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Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.01/A1

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant DC008955

Burke Medical Research Institute

Title: Differential conservation of transcription factor codes specifying mouse and human olfactory bulb interneuron phenotypes

Authors: *N. FUJIWARA[1], J. W. CAVE[1,2];
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Abstracts: The mechanisms that generate neuronal phenotypic diversity in the mammalian brain are not fully established. To better understand these mechanisms, this study examined transcription factor specification of olfactory bulb interneuron phenotypes. Most studies examining phenotypic diversity in olfactory bulb interneurons have been conducted with rodents and have indicated that individual phenotypes are specified by combinatorial co-expression of different transcription factors. To establish whether the transcription factor codes established in the mouse are conserved in the human olfactory bulb, we examined adult human and mouse olfactory bulb tissue by immunofluorescence. These studies compared the co-expression of MEIS2, SP8, FOXP2 and PAX6 transcription factors in interneurons specified by the expression of either Calretinin, Calbindin or Tyrosine Hydroxylase. In both species, FOXP2 and PAX6 were co-expressed with Calbindin and Tyrosine Hydroxylase, respectively. MEIS2 was co-expressed with both Calretinin and Tyrosine Hydroxylase in both species, but co-expression with Calbindin was only observed in mice. SP8 was co-expressed with Calretinin in both species, but in humans, it was also observed in cells containing either Calbindin or Tyrosine Hydroxylase. The co-expression of SP8 with Calbindin in humans coincided with a co-expression of Calbindin and Calretinin that was not observed in mice. The human-specific co-expression of Calbindin and Calretinin revealed a novel olfactory bulb interneuron phenotype not observed in mice. Together, the findings in this study show some significant differences in transcription factor co-expression patterns in mouse and human olfactory bulb interneurons, which may underlie differential phenotype specification between these species.

Disclosures: **N. Fujiwara:** None. **J.W. Cave:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.02/A2

Topic: A.02. Neurogenesis and Gliogenesis

Support: Jerome Lejeune Foundation

Title: Mechanisms of neuronal maturation are impaired in the developing neocortex of Mecp2 null embryos

Authors: ***F. BEDOGNI**[1], C. COBOLLI GIGLI[1], D. POZZI[2], R. ROSSI[3], L. SCARAMUZZA[1], C. ZELI[1], C. KILSTRUP-NIELSEN[4], M. MATTEOLI[2], N. LANDSBERGER[1];
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Abstracts: Rett syndrome (RTT) is a neurological disorder that affects mainly girls (1/10.000 live born female) and is characterized by autistic features, seizures, ataxia and stereotypical hand movements. Due to the timing of the onset of RTT symptoms (6-18 months of life), researchers so far mainly studied the postnatal period. However, evidences of subtle defects at birth (both in humans and RTT animal models) are now increasing, while the investigation of any possible prenatal neurodevelopment impairments has been largely neglected. We thus hypothesized that MeCP2 (the cause of roughly 90% of RTT cases worldwide) could play a role during embryonic and early postnatal development, as we detected Mecp2 expression in both progenitors and post-mitotic neurons of the cerebral cortex as early as E10. Searching for possible transcriptional impairments, possibly leading to subtle alterations during early stages of development, we performed a microarray analysis on wt and Mecp2 null embryonic cortical tissues (E15). Overall, our transcriptional screening highlighted the deregulation of a large plethora of genes typically expressed by maturing or matured neurons. This is suggestive of a delay (or possibly a stall) in the acquisition of the full mature neuronal identity, as many of these genes were accordingly deregulated at later time points (E18 and P8). Of particular interest for their involvement in activity dependent maturation, we detected defects in the transcription of several receptors and ionic channels that likely produced perturbation in down stream signalling pathways, as verified with functional experiments. Our data suggest the intriguing hypothesis that impairments during early embryonic maturation of the Mecp2 null cortex can lead to subtle pre-symptomatic features

and concur to the typical neuronal impairments in adulthood, thus opening up new perspectives on the aetiology of RTT neurological features.

Disclosures: **F. Bedogni:** None. **C. Cobolli Gigli:** None. **D. Pozzi:** None. **R. Rossi:** None. **L. Scaramuzza:** None. **C. Kilstrup-Nielsen:** None. **M. Matteoli:** None. **N. Landsberger:** None. **C. Zeli:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.03/A3

Topic: A.02. Neurogenesis and Gliogenesis

Support: NEI P30 EY022589

NEI P30 EY014081

Research to Prevent Blindness

Title: Novel regulatory mechanisms for the SoxC transcriptional network required for visual pathway development

Authors: ***X. ZHANG**[1], J. HERTZ[2], X.-L. JIN[2], B. A. DEROSA[2], J. Y. LI[2], P. VENUGOPALAN[1], D. A. VALENZUELA[2], R. D. PATEL[2], K. R. RUSSANO[1], S. A. ALSHAMEKH[2], D. VELMESHEV[3], Y. CHENG[2], T. M. BOYCE[2], A. DREYFUSS[2], M. S. UDDIN[2], K. J. MULLER[4], D. M. DYKXHOORN[3], J. L. GOLDBERG[1];
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Abstracts: What pathways specify retinal ganglion cell (RGC) fate in the developing retina? Here we report on mechanisms by which a new molecular pathway involving Sox4/Sox11 required for RGC differentiation from retinal progenitor cells (RPCs) and for optic nerve formation in mice *in vivo*, and sufficient to differentiate human induced pluripotent stem cells into electrophysiologically active RGC-like cells. We show a regulatory network where by the previously described inhibitor of RGC differentiation, REST, depends on suppression of Sox4 expression, and provide evidence for a novel soluble regulator for RGC differentiation, TGFβ superfamily member GDF-15, which also acts through Sox4 to induce RGC differentiation from

progenitor cells. Although our data suggests that Sox4 and Sox11 are independently required for RGC development, the two family members interact such that the normal SUMOylation of Sox11, which decreases its nuclear localization and suppresses its pro-RGC activity, is decreased in the absence of Sox4, allowing Sox11 to compensate for Sox4 absence. These data define novel regulatory mechanisms for this SoxC molecular network, and suggest pro-RGC molecular manipulations with potential promise for cell replacement-based therapies for glaucoma and other optic neuropathies.

Disclosures: **X. Zhang:** None. **J. Hertz:** None. **X. Jin:** None. **B.A. Derosa:** None. **J.Y. Li:** None. **P. Venugopalan:** None. **D.A. Valenzuela:** None. **R.D. Patel:** None. **K.R. Russano:** None. **S.A. Alshamekh:** None. **D. Velmeshev:** None. **Y. Cheng:** None. **T.M. Boyce:** None. **A. Dreyfuss:** None. **M.S. Uddin:** None. **K.J. Muller:** None. **D.M. Dykxhoorn:** None. **J.L. Goldberg:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.04/A4

Topic: A.02. Neurogenesis and Gliogenesis

Support: EU ZF-HEALTH 242048

EU DOPAMINET

EU mesDAneurodev

DFG SFB 780-B6

Title: Genetic control of dopaminergic neuron subtype differentiation in zebrafish

Authors: ***W. DRIEVER**, M. MANOLI, M. RATH, E. CARL, T. MUELLER, A. FILIPPI; Univ. Freiburg, Freiburg, BW 79104, Germany

Abstracts: Distinct groups of dopaminergic neurons develop at defined anatomical sites in the brain to modulate function of a large diversity of local and far-ranging circuits involved in motor control, perception, sleep and the regulation of emotion-related behavior. We use zebrafish embryos as genetic models to understand how transcriptional regulatory networks control dopaminergic subtype identity, co-transmitter phenotypes and projection behavior. Zebrafish

embryos develop dopaminergic neurons at anatomical sites similar to mammals, except for the lack of a mesencephalic dopaminergic group. We have mapped the complete projectome of all dopaminergic groups in zebrafish, and have also characterized gabaergic and glutamatergic co-transmitter phenotypes for each dopaminergic subtype. The only dopaminergic neurons in zebrafish to provide ascending projections into the subpallium/striatum are A11-type posterior tubercular dopaminergic neurons which depend on the Orthopedia transcription factor for their development. these neurons share glutamatergic co-transmission with the mammalian ascending systems. Zebrafish develop an endostriatal dopaminergic system, which provided most of the dopaminergic arborization within the striatum. this striatal system is characterized by gabaergic cotransmission, providing dopaminergic activity with two distinct co-transmitter phenotypes to the striatum. We have systematically mapped transcription factor expression to each of the anatomical dopaminergic groups in zebrafish to define their transcriptional codes, and use genetic loss- and gain-of-function approaches to functionally dissect the transcriptional networks controlling dopaminergic subtype specificity. Our data suggest specific combinations of patterning, neurogenesis and differentiation transcription factors to drive dopamine subtype specification, co-transmitter phenotypes and projection behavior.

