and importing of data. The MDDB was designed to be platform independent, based on open source software, have minimal deployment requirements, simple customization, support multiple acquisition interfaces and data formats, and present a streamlined workflow. It is important that metadata can be entered into the MDDB quickly and efficiently during experimental sessions, and that those metadata can be accessed quickly and simply when analyzing experiments. The MDDB was ultimately implemented using Drupal (open source content management system). MDIF was designed to facilitate both interactive and automatic importing, as well as to have easy and extensive logging and reporting, and built-in functions to work on common data formats. MDIF is implemented in Matlab and uses the YAML text format to define each importing job. Using MDDB has allowed our lab to significantly improve metadata capture while simplifing data management and organization. Information regarding our experiments is located in one system, is easily accessible and can be verified by multiple users. This information can then be used by MDIF to improve data importing and preprocessing, breaking down the process in simple steps. After additional testing, we plan to release and support these tools to increase scientific output in research labs.

Disclosures: **M. Novelli:** None. **L.E. Fisher:** None. **R. Gaunt:** None. **D.J. Weber:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

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Program#/Poster#: 854.04/VV54

Topic: G.07. Data Analysis and Statistics

Title: Automated calcium imaging analysis in ilastik, the interactive learning and segmentation toolkit

Authors: F. DIEGO[1,2], J. KIRKHAM[2], S. BERG[2], S. PERON[2], M. SCHIEGG[1], T. KROEGER[1], *A. KRESHUK[1], U. KOETHE[1], F. A. HAMPRECHT[1,2];
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Abstract: Calcium imaging is very powerful thanks to its ability to simultaneously monitor the neuronal activity of hundreds of cells at single cell resolution, in many brain areas and model organisms. However, the development of computational techniques for reliable extraction of neuronal activity has lagged behind experimental advances and still requires major improvements. Most existing approaches require that cells, or parts of cells, are delineated in a manual [1] or semi-automated fashion [2]. However, this procedure does not easily scale to the large datasets that are now routinely acquired. We here present an automated calcium imaging analysis workflow based on [3] and implemented in ilastik, the interactive learning and segmentation toolkit. It allows end-users to conveniently analyze their data with minimal manual effort. Its automated detection of cell centroids relies on a flexible matrix factorization that exploits the sparseness of neuronal activity in space and time. We have chosen ilastik for this implementation because it can process and visualize image data in up to five dimensions. In addition, its ability to perform calculations in a strictly lazy manner makes it especially attractive for dealing with very large datasets. We will show how this ties in with the other capabilities of ilastik that are useful for neuroscience analysis, to wit: (1) interactive pixel and object level classification, (2) convenient proof-reading, and (3) seeded segmentation. Taken together, these capabilities facilitate correlative *in vivo* imaging and 3D Electron Microscopy as in [4]. The new workflow will be made available as open source software at www.ilastik.org/sfn [1] Grewe et. al, Nature Methods 2010 [2] Huber et. al, Nature 2012 [3] Diego et. al, ISBI 2013 [4] Maco et. al, PLOS ONE 2013

Disclosures: F. Diego: None. **J. kirkham:** None. **S. Berg:** None. **M. Schiegg:** None. **T. Kroeger:** None. **A. Kreshuk:** None. **U. Koethe:** None. **F.A. Hamprecht:** None. **S. Peron:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

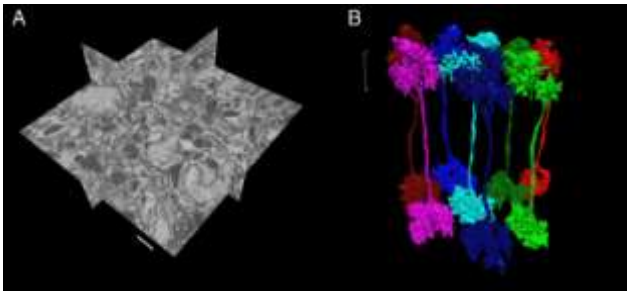
Program#/Poster#: 854.05/VV55

Topic: G.07. Data Analysis and Statistics

Title: Extracting a comprehensive map of the *Drosophila* medulla: A solution for reconstructing large EM connectomes

Authors: S. PLAZA[1], *P. K. RIVLIN[1], S.-Y. TAKEMURA[1], L. UMAYAM[1], Z. LU[2], S. C. XU[1], T. PARAG[1], D. J. OLBRIS[1], T. ZHAO[1], W. KATZ[1], J. A. HORNE[2], C. WEAVER[1], S. TAKEMURA[1], R. ANICETO[1], L.-A. CHANG[1], M. GARCIA[1], S. LAUCHIE[1], O. OGUNDEYI[1], C. SIGMUND[1], J. TRAN[1], C. LANGILLE[2], K. LE LACHEUR[2], S. MCLIN[2], A. SHINOMIYA[2], H. HESS[1], I. A. MEINERTZHAGEN[2,1], L. K. SCHEFFER[1];
[1]Fly EM Project, HHMI/Janelia Farm Res. Campus, Ashburn, VA; [2]Psychology & Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Reconstructing even small neuronal circuits from EM datasets often requires years of manual tracing (i.e., proofreading). While collaborative and crowd-sourcing efforts (Saalfeld et al. 2009, Kim et al. 2014) and machine-guided strategies (Chklovskii et al. 2010) aim to make tracing more tractable, state-of-the-art reconstructions include only a few hundred sparsely traced neurons (Denk et al. 2013) or dense tracings in very small regions, such as one medulla column (Takemura et al. 2013). We introduce a new EM reconstruction pipeline that increases throughput by roughly 5X compared to Takemura et al. 2013 with the potential for significant improvement in the future. We applied this reconstruction pipeline to seven columns of the *Drosophila* medulla extracting ~550 cells and ~350,000 synapses in ~1 year, the largest dense connectome ever derived from an EM dataset. Some of the fundamental advances in reconstruction methodology include the following: 1) imaging tissue with FIB-SEM instead of TEM giving isotropic voxels with better z resolution, and, consequently, better automatic image segmentation and easier manual proofreading, 2) segmentation algorithms that exploit non-membrane neuronal features likes mitochondria, 3) 'focused' proofreading workflows that intelligently minimize manual annotation efforts, and 4) systematic use of morphology and synaptic connectivity as quality control mechanisms. Figure: EM reconstruction of medulla. (A) Dataset with 10x10x10 nm voxel resolution (bar = 1 μm), (B) Reconstruction of eight L1 neurons exposing columnar structure of medulla (bar = 5 μm)



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Poster

854. Data Analysis and Statistics: Software Tools

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Program#/Poster#: 854.06/VV56

Topic: G.07. Data Analysis and Statistics

Support: Janelia Farm Research Campus, HHMI

Title: Analysis tools for connectomes

Authors: *L. SCHEFFER[1], C. WEAVER[1], S.-Y. TAKEMURA[1], P. RIVLIN[1], S. PLAZA[1], I. MEINERTZHAGEN[2];
[1]HHMI, Ashburn, VA; [2]Dalhousie, Halifax, NS, Canada

Abstract: A deep understanding of brain function and thence behavior faces the task of mapping the brain's actual synaptic connections, so as to yield a complete wiring diagram or connectome. The latter are now being approached using focused ion beam (FIB)-SEM reconstructions, but are too large and unwieldy to be analyzed by hand. For example, a recent connectome of seven columns from the second neuropile, or medulla, of the optic lobe in *Drosophila* melanogaster contains 550 named cells, thousands more that are partially reconstructed, and about 310,000 pre-to-post synaptic connections. We have made a first version of software designed to analyse such a connectome. Features include: (a) Comparison of neurons of the same type. Given a neuron type, such as the medulla intrinsic columnar neuron Mi1, all connections are displayed, ordered by strength and by physical location. (b) Summary of inter-column connections, ordered by direction in the hexagonal array of the medulla columns. (c) Tables ordered by connection strength, averaged over all cells of the same type. Tables such as these are useful for investigating not only the strongest connections, but also the long tail of very weak connections that seems to be typical of all wiring in this part of the brain. (d) Comparisons of specific subsets of neurons. In the medulla, 'pale' and 'yellow' columns have paired photoreceptor inputs of two different spectral subtypes and somewhat different wiring. These

tables generate the average of all columns of each type, to enable comparison between both, and reveal circuit differences. (e) Various metrics of stereotypy. It is clear from our 7-column reconstruction that the medulla is stereotyped only in a statistical sense, and not an absolute one. These tables summarize the observed stereotypy by several metrics - cell area, cell volume, and connection counts. (f) Comparisons with other reconstructions. Our group previously reconstructed a single column, from a different fly, using serial thin-section EM as opposed to FIB-SEM, and older reconstruction software. Our tables directly compare the results obtained with the two different methods. (g) Visualization of receptive fields. One of the main functions of medulla is circuits is to generate receptive fields for further processing in the deeper neuropiles, the lobula and lobula plate. To help us visualize these receptive fields, we have constructed an interactive tool that displays the inputs of selected cells weighted by their synapse number and by their physical location over the target neuron's dendritic tree.

Disclosures: **L. Scheffer:** None. **P. Rivlin:** None. **S. Takemura:** None. **S. Plaza:** None. **C. Weaver:** None. **I. Meinertzhagen:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.07/VV57

Topic: G.07. Data Analysis and Statistics

Support: NIH/NIMH grant MH094360-01A1

This project was also partially supported by CHDI foundation.

Title: Interactive registration workflows for label quantification of light-based microscopy images

Authors: ***I. BOWMAN**, M. BAY, M. Y. SONG, M. S. BIENKOWSKI, A. TOGA, H.-W. DONG;
USC INI, Los Angeles, CA

Abstract: A typical Mouse Connectome Project (MCP) study involves dozens of C57Bl/6J mouse brains, processed and analyzed toward understanding long-range neural networks. Each animal (case) is injected with two anterograde (PHA-L & BDA) and two retrograde (CTb & FG) chemical tracers, and given weeks of transport time before euthanization and brain excision.

Individual brains are cut into 50 µm coronal sections, which undergo fixation and immunostaining before being mounted onto slides for imaging. Epifluorescent scans of a single section comprise five channels (one channel for each tracer plus one channel for cytoarchitecture), each stored at up to 2 GB in size using 10x objective magnification. Opening, viewing and editing a single 2 GB scan is non-trivial even on state of the art equipment -- manually annotating the connectivity of hundreds of scans is intractable and can take a team of researchers months. At the MCP group, we therefore developed an image registration workflow for mouse brain microscopy image scans that significantly speeds up annotation and analysis. Workflow interaction is performed via a novel interface for image registration: the user observes correspondence point templates to evaluate and provide feedback to the image warping algorithm, as well as evaluate the output of subsequent processing. After warping, a segmentation method that extracts labeled cells from tissue background is applied to each scan. The segmented images provide graphical reconstructions of labeling registered to a common spatial frame. A final annotation stage employs an overlap indexing method to the segmented images. This comprehensive annotation output of our registration workflow spans multiple cases, and quantifies connectivity from numerous injection sites to their corresponding upstream or downstream regions of interest. Analyzing this data allows the user to identify clusters, hubs, motifs and other graph analysis metrics as well as visualize connectivity information. Applying our registration workflow to the MCP brain data has significantly expedited our analysis of brain connectivity, as well as furthered our ability to present these findings to the neuroscientific community.

Disclosures: I. Bowman: None. **M. Bay:** None. **M.Y. Song:** None. **M.S. Bienkowski:** None. **A. Toga:** None. **H. Dong:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.08/VV58

Topic: G.07. Data Analysis and Statistics

Support: Howard Hughes Medical Institute

Title: Mapping behavior to neural anatomy in *Drosophila* melanogaster using machine vision and thermogenetics

Authors: *A. A. ROBIE, M. RIVERA-ALBA, M. KABRA, A. EDWARDS, W. KORFF, K. M. BRANSON;
HHMI - JFRC, Ashburn, VA

Abstract: Understanding how the nervous system generates behavior requires the detailed study of the neural circuits underlying a given behavior. However, even in the well-studied model organism, *Drosophila melanogaster*, there are still areas of "terra incognita" in the nervous system where elements of these circuits are unknown. To further the understanding of the structure-function relationship of the fly's nervous system, we performed a thermogenetic activation screen using the GAL4-UAS system to target expression the cation channel dTrpa1 to small groups of neurons. We screened 2215 of the sparsest lines from the Janelia GAL4 collection and assayed the flies' behavior in an open-field walking arena. We recorded simultaneously from 8 chambers, each containing 20 flies (10 males and 10 females) for 1000 s at the permissive temperature of dTrpA1. The video, 1024x1024 at 30 fps, was recorded using a custom real-time compression algorithm that is lossless to the tracking software, and reduces file size by a factor of 80. Our analysis pipeline tracked the body and wing pose of each fly, in each of 19528 videos. From the flies' trajectories, the pipeline computed metrics of flies' locomotor movements and social interactions. We applied 14 automatic behavior classifiers that we created using JAABA (an interactive machine learning system) to these metrics. This resulted in behavioral annotations of each fly in each frame of the videos, 175 billion annotations in total. To ensure that the classifiers performed well across the entire collections of videos, we compared the automatic labels to manual labels for each behavior from a set of representative lines. The classifiers generalized well with a mean error rate of 2.7% and a maximum error rate of 6.6%. The behaviors detected by our automatic classifiers include locomotor behaviors such as walking, stopping, jumping, walking backwards or sideways, turning and wing grooming, and social interactions such as chase, touch, attempted copulation, and single-wing extension. We then extracted groups of lines with similar behavioral phenotypes based on both the annotated behaviors and raw metrics such as velocity. For these groups, we looked for overlap in the average image expression pattern of the GAL4 driver lines (imaged by the Janelia FlyLight project). The brain areas with significantly increased expression are likely to be involved in the production of the behavioral phenotype. This work results in putative brain-behavior maps, and modern genetic tools, such as intersections between two GAL4 lines, will allow further refinement of these maps to the level of single neurons.

Disclosures: A.A. Robie: None. **M. Rivera-Alba:** None. **M. Kabra:** None. **A. Edwards:** None. **W. Korff:** None. **K.M. Branson:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.10/VV60

Topic: G.07. Data Analysis and Statistics

Support: HHMI Janelia Farm Research Campus

Instituto Gulbenkian de Ciência

Title: *Drosophila* larva foraging strategies: Automatic tools for high-throughput comparative studies

Authors: M. RIVERA-ALBA[1], M. KABRA[1], C. K. MIRTH[2], *K. M. BRANSON[1];
[1]HHMI Janelia Farm Res. Campus, Ashburn, VA; [2]Development, Evolution and the Envrn., Inst. Gulbenkian de Ciência, Oeiras, Portugal

Abstract: Foraging strategies are crucial for larval growth and survival. However, little is known about how these strategies evolve or develop across species. To address this issue, we have developed a comprehensive high-throughput protocol to analyze the foraging behavior of more than 40 species of Drosophilid larvae. First, we collected a large video collection of foraging larvae at 2 developmental stages. Then, we developed a tracking algorithm to extract the trajectories of individual larvae across the collection. From these trajectories we computed more than 100 statistics describing the larval posture and dynamics. From these statistics, we used JAABA (Janelia Automatic Animal Behavior Annotator) an iterative machine learning system, to train several automatic behavior classifiers. We used these classifiers to automatically label foraging behaviors including crawling, turning, head casting, burrowing and backwards crawling in our large video collection. The foraging behavior across Drosophilid larvae shows common structure with distinct parameters for each species and stage. We found that the distribution of crawling bout lengths follows an exponential distribution for each species, suggesting memoryless events. However, the parameter of the exponential varies with species and stage. The same is true for the distribution of body angle during crawling: for all species and stages this distribution is well fit by a laplacian, but each species and stage has different parameters. We would like to use these tools to explore the question of how different two nervous systems implementing the same foraging strategy are compared to nervous systems implementing different strategies.