Disclosures: **W. Driever:** None. **M. Manoli:** None. **M. Rath:** None. **E. Carl:** None. **T. Mueller:** None. **A. Filippi:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.05/A5

Topic: A.02. Neurogenesis and Gliogenesis

Support: Medical Research Council

Title: The significance of NeuroD1 for external germinal layer formation

Authors: M. HANZEL[1], T. BUTTS[1], *R. J. WINGATE[2];
[1]MRC Ctr. for Developmental Neurobio., [2]MRC Ctr. Dev Neurobiol, King's Col. London, London, United Kingdom

Abstracts: The cerebellum has evolved elaborate foliation in the amniote lineage as a consequence of extensive transit amplification in a transient external germinal layer (EGL) mediated by the bHLH transcription factor, Atonal 1 (Atoh1). At the end of transit amplification,

granule cell precursors (GCPs) transition into mature neurons having undergone a massive expansion in number. Differentiation is associated with the down regulation of Atoh1 and upregulation of NeuroD1. To explore the characteristics of transit amplifying cells, we have examined cerebellar development in the embryonic chicken, mouse and Xenopus. Electroporation of conditional reporters *in vivo* in chick at E6-E8 and in cerebellar slice of both chick and mouse cultured at E10-14 and P1-5, respectively, has allowed us to directly observe individual granule cells from their origins at the rhombic lip to their descent into the internal granular layer (IGL) and explore the relationship between NeuroD1 and Atoh1. Our assay employs two bHLH transcription factor enhancer constructs: NeuroD1::GFP and Atoh1::Cre-recombinase. By titrating the concentration of this cre-recombinase with co-electroporated "lox-stop-lox" plasmids encoding either GFP or mCherry, we label progressively sparser cells. Using this strategy, we show that Atoh1 is expressed in granule cell precursors displaying a variety of different morphological characteristics. By contrast, the NeuroD1 enhancer construct labels GCPs as they leave the cell cycle and initiate an inward radial migration suggesting a key role for NeuroD1 in differentiation. To investigate this, we overexpressed full-length NeuroD1 within the chick GCP population and assessed the consequences for granule cell development. When expressed in early rhombic lip migrants (E5), NeuroD1 upregulation cell autonomously inhibits the formation of EGL cells. When expressed in GCPs of the EGL, NeuroD1 cell autonomously terminates proliferation, triggers radial migration and down regulates Atoh1. NeuroD1 is thus sufficient to trigger exit from the EGL. Intriguingly this regulatory relationship appears to be a relatively recent amniote innovation. In the frog (an anamniote), which has a simple unfoliated cerebellum, NeuroD1 and Atoh1 are co-expressed in a transient granule cell layer that forms on the surface of the cerebellum at metamorphosis. This external layer is nevertheless non-proliferative and thus does not comprise an external germinal layer. The evolution of transit amplification in the EGL thus required adaptation of NeuroD1, both in the timing of its expression and regulatory function, with respect to Atoh1 in amniotes.

Disclosures: **M. Hanzel:** None. **T. Butts:** None. **R.J. Wingate:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.06/A6

Topic: A.02. Neurogenesis and Gliogenesis

Title: Analyzing the role of FoxD4 in neuronal differentiation using a mouse embryonic stem cell model

Authors: *J. H. SHERMAN[1], B. KARPINSKI-OAKLEY[2], M. FRALISH[1], S. MOODY[1], A. LAMANTIA[1], T. MAYNARD[1];
[1]The George Washington Univ., Washington, DC; [2]The, Washington, DC

Abstracts: *Foxd4* is a forkhead box family transcription factor. In Xenopus embryos, *foxD4/5* serves as a master regulator of numerous neural transcription factors and plays a key role in directing embryonic stem cells towards a neural stem cell (NSC) lineage. The homologous gene in mice, *FoxD4*, is expressed in the embryonic central nervous system in a pattern similar to Xenopus. Our data indicates that this transcription factor may play similar role in promoting the development of mammalian NSCs. We used mouse embryonic stem cells (mESCs) to assess the NSC-promoting activity of Foxd4. A stable mESC line expressin a *Foxd4* targeted siRNA was used to reduce *Foxd4* expression in mESCs in a LIF/RA dependent NSC differentiation assay. Our results show clearly that this loss of *Foxd4* function diminishes the capacity of mESCs to respond to the NSC-promoting environment in the LIF/RA NSC differentiation assay. We also found that *Foxd4* knockdown does not affect mESCs pluripotency. Furthermore, FoxD4 knockdown results in decreased levels of neural differentiation markers, high levels of undifferentiated cell markers and lack of expression of mature neuronal markers. In addition, a mESC line was generated that over-expresses FoxD4 to assess the consequences of gain of function. We found that FoxD4 upregulation results in low levels of undifferentiated neuronal markers with increased expression of markers for neuronal maturation and differentiation. We also confirmed that Foxd4 (the murine gene) has similar functions to its Xenopus counterpart by injecting full length *Foxd4* into frog oocytes and assessing its effects on neural induction. As is the case for *foxD4/5*, murine Foxd4 accelerates and enhances expression of neuronal precursor and differentiation marker. Finally, localization analysis indicates that Foxd4 is expressed in the neural tube and olfactory placode epithelium at midgestation. We are currently using siRNA electroporation in an olfactory placode explant assay to assess the function of Foxd4 in developing embryonic neurogenic ectoderm. Together, our results show that Foxd4, like its Xenopus orthologue, is a key regulator of the transition from embryonic stem cell to committed neurogenic neural stem cell.

Disclosures: J.H. Sherman: None. **M. Fralish:** None. **B. Karpinski-Oakley:** None. **T. Maynard:** None. **S. Moody:** None. **A. LaMantia:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

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Program#/Poster: 588.07/A7

Topic: A.02. Neurogenesis and Gliogenesis

Support: R01 DK064678/DK/NIDDK NIH HHS/United States

R01 NS054941/NS/NINDS NIH HHS/United States

Title: Stat3 promotes motor neuron differentiation by collaborating with motor neuron-specific LIM complex

Authors: S. SEO[1], *J. C. RHEE[2], S. LEE[1], R. SHEN[3], H. CHO[3], J. LEE[3], S. LEE[3]; [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Pharmacol., SNU Pharm., Seoul, Korea, Republic of; [3]OHSU, Portland, OR

Abstracts: The motor neuron (MN)-hexamer complex consisting of LIM homeobox 3, Islet-1, and nuclear LIM interactor is a key determinant of motor neuron specification and differentiation. To gain insights into the transcriptional network in motor neuron development, we performed a genome-wide ChIP-sequencing analysis and found that the MN-hexamer directly regulates a wide array of motor neuron genes by binding to the HxRE (hexamer response element) shared among the target genes. Interestingly, STAT3-binding motif is highly enriched in the MN-hexamer-bound peaks in addition to the HxRE. We also found that a transcriptionally active form of STAT3 is expressed in embryonic motor neurons and that STAT3 associates with the MN-hexamer, enhancing the transcriptional activity of the MN-hexamer in an upstream signal-dependent manner. Correspondingly, STAT3 was needed for motor neuron differentiation in the developing spinal cord. Together, our studies uncover crucial gene regulatory mechanisms that couple MN-hexamer and STAT-activating extracellular signals to promote motor neuron differentiation in vertebrate spinal cord.