Disclosures: **M. Rivera-Alba:** None. **M. Kabra:** None. **C.K. Mirth:** None. **K.M. Branson:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.11/VV61

Topic: G.07. Data Analysis and Statistics

Support: NSF Engineering Research Center for Sensorimotor Neural Engineering (Award Number EEC- 1028725).

Title: How susceptible is the brain to the side-channel private information extraction? An experimental analysis using non-invasive brain-computer interfaces

Authors: *T. BONACI[1], J. HERRON[1], T. LIBEY[2], B. MOGEN[2], H. J. CHIZECK[1];
[1]Dept. of Electrical Enginnering, [2]Dept. of Bioengineering, Univ. of Washington, Seattle, WA

Abstract: An increasing number of Brain-Computer Interfaces (BCIs) are being developed and used in medical, marketing, and entertainment applications. BCI technology carries a great potential to improve the quality of human lives. It provides people suffering from neuromuscular disorders with a way to interact with the environment. It also enables a more personalized experience in gaming and entertainment. Currently, several neural engineering companies offer consumer-grade BCIs and software development kits. These companies have recently introduced the concept of BCI "app stores" in order to facilitate expansion of BCI applications. It is expected future BCIs will be simpler to use and will require less training time and user effort, while enabling faster and more accurate decoding of users' intentions. However, this growth in BCI application space is not without risk. Established engineering practices set guarantees on performance and reliability of BCI devices. But no standards are currently available regarding users' privacy and security. Moreover, the first BCI-enabled malicious application, referred to as "brain spyware", was introduced at the 2012 USENIX Security Symposium. This application uses a paradigm similar to the P300-speller to extract users' private information, such as credit card PINs or dates of birth, from recorded neural signals. As BCIs become widespread, other "brain malware" is easy to imagine. It may be possible to extract private information about users' memories, prejudices, religious and political beliefs. The extracted information could be used to manipulate or coerce users, otherwise harm them, or perhaps just employeed to provide targeted advertising and product purchase suggestions. In this work, we experimentally analyze how non-invasive BCI platforms, used in scenarios such as game playing or web navigation, can be misused to extract users' private information. More specifically, we analyze the feasibility of

different Event Related Potential (ERP) components for information extraction by using subliminal stimuli presented to users for approximately 7ms. In our experiment users play an EMG-controlled game and are presented with subliminal stimuli while we log their EEG to extract private information offline. Our results indicate subliminal stimuli represent a feasible way for extraction of users' private information. We observe, however, that the screen location of the stimuli, users' distance from the screen, and their attention levell all impact the way that stimuli are perceived and the type of resposnses that they elicited. In addition, familiarity with the stimuli also has an effect on extraction feasibility.

Disclosures: T. Bonaci: None. **J. Herron:** None. **T. Libey:** None. **B. Mogen:** None. **H.J. Chizeck:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.12/VV62

Topic: G.07. Data Analysis and Statistics

Title: Flokka: a document prioritization system for aiding neuroscience knowledge base curation

Authors: *K. H. AMBERT[1], A. M. COHEN[2], G. A. P. C. BURNS[3], E. BOUDREAU[2], K. SONMEZ[2];
[1]Intel Labs, Beaverton, OR; [2]Biomed. Informatics, Oregon Hlth. & Sci. Univ., Portland, OR; [3]Information Sci. Inst., USC, Marina del Rey, CA

Abstract: The importance of multi-level modeling in computational neuroscience has led to an increased need for machine-readable knowledge bases documenting knowledge at multiple levels of brain and behavior, but identifying publications containing relevant information for such collections can be laborious and expensive. Automatic machine learning-driven document classification can be a useful tool for increasing the rate at which knowledge base curators are able to identify new documents containing information of interest to them. Although using such systems to classify and prioritize documents for curation workflows is not a new idea, there are several complications associated with applying previously-described systems to the neuroscience literature base, including terminological differences between neuroscience sub-disciplines, and cross-species differences in anatomical parcellation. Here, we describe Flokka, a document classification system specifically tuned to work with neuroscience publications, and built to identify publications containing information of interest to a knowledge base of neuron-related

information. Our series of experiments compares various system configurations, and identifies several useful techniques that circumvent many of the language idiosyncrasies that can be found in neuroscience publications. We examine the differences between our best-performing system and those known to succeed in other domains, and discuss what these differences say about the characteristics of the neuroscience literature base.

Disclosures: **K.H. Ambert:** None. **A.M. Cohen:** None. **G.A.P.C. Burns:** None. **E. Boudreau:** None. **K. Sonmez:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.13/VV63

Topic: G.07. Data Analysis and Statistics

Title: Quality control software and visualization for diffusion tensor imaging

Authors: *K. J. WHITAKER;
Dept. of Psychiatry, Brain Mapping Unit, Cambridge, United Kingdom

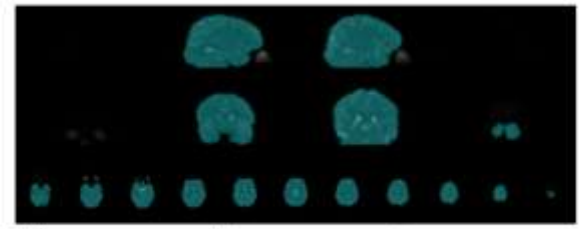
Abstract: In order to answer the plethora of questions that understanding the human brain presents, increasingly large numbers of MRI acquisitions are collected. Furthermore, the number of researchers utilizing this data also growing: within and outside of the academic institution in which data is stored, and from a widening range of research areas. In order to ensure that their work is efficient and effective, systematic quality control and assurance becomes imperative. The quality assurance code presented here allows researchers to ensure that the pre-processing and tensor fitting code that processes raw diffusion tensor imaging data has generated good enough quality images to include in group statistical modelling. The one page pdf report is easy to access, share and compare across subjects. Figures 1 and 2 are reports from example high and low quality scans. They show the original data, brain extraction mask, movement parameters, white matter segmentation, and histograms fractional anisotropy, mode of anisotropy and sum of square errors from the tensor fit. The axes for the line plots and histograms are fixed so reports can be easily compared between subjects. There is no judgement of data quality but the reports facilitate researchers in making their own decisions and in concisely describing data quality when sharing processed data with others. The code is designed to interact particularly well with FSL, is written in python and can be downloaded from https://github.com/HappyPenguin/DTI_PROCESSING.