Disclosures: **S. Seo:** None. **J.C. Rhee:** None. **S. Lee:** None. **R. Shen:** None. **H. Cho:** None. **J. Lee:** None. **S. Lee:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI 24300116

JSPS KAKENHI 23700410

MEXT KAKENHI 25123701

Title: Postmitotic dorsal spinal cord neurons are transfated into commissural neurons by induced misexpression of *Barh1*

Authors: *T. SATO[1,2], Y. MUROYAMA[3], T. SAITO[3];
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Abstracts: Mammalian *Barh1* (*Mbh1*), which is a Bar-class homeobox gene, has been shown to confer commissural neuron identity on dorsal cells in the mouse embryonic spinal cord. In our previous experiments, *Mbh1* was transfected into neural stem/progenitor cells and misexpressed in both neural stem/progenitor cells and postmitotic neurons using *in vivo* electroporation and a ubiquitous CAG promoter vector. It has not been clear whether *Mbh1* functions in neural stem/progenitor cells or postmitotic neurons during the fate change into commissural neurons. We have recently established a gene induction method by combining *in vivo* electroporation with the newest version of the Tet-On system. This method enables efficient and strict induction of gene expression in targeted postmitotic neurons in the presence of doxycycline (Dox). In the absence of Dox, leaky expression occurrs only at extremely low levels. We have applied the method to the developing mouse spinal cord to determine whether postmitotic cells would be transfated into commissural neurons by *Mbh1*. After *in vivo* electroporation, postmitotic cells that were highly labeled with BrdU, six hours before the induction of *Mbh1*, became neurons positive for Dcc, which is a netrin receptor and a marker of commissural neurons in the developing spinal cord, mimicking the phenotype of *Mbh1* misexpression under the control of the CAG promoter. This finding suggests that even postmitotic neurons are transfated into commissural neurons by *Mbh1*. This work was supported by JSPS KAKENHI grant numbers 24300116 (to Saito) and 23700410 (to Sato) and MEXT KAKENHI grant number 25123701 (to Saito).

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Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH R01 NS044080

Title: Molecular interactions between Gsx2 and Ascl1 in lateral ganglionic eminence (LGE) progenitors of the mouse

Authors: *K. ROYCHOUDHURY, M. NAKAFUKU, B. GEBELEIN, K. CAMPBELL; Developmental Biol., Cincinnati Children's Hosp., Cincinnati, OH

Abstracts: Homeobox and basic helix loop helix (bHLH) transcription factors play critical roles in progenitor maintenance vs. differentiation in the ventricular and subventricular zones of the embryonic telencephalon. Within progenitors of the lateral ganglionic eminence (LGE), Gsx2 helps maintain progenitors in the undifferentiated state while Ascl1 plays an important role in progenitor maturation. The molecular mechanisms underlying the maturation of LGE progenitors are unknown. In this study, we report a direct protein-protein interaction between Gsx2 and Ascl1 in the embryonic mouse telencephalon. We mapped the Gsx2 binding site on Ascl1 to amino acid residues in the bHLH domain of Ascl1. This interaction appears to inhibit Ascl1's ability to bind the E-box sequence. Moreover, this interaction inhibits heterodimerization of Ascl1 with E-proteins that are critical for its transcriptional activity. We are currently, establishing an E-box-driven luciferase assay to test, *in vitro*, whether the Gsx2-Ascl1 interaction inhibits transcription of Ascl1 targets. Furthermore, we will attempt to generate a Ascl1 mutant, which is incapable of interaction with Gsx2 but can still bind the E-box to examine its impact on neurogenesis, *in vivo*. Based on our data, we propose a model whereby Gsx2-Ascl1 double expressing LGE cells are primed for neurogenesis, but do not differentiate until Gsx2 is down-regulated.

Disclosures: K. Roychoudhury: None. **M. Nakafuku:** None. **B. Gebelein:** None. **K. Campbell:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 588.10/A10

Topic: A.02. Neurogenesis and Gliogenesis

Title: Identification and characterization of the genes that play important roles for the development of motor neurons

Authors: *K. SUZUKI[1], S. LEE[2], R. SHEN[1], J. W. LEE[1], S.-K. LEE[1];
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Abstracts: Motor neurons (MNs) and V2a interneurons in the vertebrate spinal cord are critical constituents of the motor circuit that coordinates locomotion. MNs project axons to muscles and control their contraction, while V2a interneurons make connections with other neurons. The transcription factors that control generation of MNs and V2a interneurons have been relatively well studied. We have previously shown that the Isl1-Lhx3 hexamer complex consisting of Islet-1 (Isl1), LIM homeobox 3 (Lhx3), and nuclear LIM interactor directs the specification of MN cell fate in the developing spinal cord. We also found that Chx10, a V2a interneuron-specific transcription factor, suppresses expression of motor neuron genes. These findings led us to hypothesize that the key genes for establishment of MN identity are induced by the Isl1-Lhx3 hexamer complex in MNs while being repressed by Chx10 in V2a interneurons. To identify the key genes for MN development, we established two embryonic stem cell (ESC) models; Isl1-Lhx3-ESCs and Chx10-ESCs, in which the expression of Is1l-Lhx3 and Chx10 are induced by doxycycline, respectively. We then identified the genes that are induced by Isl1-Lhx3 and suppressed by Chx10 using RNA-seq in the ESC models. Combined these RNA-seq datasets with ChIP-seq datasets that define the genomic binding sites for Isl1-Lhx3, we selected 17 genes for the further analysis of their possible functions in MN development. First, we investigated expression pattern of these genes in mouse developing spinal cord using *in situ* hybridization. 7 out of 17 genes are predominantly expressed in developing spinal MNs. Second, we checked whether those genes can be induced by Isl1-Lhx3 hexamer using in ovo electroporation. The expression of 4 out of 7 genes was ectopically induced by Isl1-Lhx3 in the dorsal spinal cord. Together, our study identified downstream target genes of the Isl1-Lhx3 complex, which may play important roles in the development of MNs.

Disclosures: K. Suzuki: None. **S. Lee:** None. **R. Shen:** None. **J.W. Lee:** None. **S. Lee:** None.

Poster

588. Neuronal Differentiation: Transcriptional Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Howard Hughes Medical Institute

National Eye Institute, NIH

NIH/NINDS Grant K99NS080911

Title: Cortical neuron cell types develop highly divergent epigenetic configurations

Authors: *E. A. MUKAMEL[1,2], A. MO[4], F. P. DAVIS[5], C. LUO[3], G. L. HENRY[5], S. R. EDDY[5], T. J. SEJNOWSKI[2,6], J. R. ECKER[6,3], J. NATHANS[4];
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Abstracts: Healthy cognitive function relies on the balanced interaction of tens to hundreds of excitatory and inhibitory neuronal cell types as well as glial cells. The distinct physiology of each cortical cell type emerges during pre- and post-natal brain development through epigenetic, transcriptional and morphological processes that define cells' mature state. Brain cells make use of unique mechanisms of epigenetic regulation, including abundant non-CG methylation, yet the distribution of these marks across cell types is unknown. Existing methods for isolating cell populations from mammalian tissues are difficult to use for analyses of DNA and RNA in brain tissue. We therefore developed a strategy to purify fluorescently labeled nuclei of specific cell types from fresh mouse tissue by using the cre-lox system to induce expression of an epitope-tagged nuclear membrane protein in defined cell types. Our purification procedure isolates both rare and abundant target cells with high yield and purity. We used this system to purify neocortical pyramidal neurons, parvalbumin (PV)-expressing fast-spiking interneurons, and vasoactive intestinal peptide (VIP)-expressing interneurons, followed by transcriptome and whole-genome methylome profiling (RNA-Seq and MethylC-seq). Genome-wide, base-resolution DNA methylation profiles reveal global and local cell specific patterns of methylation in both the CG and non-CG contexts. We identify thousands of differentially methylated regions (DMRs) across the three cell types, and integrate these data with published methylomes from fetal and developing brain as well as astrocytes. Regions that lose CG methylation during development of a specific neuron type (CG-DMRs) are enriched in active histone marks and cell type-specific transcription factor motifs. We also identify cell type-specific patterns of non-CG methylation that co-vary with gene transcription across cortical neuron types. Overall, our analysis of cell type-specific gene expression and DNA methylation gives insight into the genes and regulatory elements that may play a role in the maturation and maintenance of distinct neuronal identities.

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Poster

589. Oligodendrocyte and Schwann Cell Biology

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Program#/Poster: 589.01/A12

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS056427

NMSS RG3954A1/2

Title: Hedgehog and Wnt Signaling determinants of the outcome of demyelination revealed by Sox17 transgenesis

Authors: X. MING[1], *L.-J. CHEW[1], J. DUPREE[2], V. GALLO[1];
[1]Ctr. Neurosci Res., Children's Res. Inst., WASHINGTON, DC; [2]Virginia Commonwealth Univ., Richmond, VA

Abstracts: Our previous studies showed that Sox17 inhibits Wnt/beta-catenin (b-cat) signaling *in vitro* and its overexpression in CNP-Sox17 transgenic mice regulates postnatal oligodendrocyte (OL) development, ultimately preventing OL loss after focal lysolecithin (Lyso) demyelination. Our present studies investigate the effects of Sox17 on signaling pathways which attenuate myelin damage in subcortical white matter (WM) of P60 mice. Immunohistochemical analysis revealed increased bcl-2+, Gli2+, and Sonic Hedgehog+ cells, decreased FAS, and enhanced generation of new Gli2+ and Olig2+ cells in the intact CNP-Sox17 WM. These suggest that Sox17 promotes OL lineage cell survival and regeneration by inducing Hedgehog signaling. However, increased b-cat+ cells and total b-cat protein levels were also found in the adult CNPSox17 WM. The increased Gli2+ and b-cat+ cells in the CNP-Sox17 WM were both sensitive to reduction by cyclopamine-KAAD, implicating Smoothened activation by Sox17. Further focal injections of cyclopamine-KAAD in WM of BATGAL mice revealed a hierarchy which places Smoothened upstream of b-cat. Despite elevated b-cat, the levels of activated b-cat (ABC) were surprisingly not proportionately higher in the CNPSox17 WM, suggesting that Sox17 prevents changes in ABC, resulting in smaller changes in OPC maturation. This may be achieved through Axin2, which is increased in CNPSox17 WM. Focal injections of the b-cat antagonist CCT036477 (CCT) into the corpus callosum of P60 WT and CNPSox17 mice

showed, in the absence of cell death, clear reduction of ABC+ cells in both strains, but decreased total b-cat+ cells only in CNPSox17, suggesting enhanced turnover. Importantly, CCT produced significantly greater enhancement of the NG2+O4+, total O4+ cells, and reduction of NG2+O4- OPCs in WT. This also suggests that pharmacological ABC control in WT may improve OPC maturation and remyelination. Focal co-injections of CCT with Lyso in WT indeed prevented the loss of CC1+ cells, without affecting the OPC response. Interestingly, as previously observed with Lyso lesions in CNP-Sox17, CCT prevented the Lyso-induced decline in Gli2+ cells in WT. Given the role of Hedgehog in immune quiescence, it is possible that ABC control and Hedgehog signaling both regulate OL damage in CNP-Sox17. The induction by Lyso of ABC+Iba1+ microglia and ABC+Caspase3+ apoptotic cells was found to be significantly lower in CNPSox17 mice, and Gli inhibition with GANT61 in these mice abolished protection against Lyso damage. Our findings indicate that Sox17 regulates both b-cat and Hedgehog signaling in the adult WM, and the outcome of WM injury is modulated by crosstalk between these pathways.

Disclosures: **X. Ming:** None. **L. Chew:** None. **J. Dupree:** None. **V. Gallo:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.02/A13

Topic: A.02. Neurogenesis and Gliogenesis

Support: National Science Foundation - Graduate Research Fellowship Program (NSF-GRFP)

Title: Interactions of sox10 with tgf-b signaling in schwann cells

Authors: *J. RODRIGUEZ-MOLINA[1], J. MORAN[2], J. SVAREN[3];
[1]Cell. and Mol. Pathology, [2]Waisman center, [3]Comparative Biosci., Univ. of Wisconsin, Madison, WI

Abstracts: Schwann cell development is regulated by integration of intrinsic gene expression programs and external cues that affect Schwann cell proliferation and differentiation. One regulator of Schwann cell development is TGF beta signaling, which controls proliferation and apoptosis of embryonic Schwann cells, and Schwann cells themselves can also synthesize TGFbeta molecules. However, as Schwann cells mature, they become less responsive to TGF beta signaling. Sox10, an SRY-related HMG-box transcription factor, is crucial for embryonic

development and ultimate differentiation of Schwann cells. While Sox10 has been shown to activate a number of molecules in Schwann cell development, we explored the possibility that Sox10 may also directly repress certain genes. Microarray analysis of Schwann Cells treated with an siRNA targeting Sox10 identified several genes that become induced. The induced genes were analyzed and found that several of the Sox10-repressed genes are involved in the TGF-B pathway. Reduction of Sox10 also lead to induction of a Smad 2/3-responsive (SBE) reporter plasmid, and activation of the reporter plasmid by TGFb1 is amplified when Sox10 is ablated. Furthermore qRT-PCR studies showed TGF-B target genes induced by Sox10 downregulation and this induction was further augmented under TGF-B ligand treatment. Together, these data suggest that Sox10 represses the TGF-B pathway in SCs. However, Sox10 ChIP-Seq data did not show any binding sites for Sox10 around TGFbeta target genes, suggesting that Sox10 do not repress TGFbeta components directly. Overall, these results suggest that Sox10 inhibits TGFbeta pathway activation in Schwann cells, although the mechanism by which Sox10 represses the pathway is not yet known.

Disclosures: **J. Rodriguez-Molina:** None. **J. Moran:** None. **J. Svaren:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.03/A14

Topic: A.02. Neurogenesis and Gliogenesis

Support: NHLBI Grant R01HL104173

NINDS Grant P01NS062686

Title: Hypoxia-induced alterations in white matter-producing cells of the perinatal piglet

Authors: ***P. D. MORTON**[1,2], N. ISHIBASHI[1,2], L. KOROTCOVA[1,2], K. AGEMATSU[1,2], R. A. JONAS[1,2], V. GALLO[2];
[1]Cardiovasc. Surgery, [2]Ctr. for Neurosci. Res., Childrens Natl. Med. Ctr., Washington, DC

Abstracts: Previous and ongoing studies demonstrate that a majority of preterm birth patients display significant neurological deficits. A primary etiology of these deficits involves desaturated or reduced cerebral blood flow, resulting in restricted oxygen supply. Since glial cells are sensitive to hypoxic environments, white matter injury represents a major cause underlying

neurological deficits in preterm patients. The subventricular zone (SVZ) generates neural stem/progenitor cells (NSPCs) that replenish damaged glia in the brain via endogenous gliogenesis throughout the human lifespan. Because the SVZ is highly vascularized, and a majority of SVZ NSPCs are in direct contact with blood vessels, hypoxia-induced, pathological alterations in gliogenesis from subventricular zone (SVZ)-derived NPSCs represent crucial cellular/molecular mechanisms underlying neurological impairments in preterm births. The porcine brain, including the SVZ, shares significant anatomical/structural similarities to its human counterpart. Here, our preliminary data show that chronic hypoxia induces a significant boost in proliferating Olig2+ cells in the SVZ of perinatal piglets. Additionally, we see an increase in gliogenesis in the periventricular white matter underlying the frontal cortex. Taken together, these findings suggest that hypoxia induces gliogenesis in order to repopulate damaged white matter.

Disclosures: P.D. Morton: None. **N. Ishibashi:** None. **L. Korotcova:** None. **K. Agematsu:** None. **R.A. Jonas:** None. **V. Gallo:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.04/A15

Topic: A.02. Neurogenesis and Gliogenesis

Support: Parkinsonfonden

Multipark strategic area

Jeanssons Stiftelser

Crawford Fondation

KocKs Foundation

Segerfalk Foundation

Title: Alpha-synuclein expression in rodent and human pluripotent stem cell-derived oligodendrocytes: Implications for Multiple system atrophy

Authors: *M. DJELLOUL[1], C. VIGNON[1], K. FOG[2], L. ROYBON[1];
[1]EMV, Lund Univ., Lund, Sweden; [2]H. Lundbeck A/S, Neurodegeneration-1, Copenhagen,, Denmark

Abstracts: Multiple system atrophy (MSA) is an adult-onset, rapidly progressing, fatal neurodegenerative disease of unknown etiology. The disease involves a combination of atypical Parkinsonism, cerebellar ataxia, and pyramidal dysfunction, and at later stage autonomic failure. MSA is classified as a synucleinopathy. The hallmark of the disease is the presence of alpha-synuclein (α-syn)-rich glial cytoplasmic inclusions (GCIs) in the oligodendrocytes. α-syn is an intracellular protein predominantly synthesized in neurons of the central nervous system. Adult mature oligodendrocytes do not express α-syn and studies performed using postmortem tissue from MSA patient failed to significantly show increased abundance of α-syn mRNA in GCIs-containing oligodendrocytes. Thus, the source of α-syn present in the GCIs is still of debate. One possibility that could explain the presence of α-syn in the GCIs relies on transfer of α-syn from the extracellular environment into the oligodendrocytes. Another relies on a maintained or de novo expression of the SNCA gene encoding for α-syn in oligodendrocytes. Whether oligodendrocytes express SNCA remained to be determined. Here, we generated rodent and human pluripotent stem cells, which we differentiate into oligodendrocytes and assessed SNCA expression in these cells. We first defined the temporal expression of oligodendrocytes markers NKX2.2, OLIG2, SOX10, PDGFR-α, CNPase and O4; we could then determine that a significant proportion of oligodendrocyte progenitors defined by the expression of O4 and SOX10 stained positively for α-syn. This novel finding will be of high interest to those studying the origin of α-syn in GCIs, and open new research avenues using patient-derived pluripotent stem cells for assessing the consequences and functional role of α-syn in oligodendrocytes under physiological and pathological conditions.