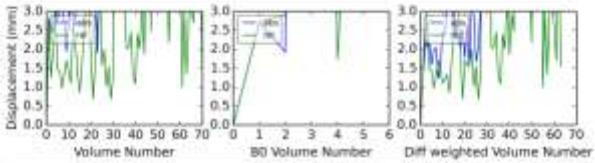
Diffusion Tensor Imaging Quality Report

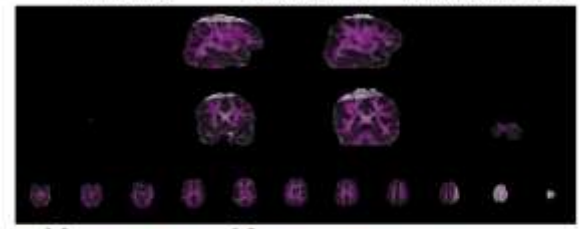
SubID:_____ Date:_____

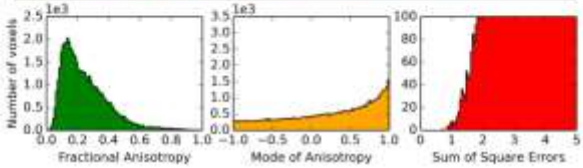
Pass ☐

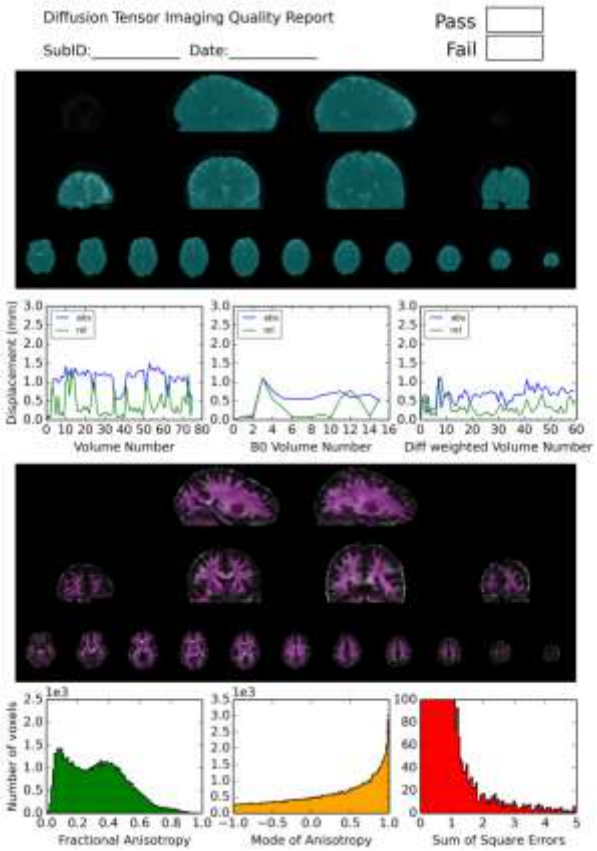
Fail ☐











Disclosures: K.J. Whitaker: None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.14/VV64

Topic: G.07. Data Analysis and Statistics

Title: Automatized depth electrode for electroencephalographic recording in epilepsy using alternative software

Authors: *J. R. BELTRAN[1,3], M. MACIEL ARELLANO[2], G. GARCIA TORALES[2]; [1]Univ. Guadalajara, GUADALAJARA, Mexico; [2]Univ. Guadalajara, Guadalajara, Mexico; [3]Mecatronica, Ctr. de Enseñanza Técnica Industrial, Guadalajara, Mexico

Abstract: Epilepsy is a neurological alteration characterized by the recurrent appearance of spontaneous seizures due to neuronal hyperactivity in the brain. Approximately 10% of the general population experience at least one seizure during their lifetime and 1% have epilepsy. The electroencephalographic technique offers the advantage to monitor the seizures in different experimental epilepsy models this depends on the quality of the electrode implantation surgery. The epilepsy animal models are a good tool to study the basic mechanism for the epileptiform activity. We used an automatized system to avoid the direct animals (rats) manipulation, this method prevents noise in the electroencephalography (EEG) and offers the option of doing behavioral probing along with the EEG. This investigation purpose is to analyze these animals behavior during epileptic seizures with the EEG register, using the automatized equipment that has no noise signals generated by a human being, and the dynamic control position of the implanted electrodes, the mobile electrodes provide a better signal and greater area of study. For this purpose intracranial EEG recordings from hippocampus, were made in Wistar rats with motorized microdrive and alternative software. Seizures were induced by intracerebroventricular 4-Aminopyridine (4-AP) which is a potent convulsive drug that blocks voltage-activated K+ channels in a wide variety of cells types, including neurons, cardiac muscle, skeletal and smooth muscle. The systemic or intracerebral administration of 4-AP induces generalized tonic convulsions in man and other species. The alternative presented in this project is the technological development, which may make the electroencephalogram wireless and with the use of the depth automatized electrodes will be able to be moved automatically, by a long-distance control in these experiments was evaluated the normal and pathological brain activity. These results show that to use this registration system allows a higher precision and one option for the EEG in behavior experiment.

Disclosures: J.R. Beltran: None. **M. Maciel Arellano:** None. **G. Garcia Torales:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

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Program#/Poster#: 854.15/VV65

Topic: G.07. Data Analysis and Statistics

Support: German INCF Node (BMBF grant 01GQ1302)

European Regional Development Fund (ERDF), Project "NTIS - New Technologies for Information Society", European Centre of Excellence, CZ.1.05/1.1.00/02.0090.

Title: Mobile applications for acquiring experimental metadata: Supporting data description/reproducibility at the bench

Authors: *Y. LE FRANC[1,2], D. GONZALEZ[2], I. MYLYANYK[2], J. GREWE[2,3], P. JEŽEK[4], R. MOUčEK[4], T. WACHTLER[2];
[1]Theoretical Neurobio., Univ. of Antwerp, Wilrijk, Belgium; [2]Dept. Biologie II, Ludwig-Maximilians-Universität München, Munich, Germany; [3]Eberhard Karls Univ. Tübingen, Tübingen, Germany; [4]Dept. of Computer Sci. and Engineering, New Technologies for Information Society, Univ. of West Bohemia, Plzeň, Czech Republic

Abstract: Enforcing data reproducibility, sharing and reuse requires designing solutions tailored for scientists and allowing them to cope on a daily basis with the complexity of experimental paradigms in neuroscience and of the associated recorded data and metadata. The current situation poses a challenge to capture metadata in a computer readable format as often such crucial experimental information is hand-written in lab notebooks. The process of converting this information in various text files or spreadsheets is time-consuming and error prone. Furthermore, it leads to an explosion of incompatible metadata formats, which hampers the development of common software solutions to further manage and re-use the metadata. A flexible data model for metadata, odML, was proposed (Grewe et al., 2011) to support efficient organization of experimental metadata. In order to enable scientists to seamlessly and efficiently acquire their metadata in such machine readable format, we here present a standalone mobile app that can be used at the bench, independent of lab environment, or even outside in the field. The tool was inspired by, and is similar in concept to, the pioneering solution for clinical assessment, CARAT (Turner et al., 2011), but uses odML as a standard data model. This standalone app runs on iOS and Android platforms and provides an intuitive and simple user interface for designing metadata templates and managing metadata structures. Through this interface, researchers are able to build metadata forms that can be either directly filled in with acquired values, or saved as empty templates for re-use. Moreover, the user can add constrains on the forms such as defining required fields, the order of the fields. Thus, one can design fillable forms that are adapted to the user's experiment and can be re-used in later experiments. Experimental records are stored as odML files and therefore can be integrated with other experimental metadata. This mobile application provides an important element in a tool chain for metadata management that facilitates the recording of metadata in machine-readable form. The strengths of this approach is 1- to hide the complexity of odML structures from the user while providing the necessary control over the data entry process and 2- to rely on a general and efficient model to acquire metadata from various experiment types and scientific domains. References: Grewe, J., Wachtler, T., & Benda, J. (2011). A Bottom-up Approach to Data Annotation in Neurophysiology. Frontiers in neuroinformatics, 5, 16. Turner JA, Lane SR, Bockholt HJ and Calhoun VD (2011) The clinical assessment and remote administration tablet. Front. Neuroinform. **5**:31.

Disclosures: **Y. Le Franc:** None. **D. Gonzalez:** None. **I. Mylyanyk:** None. **J. Grewe:** None. **P. Ježek:** None. **R. Mouček:** None. **T. Wachtler:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.16/VV66

Topic: G.07. Data Analysis and Statistics

Title: What is a complete record of observational neuroscience data?