Disclosures: **M. Djelloul:** None. **C. Vignon:** None. **K. Fog:** None. **L. Roybon:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.05/A16

Topic: A.02. Neurogenesis and Gliogenesis

Support: Funds for intramural research NICHD

ANR and ARSEP grants, France

Title: Non-synaptic junctions on myelinating glia promote preferential myelination of electrically-active axons

Authors: *R. D. FIELDS[1], H. WAKE[2], F. C. ORTIZ[3], P. R. LEE[4], M. C. ANGULO[3]; [1]NICHD, NIH, BETHESDA, MD; [2]National. Inst. Basic Biol., NINS, Japan, Japan; [3]INSERM, Paris, France; [4]NICHD, Bethesda, MD

Abstracts: The myelin sheath on vertebrate axons is critical for neural impulse transmission, but whether electrically active axons are preferentially myelinated by glial cells, and if so, how, are long-standing questions of significance to nervous system development, plasticity, and disease. The surprising discovery of synapses formed on glial progenitors, oligodendrocyte progenitor cells (OPCs, also called NG2 cells) has remained enigmatic for over a decade, but it has been hypothesized that these synapses could promote myelination of electrically active. We find that oligodendrocytes do preferentially myelinate electrically active axons, but synapses from axons onto myelin-forming oligodendroglial cells are not required. Instead, vesicular release at non-synaptic axo-glial junctions induces myelination. These non-synaptic functional junctions induce a local rise in cytoplasmic calcium in glial cell processes in contact with active axons and this signaling stimulates local translation of myelin basic protein to initiate myelination of axons releasing neurotransmitter from vesicles. Preferential myelin induction on electrically active axons would have profound effects on circuit function by the resulting increased conduction velocity, and thus provide another mechanism of plasticity complementing synaptic plasticity.

Disclosures: R.D. Fields: None. **H. Wake:** None. **F.C. Ortiz:** None. **P.R. Lee:** None. **M.C. Angulo:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.06/A17

Topic: A.02. Neurogenesis and Gliogenesis

Support: Japan MEXT Grant-in-Aid for Scientific Research on Innovative Areas

Title: Function of CD44 in NG2 progenitor cell differentiation and the potential of CD44 as the therapeutic target for multiple sclerosis

Authors: *T. UEKI[1], S. IDE[1], M. MURANO[1], K. SHIBASAKI[1], S. MORIKAWA[1], Y. OUCHI[2];
[1]Dept. of Anat. and Neurosci., [2]Photon Med. Res. Ctr., Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan

Abstracts: Recent investigation has revealed that glial cell lineage is not simply classified into three types of cells, astrocyte, oligodendrocyte and microglia, as classically described, but another new population, NG2 cell, should be taken into consideration as a member of glial family. Among functions of NG2 cells shown by previous physiological studies, modification of synaptic transmission, generation of new neuron and oligodendrocyte to repair the injured CNS, and regulation neural functions via neuron-NG2 cell synapse are included, but molecular machinery underlying various functions of NG2 cell is still to be solved. Interestingly, prominent evidence demonstrated the high expression level of CD44 in NG2 cell. CD44 is a membrane protein, which is cleaved by γ-secretase related to the etiology of Alzheimer's disease. Because the involvement of CD44 in NG2 cell's function has not been appreciated well yet, the present study investigated how CD44 works to accomplish multiple roles of NG2 cell in the CNS by newly developing molecular imaging system on the basis of FRET technology, where functional fluorescent probe switches on and emits fluorescence upon the shedding of membranous CD44 by activated γ-secretase. Utilizing the newly developed imaging method, the generation of intracellular fragment of CD44 could be visualized under fluorescent microscope, and the present results demonstrated that the activation of γ-seceretase induced astrocytic differentiation of NG2 cell, whereas the inhibition of γ-secretase arrested NG2 cells in immature status. These data propose the possibility that membranous CD44 of NG2 cell should be therapeutic target to treat demyelinated disorders such as multiple sclerosis, in which immature NG2 cells are accumulated in the affected brain. Further investigations are required to identify the transcriptional target of the intracellular domain of CD44 in NG2 cell, and also to appreciate the precise implication of γ-secretase in glial development and interaction with neuron.

Disclosures: T. Ueki: None. **S. Ide:** None. **M. Murano:** None. **K. Shibasaki:** None. **S. Morikawa:** None. **Y. Ouchi:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.07/A18

Topic: A.02. Neurogenesis and Gliogenesis

Support: R01 NS074870

Title: Identification and characterization of novel intronic enhancer elements of the mouse Cspg4 gene in oligodendrocyte lineage cells

Authors: *H. GOTOH[1,2], T. NOMURA[1], K. ONO[1], A. NISHIYAMA[2];
[1]Dept. of Biol., Kyoto Prefectural Univ. of Med., Kyoto, Japan; [2]Dept. of Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstracts: NG2 cells are proliferating oligodendrocyte precursor cells and are characterized by the expression of NG2 encoded by the Cspg4 gene. NG2 is an integral membrane protein that regulates proliferation and differentiation of NG2 cells and is downregulated as they undergo terminal differentiation. However, the cis-regulating elements and trans-activating factors that are critical for the regulation of Cspg4 transcription in NG2 cells have remained unknown. In order to identify the regulatory sequences of Cspg4, we screened the promoter and enhancer sequences of the C57BL/6 mouse Cspg4 contained in the bacterial artificial chromosome plasmid that had been reported to recapitulate endogenous NG2 expression. When we tested a 1.5-kb promoter sequence, it directed transcription in neurons and astrocytes but not in NG2 cells when tested on primary cultures from neonatal mouse brains. Additional screening identified a novel 1.4-kb enhancer region in the first intron of the Cspg4 gene. We next established a transgenic mouse line that expressed enhanced green fluorescent protein (GFP) under the regulation of the intronic enhancer. Interestingly, GFP signals were observed in oligodendrocyte lineage cells, with strong expression in NG2 cells and weaker expression in oligodendrocytes but not in astrocytes or neurons. Interestingly, GFP was not detected in vascular pericytes that also express NG2 protein in the CNS. This observation suggests that Cspg4 transcription in NG2 cells is differentially regulated from that in pericytes. Furthermore, luciferase assays were used to test the effects of key oligodendrocyte transcription factors on the activity of the Cspg4 enhancer. We found that Sox9, Sox10, Olig2, and Mash1 positively regulated the activity of the Cspg4 enhancer. In addition, we found that Sox family transcription factors and bHLH transcription factors cooperated on the intronic enhancer to upregulate the activity. These findings provide novel insight into glial cell-specific transcription of Cspg4 as well as provide a useful tool for targeting genes specifically to oligodendrocyte lineage cells.

Disclosures: H. Gotoh: None. **T. Nomura:** None. **K. Ono:** None. **A. Nishiyama:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.08/A19

Topic: B.11. Glial Mechanisms

Support: NSF Graduate Research Fellowship

National Institute of General Medical Sciences

Title: Role of myosin light-chain kinase in schwann cell cytoskeletal organization, differentiation, and myelination

Authors: *T. TOSEEF, C. V. MELENDEZ-VASQUEZ;
Hunter College, CUNY, New York, NY

Abstracts: Myelination in the peripheral nervous system is carried out by Schwann cells (SC), which surround all axons. Non-muscle myosin II (NMII), an actin-binding motor protein, is a key regulator of cytoskeleton dynamics necessary for interactions between SC and axons that lead to normal myelination. NMII activity is regulated by the phosphorylation of its regulatory light chain (MLC). Previous data suggests that one of the kinases that phosphorylates MLC, myosin light chain kinase (MLCK) may be involved in pathways that are activated by axonal signals at the onset of myelination. The purpose of this work is to identify proteins that may regulate the activity of MLCK in SC during myelin formation and repair. Firstly, using co-immunoprecipitation of MLCK from primary SC line, we will identify other proteins that are regulating the activity of MLCK. Secondly, we will characterize changes in MLCK expression after a peripheral nerve injury and evaluate the role of MLCK and its binding partners in promoting myelin repair and remyelination. The results from this work will provide insight into mechanisms that SC use to differentiate during development and dedifferentiation after injury, which is a prerequisite for successful nerve repair.

Disclosures: T. Toseef: None. **C.V. Melendez-Vasquez:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.09/A20

Topic: A.02. Neurogenesis and Gliogenesis

Title: A critical role of tuberous sclerosis complex-1 (tsc1) for myelination in the cns

Authors: *M. JIANG[1], L. LIU[2], X. HE[1], Q. R. LU[1];
[1]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]West China Med. Univ. of Sichuan Univ., Chengdu, China

Abstracts: Oligodendrocyte development and myelination is a highly regulated process controlled by intrinsic and extrinsic signaling. Tuberous Sclerosis Complex-1 (TSC1) is an upstream negative regulator of mTOR, the mammalian target of rapamycin complex (mTOR) that is a master regulator of cell growth and proliferation. Tsc1 deficiency can lead to mTOR hyperactivation. Previous studies have shown that mTOR deficiency causes a defect in myelination. In this study we assessed the function of activated mTOR in myelination by deleting Tsc1 in oligodendrocyte lineage. We hypothesize that the increase of mTOR signaling would promote oligodendrocyte precursor differentiation. To our surprise, ablation of TSC1 in oligodendrocyte progenitors causes a severe defect in oligodendrocyte differentiation and myelination. Intriguingly, the number of oligodendrocyte precursor cells (OPC) is reduced in the TSC1 mutant brain. Our data suggest that TSC1 deficiency causes an increase of oligodendrocyte cell death *in vivo* and *in vitro*. Gene expression profiling analysis of the control and TSC mutants reveals a decrease of myelination-related genes and a concomitant increase of death-inducing signaling complex. Moreover, deletion of TSC1 in post-mitotic oligodendrocyte lineage cells impairs oligodendrocyte myelination but not OPC expansion. Our results demonstrate a stage specific function of TSC1 in regulation of oligodendrocyte lineage progression and myelination, and indicate that balancing of mTOR activity is critical for proper development of oligodendrocyte and myelination.

Disclosures: **M. Jiang:** None. **L. Liu:** None. **X. He:** None. **Q.R. Lu:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.10/A21

Topic: A.02. Neurogenesis and Gliogenesis

Support: NSFC/RGC:N_HKU741/11

SK Yee Medical Research Fund

Title: Juxtacrine signalling via Notch and ErbB receptors in the switch to fate commitment of bone marrow-derived Schwann cells

Authors: *D. K.-Y. SHUM[1], E. W. Y. TAI[2], G. K. H. SHEA[1], A. Y. P. TSUI[1], K. H. Y. LEUNG[1], Y. S. CHAN[2];
[1]Dept. of Biochem., [2]Dept. of Physiol., Fac Med, The Univ. of Hong Kong, Hong Kong, China

Abstracts: Our strategy of deriving Schwann cells from bone marrow stromal cells exploits purified dorsal root ganglia (DRG) neurons to provide Schwann cell-like cells (SCLCs) with juxtacrine signals that mediate commitment to the Schwann cell fate. In search for the signals, we found both Notch ligands and neuregulin-1 type III localized on the surface of DRG neurons. Immunopositivity for cell surface Notch-1 receptor was detectable on bone marrow-derived SCLCs but the ErbB2/3 heterodimeric receptors of neuregulin-1 were barely detectable. In co-cultures, the Notch ligands on DRG neurons triggered nuclear translocation of Notch intracellular domain (NICD), corresponding increase in ErbB2/3 expression in the SCLCs and attainment of the Schwann cell fate. Treatment of co-cltures with DAPT or a DLL-1 blocking peptide to perturb Notch signaling deterred not only the increase in ErbB2/B3 expression in SCLCs but also the progress of SCLCs to the Schwann cell fate. We conclude that juxtacrine signalling via Notch plays a key role in the upregulation of ErbB receptors for neuregulin-driven commitment of SCLCs to the Schwann cell fate.

Disclosures: D.K. Shum: None. **E.W.Y. Tai:** None. **G.K.H. Shea:** None. **A.Y.P. Tsui:** None. **K.H.Y. Leung:** None. **Y.S. Chan:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.11/A22

Topic: A.02. Neurogenesis and Gliogenesis

Support: NSFC Grant Y12011

Title: Optogenetic modulation of endogenous adenylate cyclase level in cultured schwann cells

Authors: *X. FENG, W. XIE, Z. WANG, Z. ZHOU, Y. ZHOU, Y. QIU, L. WANG;
Shenzhen Inst. of Advanced Technol., Guang Dong, China

Abstracts: Recently, optogenetics has brought innovative insight into neuroscience. A photoactivated adenylyl cyclase (bPAC) was combined as a kind of light driven enzyme with high cyclic adenosine monophosphate (cAMP) synthesis ability. In Schwann cells (SCs), cAMP

plays an essential role to initiate myelination and deeply involves both in proliferation and differentiation. Forskolin administration into cultured SCs can increase the cAMP level and induce myelination in SC/neuron co-culture system. In order to avoid nonselective effect of this traditional method, we delivered photoactivated bPAC gene by lentiviral vector with MBP promoter into SCs and 455nm blue light was used to trigger the transfected cells. It was proposed the transfected SCs would started the myelination procedure after light stimuli. As expected, 2-min light pulse light stimuli indeed induced a giant 500-fold increase of intracellular cAMP level; and the extent of cAMP rise had significant time-dependent trend. However surprisingly, single 2-min light pulse stimuli failed to upregulate the expression of mRNA or proteins associated with myelination such as P0, Krox-20, MBP, MAG, or downregulate of non-myelinating SC phenotype. We also tried to extend light stimuli to once a day for 5 days. Our results showed long time light stimuli couldn't induce myelination-like morphological change similar to previous single stimuli. Moreover, we found after single 30-min light flash, SCs gradually presented a form similar to apoptosis. Our studies indicated that light stimuli triggered time-dependent cAMP remarkable elevation in SCs resulted in apoptosis tendency rather than inducing proliferation or differentiation. It is suggested that a steady and appropriate cAMP level is essential for cellular functional modulation in SCs.

Disclosures: X. Feng: None. **W. Xie:** None. **Z. Wang:** None. **Z. Zhou:** None. **Y. Qiu:** None. **Y. Zhou:** None. **L. Wang:** None.