Authors: *P. S. PENNEFATHER[1], W. SUHANIC[2];
[1]Univ. Toronto, Toronto, ON, Canada; [2]gDial Inc, Toronto, ON, Canada

Abstract: Neuroscience knowledge of how brain cells, genes and circuits interact remains in a constant state of flux. Since that knowledge is justified through interpretation of experimental observations, records of those observations need to be amenable to re-interpretation and re-consolidation in ways that accommodate new perspectives and previously unappreciated information. Observational data, whatever their origin, can now be precisely preserved digitally independent of the reasons and methods governing its collection. That data may become meaningless if separated from the rationalizations used to justify their collection. But, although those rationalizations may come in time to be recognized as deficient, that evolution of understanding need not devalue the recorded data provided re-interpretation is possible. Such inevitable re-interpretation can be facilitated by a complete record of how the observational data were carried out and recorded in the first place. Here we explore a work flow for characterizing a complete record of the process responsible for acquiring and digitally recording any given set of observational neuroscience data. We use the case of imaging live-cell dynamics captured using imaging sensors that generate raw image files-(which are minimally formatted)-while enabling its storage in a computer readable format for subsequent display and analysis. We have previously described how our BioTIFF/Intercase platform can enable wrapping and then importing that raw image data into an augmented .tif format to serve as an indexable repository of observational data. Here we extend and justify the concept of a complete record to include recording the configuration of the operating system that enabled the digital data to be recorded. When the BioTIFF recording is accomplished using, for example, an open source operating system like Linux, all elements of the operating system can be registered in the record. We will discuss how other input settings associated with any given observational event can and should be recorded and linked to expected outputs. Subsequent recognition of outcomes in need of re-interpretation can then be more effectively analyzed to determine what assumptions are responsible for experimental outputs deviating from expectations. We propose that raw research

object outputs of any publicly funded research should be recorded in as complete a manner as possible to enable re-use and re-interpretation.

Disclosures: **P.S. Pennefather:** Other; The authors are owners of a two person strat up called gDial seeking to deliver complete record services. **W. Suhanic:** Other; The author is CEO of gDial Inc.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

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Program#/Poster#: 854.17/VV67

Topic: G.07. Data Analysis and Statistics

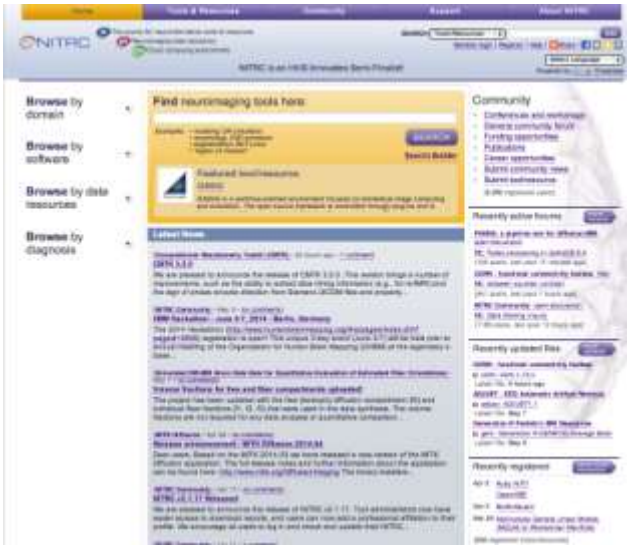
Support: Contract No.: N02-EB-6-4281 NIH Blueprint for Neuroscience Research.

Title: Neuroimaging informatics tools and resources clearinghouse (nitrc): A resource for education in neuroimaging techniques

Authors: *D. N. KENNEDY, C. HASELGROVE;
Psychiatry, U. Massachusetts Med., WORCESTER, MA

Abstract: We report on a knowledge environment that is suited to education in neuroimaging informatics recently expanded from MR to PET, EEG, MEG, SPECT, CT and optical neuroimaging tools and resources: Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC). Funded by the NIH Blueprint for Neuroscience Research, NIBIB, NIDA, NIMH, and NINDS, NITRC fosters a user-friendly clearinghouse environment for the neuroimaging informatics community. NITRC's goal is to support researchers dedicated to enhancing, adopting, distributing, and contributing to the evolution of previously funded neuroimaging analysis tools and resources for broader community use. Located at www.nitrc.org, NITRC promotes software tools, workflows, resources, vocabularies, test data, and now, pre-processed, community-generated data sets (1000 Functional Connectomes, ADHD-200) through its Image Repository (NITRC-IR). NITRC gives researchers greater and more efficient access to the tools and resources they need; better categorizing and organizing existing tools and resources via a controlled vocabulary; facilitating interactions between researchers and developers through forums, direct email contact, ratings and reviews; and promoting better use through enhanced documentation. In Summary, NITRC facilitates access to a growing number of neuroimaging tools and resources (~655), and supports (~1 mil. hits monthly by ~142,750

unique visitors, initiating ~450,000 downloads). NITRC has established itself as a key resource for the training of and advancement in neuroimaging research.



Disclosures: **D.N. Kennedy:** None. **C. Haselgrove:** None.

Poster

854. Data Analysis and Statistics: Software Tools

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.18/VV68

Topic: G.07. Data Analysis and Statistics

Support: ICT-FET FP7/2007-2013, FET Young Explorers 284772

Title: A bi-directional neuro-robotic system for the study of computational properties of neuronal assemblies

Authors: J. TESSADORI, M. BISIO, I. NAVA, *M. CHIAPPALONE;
Neurosci. and Brain Technologies, ISTITUTO ITALIANO DI TECNOLOGIAA, Genova, Italy

Abstract: Many features of the brain are currently impossible to replicate in a technological system, such as quick processing of sensory information and subsequent generation of complex motor commands. Hybrid model systems have been developed to study sensori-motor feedback loops with respect to a supposed behavior (e.g., obstacle avoidance). We present our neuro-

robotic architecture based on a neural controller bi-directionally connected to a virtual robot, characterized by proximity sensors and wheels. As neural controller, we used cortical and hippocampal cultures dissociated from embryonic rats (E18) and kept alive over Micro Electrode Arrays (MEAs) for 3-4 weeks. Proximity sensor readings are coded as square-wave impulses sent to the network, while two sets of electrodes are defined as left and right output areas of the robot. Repetition rate of input events is the feature used to code information. For decoding, each time a given event is detected in one of the output electrodes, the speed of the corresponding wheel is increased, while it decreases exponentially in time. We tested uniform and patterned networks for the impact of geometry on responses; we tested decoding schemes in which bursts and isolated spikes had different weights to understand the role of these events in information coding; we applied tetanic stimulation as a possible learning cue and analyzed the induced changes in functional connectivity. We verified an exchange of information by comparing the navigation performances of the robot in closed-loop and in two different control conditions. Partial confinement of networks lead to different patterns of activity that can be used in neural control tasks. Tetanic stimulation caused remarkably precise changes in the temporal response patterns to stimulus, with different effects on hippocampal and cortical cultures. These changes tended to be unpredictable from the spatial point of view and could not be used to strengthen or weaken connections at a specific site. Testing different decoding schemes indicate that, at least in cultures, bursts do not code for specific features of the stimulus, but this result might depend on the distribution of the adopted stimuli train. We plan to take advantage of our experimental setup to investigate the interplay between spontaneous and evoked activity in neuronal assemblies and the resulting properties.

Disclosures: **J. Tessadori:** None. **M. Bisio:** None. **I. Nava:** None. **M. Chiappalone:** None.