Poster
(Unable to Attend)

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.12/A23

Topic: A.02. Neurogenesis and Gliogenesis

Support: NMRC CBRG (NMRC/CBRG/0002/2012)

Title: Nanofiber-mediated microRNA delivery for enhanced oligodendrocyte differentiation

Authors: *S. CHEW[1], H. DIAO[1], W. LOW[2], U. MILBRETA[3], R. Q. LU[4];
[2]Ch, [3]Chem. & Biomed. Engin., [1]Nanyang Technological Univ., Singapore, Singapore; [4]Dept. of Pediatri, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstracts: MicroRNAs, miR-219 and miR-338, regulate oligodendrocyte differentiation and myelination. Independently, recent studies have also demonstrated the ability of fiber constructs to modulate oligodendocyte myelination. Therefore, nanofiber-mediated delivery of miR-219 and/or miR-338 may have promising applications in treating traumatic central nervous system injuries by controlling oligodendrocyte behavior. In this study, electrospun, aligned poly-ε-caprolactone (PCL) nanofibers of three different average fiber diameters (300nm, 700nm & 2.0 um) were modified with DOPA-melanin coating and adsorbed with miRNAs/ TransIT-TKO® (miRNA/TKO) complexes to evaluate the synergistic effects of fiber diameter, miR-219, and miR-338 on the differentiation and maturation of seeded oligodendrocyte progenitor cells (OPCs). Randomly-oriented 2.0 um fibers were also included to study the fiber orientation effect on OPCs behavior. The loading efficiency of miRNA/TKO complexes was more than 86% for all scaffolds. Four days post-transfection, miR-219 treatment alone or in combination with miR-338 (denoted as 'miR-219+338') successfully silenced the negative regulators of oligodendrocyte differentiation (Sox6, FOXJ3, ZFP238 and PDGFR-alpha) on 300 nm aligned fibers. A higher proportion of MBP^+ (mature marker) and RIP^+ (intermediate marker) oligodendrocytes were observed with miR-219 and miR-338 treatment. Enhanced OPC maturation was also observed with larger fiber diameter (2 um >700 nm>300 nm). On 300 nm and 700 nm fibers, the effects of miR-219 and miR-219+338 treatment were more prominent, inducing significant OPCs differentiation to MBP^+ oligodendrocytes as compared to negative microRNA controls (miR-NEG) ($p=0.037$, ANOVA). Altogether, nanofiber-mediated microRNAs delivery enhanced OPCs differentiation to oligodendrocytes. Such constructs may serve as potential platforms for controlling OPC differentiation through synergistic topographical and biochemical signaling.

Disclosures: S. Chew: None. **H. Diao:** None. **W. Low:** None. **U. Milbreta:** None. **R.Q. Lu:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

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Program#/Poster: 589.13/A24

Topic: A.02. Neurogenesis and Gliogenesis

Support: National MS Society USA (RG4706A4/2 to V. Gallo and B. Nait-Oumesmar)

French MS foundation - ARSEP

Title: Sox17 affects oligodendroglial cell lineage progression

Authors: M. FAUVEAU, B. WILMET, C. KERNINON, C. DEBOUX, *B. NAIT-OUMESMAR;
ICM, Inserm-Upmc UMRS-1127, CNRS UMR 7225, Paris, France

Abstracts: In the central nervous system (CNS), myelination is a crucial process, timely regulated by oligodendroglial cell lineage progression. During development, the transition from proliferative/migrating oligodendrocyte precursor cells (OPCs) stage towards myelinating oligodendrocytes occurs through OPC cell cycle exit and differentiation. SOX transcription factors are key regulators of oligodendroglial cell proliferation and differentiation. Among them, SOX17 (subgroup F) was recently identified as a new regulator of oligodendrocyte development. In the developing CNS, SOX17 expression is detected in oligodendroglial cells and is highest in differentiating oligodendrocytes. Furthermore, in primary OPC cultures, SOX17 expression peaks at the pre-myelinating stage, correlating with the transition between OPC proliferation and differentiation. These findings suggest that SOX17 may play critical functions in OPC cycle exit and differentiation. To investigate the functional role of SOX17 *in vivo*, we generated a transgenic mouse model overexpressing SOX17 and the EGFP reporter in SOX10+ oligodendroglial cells, in a doxycycline (DOX) inducible manner (Tet-ON system). To trigger SOX17 overexpression in all oligodendroglial subpopulations, DOX treatment was initiated from embryonic day 12.5 (E12.5) to post-natal day 15 (P15). In DOX-treated transgenic mice, SOX17 gain-of-function was specifically detected in OLIG2+ cells, PDGFRα+ OPCs and CC1+ oligodendrocytes. Interestingly, we found that SOX17 overexpression enhanced OPC proliferation and decreased the proportion of CC1+ differentiated oligodendrocytes. Nevertheless, the pool of OLIG2+ and TUNEL+ apoptotic cells were not significantly modified in SOX17 transgenic mice compared to controls. MBP immunohistochemistry and electron microscopy analysis revealed a severe defect of myelination in SOX17 transgenic mouse spinal cord. At later developmental stages, the differentiation impairment was compensated. However, according to g-ratio analysis, myelin sheaths remained thinner in SOX17-overexpressing mice. Altogether, our data reveal critical functions of SOX17 in OPC maintenance and lineage progression.

Disclosures: M. Fauveau: None. **B. Nait-Oumesmar:** None. **B. Wilmet:** None. **C. Kerninon:** None. **C. Deboux:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Shriners Hospital for Children

National MS Society

RO1 NS025044

Title: Wnt effector TCF7l2 regulates oligodendrocyte differentiation independent of Wnt signaling

Authors: *F. GUO[1,2], J. LANG[1], Y. MAEDA[1], M. POPAL SAEED[1], E. M. HAMMOND[2], D. PLEASURE[1,2];
[1]UC Davis Sch. Med., SACRAMENTO, CA; [2]Inst. of Pediatric Regenerative Medicine, Shriners Hosp. for Children, Sacramento, CA

Abstracts: The role of Wnt effector TCF7l2 in controlling postnatal oligodendrocyte (OL) development is unknown, though prior important studies suggest that TCF7l2 negatively regulates OL differentiation through TCF7l2-mediated Wnt activity in oligodendroglial progenitor cells (OPCs). However, no genetic data have been reported thus far to support this hypothesis. Our prior study reports that APC ablation leads to fewer differentiated oligodendrocytes (OLs), aberrant activation of Wnt signaling and down-regulation of TCF7l2, the two latter of which are seemingly conflict each other. Here we found that APC disruption stalled OPCs in a population of NG2-/PDGFRa+/Ki67- status where another member of Wnt effectors LEF1 was significantly induced to subsequently mediate the aberrant activation of Wnt signaling we have observed. These data have three novel implications of TCF7l2 biology we proposed here: (1) TCF7l2 is upregulated normally in differentiated OLs but not OPCs - thus its' reduction reflecting fewer differentiated OLs in APC-KO; (2) TCF7l2 promotes rather than inhibits postnatal OL differentiation - its' conditional ablation leading to significant fewer OLs; (3) TCF7l2's role of OL differentiation is independent of Wnt activation - disruption of TCF7l2 not altering Wnt signaling activity. Using immunohistochemistry and mRNA *in situ* hybridization, we demonstrated that TCF7l2 was exclusively expressed in post-mitotic Mbp and Cnp-mRNA and protein-expressing, newly differentiated OLs in the central nervous system except for neurons in forebrain thalamus. Using Wnt reporter mice BAT-lacZ, we showed that the expression and upregulation of TCF7l2 in OLs were not correlated with the activation of Wnt/b-catenin signaling. Using an inducible Cre-loxP system, we found that TCF7l2 ablation in oligodendroglial lineage cells inhibited OL differentiation, without affecting the OPC proliferation and apoptosis. We also found that TCF7l2 was induced in post-mitotic newly differentiated OLs after myelin damage in the cuprizone-elicited demyelinative/remyelinative

model and in the autoimmune-induced myelin oligodendrocyte glycoprotein (MOG35-55)-peptide EAE model. Genetic ablation of TCF7l2 in oligodendroglial lineage cells inhibited OL differentiation and timing remyelination in cuprizone model. Furthermore, the induction of TCF7l2 in oligodendroglial lineage cells was not associated with the activation of Wnt/b-catenin signaling after myelin damage. Collectively, our study suggests that TCF7l2 is a positive regulator for OL differentiation, promoting OL differentiation independent of Wnt/b-catenin signaling.