Poster

854. Data Analysis and Statistics: Software Tools

Location: Halls A-C

Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.19/VV69

Topic: G.07. Data Analysis and Statistics

Support: NSF Grant ECS-1002391

Title: Improved M-Sorter with unsupervised clustering

Authors: ***W. MA**, J. SI;
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Abstract: We first introduced our M-Sorter package in 2012. The M-Sorter aimed at providing automatic action potential (spike) detection and classification with high accuracy. The first M-Sorter was based on the multiple correlation of wavelet coefficients (MCWC) algorithm for spike detection. In this study we propose a new version of an improved M-Sorter, or the M-Sorter2. An important new feature of M-Sorter2 is that it replaces the semi-automatic K-means clustering method with our novel accurate robust expectation-maximization (AREM) spike classification method. This approach automatically generates the number of clusters and assigns detected spikes to each cluster. The M-Sorter2 consists of three major steps: 1) the same spike detection using MCWC as in the original M-Sorter (Yuan et. al, 2012); 2) principal component analysis (PCA) based feature extraction; and 3) automatic clustering by AREM. Only two free parameters are needed in M-Sorter2: noise floor level τ and spike quality level S. A high τ value is preferred if signal to noise ratio is low and a high S value is correlated with tightened spike detection condition, i.e., only high quality spikes are identified for further classification. The performance of the M-Sorter2 was tested using 3 types of datasets: artificial dataset, labelled real dataset and unlabeled real dataset. The artificial dataset is from the Wave Clus software package, which consists of 4 groups of datasets. The real dataset has 8 subsets of extracellularly recorded neural waveforms from rat's motor cortices of our lab. One of the real datasets was manually labelled by an expert to provide a labelled real dataset. The same dataset was then verified by another expert. The M-Sort2 was compared with Wave Clus (Quiroga et al., 2004), as well as Signal Energy detection plus T-Distribution EM classification in Plexon's Offline Sorter (Plexon Inc.). The threshold and temperature in Wave Clus, the energy threshold and Multi. DOF in Offline Sorter, as well as τ and S in M-Sorter2 are determined by the user for optimal sorting performance. According to the test results from the artificial dataset, M-Sorter2 outperformed the other two sorters in terms of low false positive and false negative rates, and high classification accuracy even under conditions of low SNR, high similarity among clusters, and overlapping spikes. The test results from the labelled real data also provided similar conclusion that M-Sorter2 not only had a better overall sorting performance, but also required less dependence on the design parameters. For the real datasets, we inspected spike counts, waveform shapes, and waveform variances of sorted spikes for all 3 sorters.

Disclosures: W. Ma: None. **J. Si:** None.

Poster

854. Data Analysis and Statistics: Software Tools

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Time: Wednesday, November 19, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 854.20/VV70

Topic: G.07. Data Analysis and Statistics

Title: Google glass display for a brain-computer interface

Authors: *R. E. ALCAIDE[1,1], D. BROWN[2], X. MA[3], A. AREF[4], J. HUGGINS[1];
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Abstract: Brain-computer interfaces (BCIs) offer the hope of communication and technology control without physical movement. Instead, voluntary brain activity is interpreted as commands to control technology. For this reason BCIs may soon help people with degenerative movement disorders, like amyotrophic lateral sclerosis (ALS). ALS leads to degeneration of motor neurons and with time can leave a person unable to move or communicate. For these people, the simple act of telling their spouse, "I love you", turning on a TV or posting a message on a social media site has indescribable value. One of the hurdles for practical everyday brain-computer interface (BCI) use is the large, cumbersome display, which interferes with sight lines and presents challenges to portability. Further, such a display requires adjustment every time the user is repositioned. Google Glass (Google inc. Mountain view, CA) is a transparent, head-mounted display that offers a high degree of portability and customization. We have integrated Google Glass as a BCI display for typing and launching of android-based applications using only brain activity. The Google Glass BCI system was also integrated with an Arduino (Sparkfun Inc. Boulder, CO) to allow control of external devices such as a home automation system and a television. BCI 2000 was adapted to control the Google Glass display though a Wi-Fi connection and a custom built android environment. These custom developed tools circumvented restrictions of the default Google Glass development tools. In future work we plan to further integrate the BCI and Google Glass system. For example we are integrating social media applications such as Twitter and Facebook. We are also developing tools to allow the Google Glass BCI user to search and view YouTube videos. We are also exploring smartphone functionalities to create context-specific BCI control options. For example, using the GPS libraries of the Android Smartphone, we will be able to provide location dependent commands. We also plan on further integrating the Google Glass BCI to other external devices such as wheelchairs. We believe our developments may help bring BCIs outside the laboratory and into the homes of people in a locked-in state from conditions such ALS.

Disclosures: R.E. Alcaide: None. **D. Brown:** None. **X. Ma:** None. **A. Aref:** None. **J. Huggins:** None.

Poster

854. Data Analysis and Statistics: Software Tools

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Program#/Poster#: 854.21/VV71

Topic: G.07. Data Analysis and Statistics

Support: Technological validation of the results of research and development
CZ.1.05/3.1.00/14.0298

Title: Prototypes of software portal and stimulation device for electrophysiological research

Authors: ***R. MOUCEK**, P. JEŽEK, P. MAUTNER, J. NOVOTNÝ, M. BYDŽOVSKÝ, J. RINKES, T. PROKOP, J. ŠTěBETÁK, P. BRůHA;
New Technologies for the Information Society, Univ. of West Bohemia, Plzen, Czech Republic

Abstract: This abstract focuses on recent conceptual improvements of two software and hardware prototypes (as foreseen in Mouček et al., 2014) that can be easily integrated into various electrophysiological laboratory systems: the EEG/ERP Portal (EEGbase) and a stimulation device for cognitive research. The EEG/ERP Portal is a web based application for long-term storage, management, processing and sharing of electrophysiological data. From the beginning it was designed and developed using robust open source technologies and semantic web resources to ensure future extensibility of the application and sustainability of stored data/metadata. On the other hand, the Portal suffered from low flexibility with respect to the scope of the electrophysiological domain and covered a small number of laboratory needs in terms of the variety of stored metadata and customization of the user interface. As a solution, we left the idea of using high level concepts of the Semantic Web and individually applied selected best practices of this approach. This also includes the use of a non-relational database for storing and retrieving metadata. This change has led to higher flexibility of the metadata model, easier application of full-text search, and overall user acceptance. The stimulation device for cognitive research is an ARM based Cortex microcontroller including firmware and optional control software for creating various experiments in which the subject is stimulated by visual and/or auditory stimuli. It works in three modes: 1- sequential stimulation mode in which stimuli are presented sequentially respecting their probability (eligible for evoking ERP components in cognitive research), 2- simultaneous stimulation mode in which stimuli are presented simultaneously with corresponding frequencies (eligible for evoking VEP/SSVEPs), 3- stimulation mode with waiting for pressing a response button (eligible for experiments that include reaction time measurements). Stimulation outputs can be e.g. simple LEDs, LED panels, patterns presented on small LCD displays, simple tones of various frequencies and lengths, or sounds stored in wav files. The stimulator designed for research laboratories is mobile, enables synchronization with recording devices and can be connected to conventional equipment. Both prototypes are tested and their operation is continuously evaluated during the experimental work.
References: Mouček R, Ježek P, Vařeka L, Řondík T, Brůha P, Papež V, Mautner P, Novotný J,

Prokop T and Štěbeták J (2014) Software and hardware infrastructure for research in electrophysiology. Front. Neuroinform. 8:20. doi: 10.3389/fninf.2014.00020

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Poster

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Topic: G.07. Data Analysis and Statistics

Support: ERDF, Project NTIS CZ.1.05/1.1.00/02.0090

Title: Complete process for collecting of well-described EEG/ERP experiments using mobile devices and their storage in remote databases

Authors: ***P. JEŽEK**, V. PAPEŽ, R. MOUčEK;
Computer Sci. and Engineering, New Technologies for Information Society, Univ. of West Bohemia, Plzen, Czech Republic

Abstract: Penetration of computers into medicine has formed a new scientific discipline, neuroinformatics. This discipline, which links computer sciences with medicine, significantly lowers execution of time consuming tasks and allows for collection of large data sets. A crucial task in experimental research is data and metadata management. The size and complexity of data is increasing with the number of experiments. As a solution so called neuroinformatics databases are gradually being developed. Because of heterogeneous nature of electrophysiological data, these databases must be robust and flexible. Especially in the EEG/ERP domain, we are developing a complex software platform for collection EEG/ERP experiments- the EEG/ERP Portal. The EEG/ERP Portal is software platform whose aim is to contribute to sharing knowledge from EEG/ERP research to community by providing a complex data storage, knowledge base, signal processing methods or integration with supplementary tools. The data are managed through several user interfaces; a web based interface for human readers, a semantic web interface for automatic reasoners, or a web-service for third party tools. All user interfaces provide a functionality to upload, download and manage experiments. A wizard facilitates experiments upload by forms that guide users to fill all necessary metadata. The internal data storage ensures sufficient flexibility for heterogeneous metadata and integrity for data by a

combination of non-relational and relational databases. An authorized access is ensured by user accounts, user groups and data anonymization. Because of a lot of situations when internet connection is not available exist we are developing a mobile system that is able to re-use a server layout generated from the data layer. Once the layout is generated, the mobile tool can work offline. The user fills metadata using pre-generated forms. The data are synchronized with the server when the system gets online. This approach is useful in environments such as hospitals or prisons where a wireless connection is usually not permitted. In addition, the usage of mobile devices can reduce a usage of error prone paper forms. Combination of server-based system and a mobile device is practical solution for future work that includes a complex monitoring of electrophysiology potentials from a human body using a set of wireless sensors. It brings a complete way from human body data gathering, through their description by metadata to an online synchronization with a remote storage. Such data are fully prepared to be shared with the community.

Disclosures: **P. Ježek:** None. **V. Papež:** None. **R. Mouček:** None.

Poster

854. Data Analysis and Statistics: Software Tools

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Topic: G.07. Data Analysis and Statistics

Support: ERDF, Project NTIS CZ.1.05/1.1.00/02.0090

Title: Effects of personal electronic health record system on reasoning in neurophysiology experiments

Authors: *V. PAPEŽ[1,2], R. MOUčEK[2], P. JEŽEK[2], T. ŘONDÍK[2];
[1]Dept. of Computer Sci. and Engin., Univ. of West Bohemia, Czech Republic; [2]NTIS - New Technologies for Information Society, Univ. of West Bohemia, Plzen, Czech Republic

Abstract: Bioinformatics and assistive technologies are more present in common households. Thermometers, bathroom scales, heart rates sensors, accelerometers, pressure gauges, respiration sensors, and even mobile EEG devices are more affordable. We can gather medical data 24/7. The question is: What to do with these data? Our neuroinformatics research group established a new project following the ideas realized during the development of the EEG/ERP Portal (EEGBase - http://eegdatabase.kiv.zcu.cz/). EEGBase is a software solution for storing, sharing

and analyzing data from neurophysiological experiments. These data get their meaning from related sets of metadata that cover only a small subset of subjects' health condition. Therefore, an idea of advanced personal electronic health record system (hereafter PEHR) appeared. The PEHR's concept is modular (each module represents one health domain - ECG, EEG, etc.) including advanced analysis cross over the modules. The system is able to store and analyze data from various resources (sensors, external data sources, user's manual inputs, etc.) and EEGBase is a proof-of-concept resource. Data have an unambiguous meaning when they are related to verified ontologies and terminologies. Therefore, the system is designed to respect openEHR (http://www.openehr.org/) standards. Since there are no openEHR archetypes for neurophysiology, these are based on existing domain ontologies. Basic usability of EHR systems in general depends on whom it is built for (physician, patient, hospital etc.). The PEHR is focused mainly on patients (they can observe their conditions and prevent potential problems), but it can also help researches to answer non-trivial questions using cross analysis. We can ask, for example, the following questions: Is driver's attention better or worse after two weeks of meat diet? Does waking up in the third REM sleep stage affect concentration/meditation during the next day? To try answer these questions, the PEHR can established a proper knowledge base (patient's diet, sleep duration, physical activity, etc.). Moreover, many of the questions emerge when associating data that are not apparently related.

Disclosures: **V. Papež:** None. **R. Mouček:** None. **P. Ježek:** None. **T. Řondík:** None.

Poster

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Topic: G.07. Data Analysis and Statistics

Support: European Regional Development Fund (ERDF), Project "NTIS - New Technologies for Information Society", European Centre of Excellence, CZ.1.05/1.1.00/02.0090

Title: Solution for batch processing of electrophysiological data

Authors: ***T. RONDÍK**[1,2], V. PAPEŽ[2], R. MOUčEK[2];
[1]Dept. of Computer Sci. and Engin., Univ. of West Bohemia, Czech Republic; [2]NTIS – New Technologies for the Information Society, Univ. of West Bohemia, Pilsen, Czech Republic

Abstract: Our neuroinformatics research group at the University of West Bohemia works in electrophysiological data processing, with an emphasis on electroencephalography and event-related potentials (ERP), since 2003. During that time, we have built a completely equipped experimental laboratory. Based on our experience, we are designing and developing a complex solution for electrophysiological data processing and analysis. We split the development into four phases and currently we entered the third phase. In the first phase, we identified workflows and methods (including their parameters which can be identified from input data) for the most common scenarios when the EEG/ERP signal is processed. The scenarios cover detection of basic brain rhythms and detection of steady state visually evoked potentials (SSVEP) in the continuous EEG signal, and detection of occurrence of ERP components in EEG/ERP signal. In the second phase, we implemented workflows and methods identified in the first step. The result is a three-layer application for batch processing of electrophysiological data. The input/output layer contains methods for reading from/writing into the formats most widely used for storing EEG/ERP data. The application layer contains methods for processing and analyzing EEG/ERP signals. Most of the methods are already implemented in signal processing libraries. However, some methods were implemented from scratch because their open-source implementation was not available. Both input/output layer and application layer contain an interface for adding new formats and methods. We designed and implemented the core of the application for future possible extension with common biosignals. Currently, in the third phase, we are working on an application interface which allow using the application as a service. In the fourth phase, we will implement a web-based front-end application and offer our workflows and methods as a cloud service.

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Poster

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ITMAT

Alfred P. Sloan Foundation

Title: Characterizing modular structure in neuroimaging data: The network community architecture toolbox

Authors: D. BAKER[1], S. FELDT MULDOON[1], S. GU[1], A. KHAMBHATI[1], M. MATTAR[1], Q. TELESFORD[1], M. YANG[1], *D. S. BASSETT[2];
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Abstract: Recent work applying measures of network theory to neuroimaging data has revealed that the brain can be viewed as a complex network that, like many other systems in biological, social, technological, and physical fields, displays community structure. A common notion of a community is a group of network nodes that displays strong and dense connectivity, more so than expected under the assumptions of an appropriate null model. Brain networks extracted from large-scale structural, diffusion, or functional neuroimaging data show clear community structure in which network communities tend to map to known cognitive systems, including sensorimotor, default mode, executive, visual, auditory, salience, and attention systems. However, tools for examining the statistical significance, organization, dynamics, and spatial features of these communities remain sparse. Here we report the development of a freely downloadable MATLAB toolbox devoted to characterizing community architecture in arbitrary networks extracted from any neuroimaging data set and composed of any number of nodes or edges. The toolbox offers code to compute network diagnostics in 4 principle areas: (i) static community architecture (including community size and strength), (ii) dynamic community architecture (including flexibility and stationarity), (iii) community significance in comparison to a family of null models, and (iv) spatial organization (including radius and hemispheric laterality). We illustrate the use of these tools using an existing fMRI data set acquired from 20 healthy individuals as they learn a new motor skill over the course of 6 weeks of training. These tools enable the characterization of communities within the context of the wider network, supporting the distinction of different communities in structural or cognitive roles. We anticipate this toolbox to be of use to researchers investigating network structure from the perspectives of both cognitive and clinical neuroscience.

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Poster

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Topic: G.07. Data Analysis and Statistics

Support: Merz Pharma

Title: Kinematic assessments effectively guide longitudinal treatment of upper limb tremor in Parkinson's disease

Authors: *J. LEE[1,2], F. RAHIMI[2], O. SAMOTUS[2], M. JACKMAN[2], M. JOG[2];
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Abstract: Themes: Disorders to nervous system - movement disorders - PD **Body:** Parkinson disease tremor (PDT) is a disabling visible symptom and treatment of tremor is currently inadequate. Focal treatment such as botulinum neurotoxin type A (BoNT-A) injections may be useful by better characterization of tremor biomechanics. Kinematic sensor technology objectively captures the biomechanics of tremor movements at the wrist, elbow and shoulder arm joints. Longitudinal efficacy and safety of BoNT-A injections for PDT has not yet been studied. 13 PD patients underwent kinematic assessment over 96 weeks receiving BoNT-A injections every 4 months and a follow-up six weeks following a treatment. Recordings were taken with patients in rest, posture and in weight holding states. Degrees of freedom (DOF) at each arm joint were: flexion-extension, pronation-supination, radial-ulnar at wrist, flexion-extension at elbow, and flexion-extension, abduction-adduction at shoulder. Dosing of BoNT-A and muscle selection for injection were determined based on kinematic data and the physician's clinical experience. Over the 96-week study, seven injection treatments were administered. Total tremor angular amplitude, representing tremor severity, did not return to baseline severity throughout the treatment course. Tremors during rest and postural states, found to be most bothersome by patients, were significantly reduced by 87% in the wrist and 90% in the elbow and shoulder arm joints following the 7th treatment. Clinical ratings such as Unified Parkinson's Disease rating scale rest tremor scores improved by 48% and Fahn-Tolosa-Marin tremor rating scores improved by 37% at week 96. Maximal grip strength was not significantly reduced and disabling side-effects such as arm weakness was limited with no effect on arm function. The ability of kinematics to decompose tremor provides physicians an objective tool to determine injection parameters, improving treatment of PDT. This study has been extended for an additional two years involving nine injection cycles to determine BoNT-A longitudinal outcomes.

Disclosures: J. Lee: None. **F. Rahimi:** None. **O. Samotus:** None. **M. Jackman:** None. **M. Jog:** 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.; Grant: Merz Pharm.

Poster

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Topic: G.07. Data Analysis and Statistics

Title: The next step: A wearable device for longitudinal data collection of sleep and movement

Authors: *R. S. KALMAR, M. DIAMOND, S. DIAMOND, S. IYENGAR;
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Abstract: Background: Understanding individual patterns of physical activity and of sleep are fundamental to our ability to identify disease states, as well as baseline states of health. However, despite significant advances in our understanding of underlying neurobiology, the majority of this research has been in the context of laboratory settings. Previously, adoption of wearable activity monitors for large-scale or longitudinal research was limited by technical and practical constraints. These devices were bulky, required regular removal for battery charging, bathing, or swimming, and generally were not designed for everyday use. However, recent advances in technology, including the miniaturization and increased efficiency of electronics, have made it possible to design an activity monitor that can be easily worn continuously for months or years. Methods: Collaborating with a team of engineers, physicians, and designers, we iteratively designed, tested, and manufactured an activity monitor optimized for wearability and data collection over extended periods of time. We developed and validated activity tracking algorithms using video recordings and comparable devices, across multiple wearing positions (wrist, waist, chest, foot, head) and across multiple types of activity (walking, running, biking, swimming, sleep, etc.). Discussion: We developed a wearable activity monitor optimized for day-to-day usability, battery life, durability, and accurate over a range of different activities and wearing positions. Combining a high-resolution 3-axis accelerometer, data transfer and communication via low energy Bluetooth (4.0), 4-6 month battery life, waterproof construction, and a small form factor (thinner than 8.5mm, lighter than 10 grams), our device is designed for use by both consumers and researchers. These properties make our device popular as a consumer device, but also well-suited for longitudinal collection of sleep and movement data. Summary: Collection of objective, quantitative data about physical activity during natural behavior has applications for many research investigations. Previous technology was inadequate for capturing this activity across many users or over long periods of time. Leveraging advances in mobile and consumer technology, we created an activity monitoring system that measures physical activity and sleep continuously for at least 3 months. Our device is ready for use in studies of physical

activity, sleep, or clinical applications. The data collected by these devices promises an unprecedented understanding of how individual and population patterns of activity underlie healthy and disease states.

Disclosures: **R.S. Kalmar:** A. Employment/Salary (full or part-time):; Misfit Wearables. **M. Diamond:** A. Employment/Salary (full or part-time):; Misfit Wearables. **S. Diamond:** A. Employment/Salary (full or part-time):; Misfit Wearables. **S. Iyengar:** A. Employment/Salary (full or part-time):; Misfit Wearables.

Poster

854. Data Analysis and Statistics: Software Tools

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Topic: G.07. Data Analysis and Statistics

Support: MH096093

Beckman Institute

R25GM075149

Title: Automation of mouse brain extraction for computational neuroimage analysis

Authors: A. DELORA[1], A. GONZALES[1], *E. L. BEARER[1,2];
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Abstract: Magnetic resonance imaging (MRI) is valuable technique in neuroscience with years of research and development. Nearly all of the advances in MR image analysis and processing have focused on human MR images, leaving animal neuroimagers bereft of time-saving automations. Here, we present a novel time-saving methodology for template-based brain extraction of rodent images that is built with common neuroimaging tools (FSL, NiftyReg) and is applicable to T1- and T2-weighted MR images. In this research, we focus our efforts on mouse brain MRI, but the program works on images from other mammalian brains as well. It is anticipated that once a short report is published, the software will be made available on the authors' websites for public download. Supported by MH096093 (ELB) and the Beckman Institute (REJ).

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Poster

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Topic: G.07. Data Analysis and Statistics

Title: Pyoperant: An open source python package for operant conditioning

Authors: *T. GENTNER[1,2], J. T. KIGGINS[2], M. THIELK[2];
[1]Psycholgy, UCSD, La Jolla, CA; [2]Neurosciences, UC San Diego, San Diego, CA

Abstract: Operant conditioning is a simple form of behavioral training that has been used to study associative learning and otherwise control behavior for many years. Logically, it consists of presenting a stimulus and linking the subject's response to that stimulus to either a rewarding or aversive outcome, typically over the course of many stimulus-response-outcome instances (trials). In practice, however, error checking, data storage, and machine-specific hardware interactions often obfuscate the simplicity of the task, limiting its flexibility and power. This limitation becomes increasingly apparent when deploying high-throughput behavioral experiment control systems, transferring subjects from a training panel to an awake behaving electrophysiology panel, or simply trying to share behavioral protocols. To deal with these challenges, we present an open-source python package, PyOperant (http://github.com/gentnerlab/pyoperant), which we have developed for the control of operant experiments. The package provides a cross-platform object-oriented framework to easily construct, conveniently share, and rapidly iterate on new operant behavior paradigms. This is achieved principally through abstracting physical component manipulation from low-level hardware manipulation and by defining behavioral protocols as classes which can be extended through object inheritance. Further, experimenters are able to integrate their behavioral protocols with other Python packages for online data analysis or experimental control. We also present a real-world example of PyOperant deployed to control 38 operant panels including two awake behaving electrophysiology acquisition machines.

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