Disclosures: F. Guo: None. **J. Lang:** None. **Y. Maeda:** None. **M. Popal Saeed:** None. **D. Pleasure:** None. **E.M. Hammond:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.15/A26

Topic: B.11. Glial Mechanisms

Support: NIH grant NS051509

NIH NRSA NS076098

Title: Oligodendrocyte dynamics in the adult brain

Authors: *E. G. HUGHES, D. E. BERGLES;
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Abstracts: Oligodendrocytes facilitate rapid communication between neurons by accelerating the propagation of action potentials, reducing the energetic cost of information transfer, and providing metabolic support to axons. Although the majority of myelination occurs during young adulthood, oligodendrocyte precursor cells (OPCs) continue to generate oligodendrocytes in the adult brain, raising the possibility that myelination is a dynamic process that can be modified by experience. However, it is not known whether oligodendrogenesis in the adult brain represents *de novo* production of new oligodendrocytes or oligodendrocyte replacement due to natural turnover. It is also unclear whether these new cells fill gaps along myelinated axons (e.g. internode replacement or addition) or myelinate previously unmyelinated axons. To study oligodendrogenesis and myelination in the adult CNS, we performed *in vivo* two-photon time-lapse imaging in the somatosensory cortex of transgenic mice in which EGFP is expressed

specifically in mature oligodendrocytes (*MOBP-EGFP* mice). Histological analysis revealed that EGFP is expressed by nearly all myelinating oligodendrocytes in the CNS of *MOBP-EGFP* mice, allowing visualization of cell somata and internode processes *in vivo*. To determine the extent of oligodendrocyte generation and the stability of internode segments, we repeatedly imaged oligodendrocytes through a cranial window for up to two months in mice > 5 months-old. Our studies indicate that the majority of oligodendrocytes are stable, with the small number of oligodendrocytes formed in cortical gray matter adding to the total population. Although most oligodendrocyte internode segments were also stable, some increased in length while others retracted, indicating that myelin segments along some axons are remodeled. These results suggest that adult oligodendrogenesis represents *de novo* myelination rather than a response to oligodendrocyte turnover and that the myelination state of axons is not fixed. To monitor differentiation of OPCs *in vivo*, we generated triple transgenic mice (*Olig2-CreER*; *R26-lsl-tdTomato*; *NG2-mEGFP*) in which membrane-anchored EGFP is expressed by OPCs, and all stages of the oligodendrocyte lineage express tdTomato. Fate tracing studies in these mice revealed that the majority of OPCs that differentiated died in the premyelinating stage, with only a small proportion maturing into myelinating oligodendrocytes. This ongoing cycle of OPC differentiation, death and replacement in the adult CNS, may facilitate rapid regeneration of oligodendrocytes following demyelination and help alter the pattern of myelination with life experience.

Disclosures: E.G. Hughes: None. **D.E. Bergles:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

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Program#/Poster: 589.16/A27

Topic: B.11. Glial Mechanisms

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Title: Terminal Schwann cells sense the spillover of ACh at the neuromuscular junction by muscarinic and α7 nicotinic receptors

Authors: *E. KREJCI[1], E. GIRARD[2], C. COLASANTE[3], V. BERNARD[4], N. LENIZ[5], D. SAMIGULLIN[5], J. LEROY[1], B. PLAUD[1], E. NIKOLSKY[6], K. PETROV[6];
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Abstracts: The terminal Schwann cells (TSC) sense the synaptic activity but it remains unclear how. Acetylcholine (ACh) is one of the signal at the mammalian neuromuscular junction (NMJ). The action of ACh is well controlled by acetylcholinesterase (AChE) that is clustered by ColQ in the basal lamina that runs between the nerve terminal and the muscle fiber. AChE prevents the repetitive binding of ACh on the muscle nicotinic receptors (nAChR). In addition to AChE, butyrylcholinesterase (BChE) is also abundant at the NMJ. In contrast to AChE, BChE is specifically anchored at the surface of the TSC by the small protein PRiMA, the transmembrane protein anchor of brain AChE. We indeed found by using a specific monoclonal antibody against mouse BChE that BChE is localized at the surface of the TSC in wild type NMJ but retained inside the TSC in PRiMA KO mice. Inhibition of BChE did not change directly the activation of the muscle nAChR but depressed significantly the release of ACh. This depression was unchanged after treatment with 1μM atropine to block the all muscarinic ACh receptors (mAChR) or in β2 or β4nAChR KO mice but was prevented in α7 nAChR KO mice or after treatment with MLA (a specific blocker of α7 nAChR). We have then used antibodies against α7 nAChR to localize the receptor and we observed a weak labelling at the TSC in WT but not in α7 nAChR KO mice. Moreover, we found pattern of nerve stimulations that triggered calcium waves in the TSC that were abolished after MLA treatment. On the other hand, different experiments have shown that the activation of mAChR may also trigger calcium waves in the TSC, supporting the presence of mAChR at the surface of the TSCs. The presence of α7 nAChR on the surface of TSC together with mAChR challenges the unifying hypothesis that slowly desensitizing high-affinity receptors determines both the selectivity and the sensitivity of glial activation. We also established that BChE controls the spillover of ACh even when AChE is active supporting a specific role of BChE.

Disclosures: E. Krejci: None. **E. Girard:** None. **C. Colasante:** None. **V. Bernard:** None. **N. Leniz:** None. **E. Nikolsky:** None. **B. Plaud:** None. **J. Leroy:** None. **K. Petrov:** None. **D. Samigullin:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.17/A28

Topic: B.11. Glial Mechanisms

Title: Differential regulation of non-myelinating & immature gene expression profiles in schwann cells from Cx32-null mice

Authors: *M. M. FREIDIN[1], C. K. ABRAMS[2];
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Abstracts: Connexin32 (Cx32) is found in the non-compact myelin of the paranodes and Schmidt-Lanterman incisures in myelinated peripheral nerve. Myelinating Schwann cells (SCs) express Cx32 in coordination with other myelin genes in mature and developing peripheral nerves. Recent studies have demonstrated that Cx32 is also regulated in non-myelinating SCs during development and in regenerating peripheral nerve following injury. Myelinating and non-myelinating SCs represent the terminal step of SC differentiation *in vivo*. The present studies use real time PCR expression profiling to determine whether disruption of Cx32 influences SC expression of genes identified with non-myelinating and de-differentiating phenotypes in proliferating and regenerating SCs from Wild Type (WT) and Cx32-null (32KO) mice. Based on analysis of a large microarray data set comparing uninjured and injured sciatic nerves from WT and 32KO mice at 5, 7, and 14 days post crush, a panel of genes associated with mature myelinating and de-differentiated immature SCs were used to screen cultured SCs and samples from uninjured and axotomized sciatic nerves from WT and 32KO mice. Levels of p75NGFR, a marker for non-myelinating and immature SCs, were increased in uninjured nerves and cultured SCs from 32KO mice. Other key regulators of myelin gene expression were significantly altered in 32KO SCs; suggesting that loss of functional Cx32 leads to expression of genes associated with immature and injured SCs and dysregulation of pathways required for the maintenance of normal peripheral nerves and re-differentiation to mature myelinating phenotypes in regenerating nerves following injury.

Disclosures: M.M. Freidin: None. **C.K. Abrams:** None.

Poster

589. Oligodendrocyte and Schwann Cell Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.18/A29

Topic: B.11. Glial Mechanisms

Title: Unravelling the PLPnull phenotype: Identification of SIRT2 targets in oligodendrocytes

Authors: K. KUSCH, O. JAHN, I. TZVETANOVA, H. B. WERNER, J. M. EDGAR, B. KASAPOGLU, *K. .-A. NAVE;
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Abstracts: Absence of the NAD+ dependent protein deacetylase, sirtuin 2 (SIRT2), in the myelin fraction was the most prominent finding in analysis of the proteolipid protein (Plp1) knock out mouse, a model for spastic paraplegia type 2 (Werner et al. 2007). In order to investigate the pathology in this model, we focus on functions of SIRT2. While deletion of Sirt2 leads to no pathological phenotype, SIRT2 deficiency in the context of a Cnp knockout results in axonal swellings, neuroinflammation and premature death. The role of SIRT2 as an oligodendroglial disease modifier is supported by an oligodendrocyte specific conditional Sirt2xCnp double knockout, mimicking the phenotype of the full double knockout. For SIRT2 target protein identification, we performed a proteomic screen comparing cytosolic CNS protein fractions derived from SIRT2null or WT mice, with respect to alteration in protein net charge. A verification by IEF-immunoblot proved SIRT2 dependent PTM changes in carbonic anhydrase 2 (CA2) and glucose-6-phosphate-isomerase (G6PI). Acetylation sites of CA2 were identified using Ion Mobility-Enhanced Mass spectrometry. Acetylated lysine residues might interfere with protein interaction of CA2 and monocarboxylate transporter 1 (MCT1) (Klier et al. 2014) in oligodendrocytes and thereby efficient axonal metabolic support with lactate.

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Poster

589. Oligodendrocyte and Schwann Cell Biology

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Program#/Poster: 589.19/A30

Topic: B.11. Glial Mechanisms

Support: Sardinia Region Research **Support:** Regional Law 7-CRP-25879/2011

Title: Tubulin post-translational modifications in Schwann cells

Authors: *S. D. GADAU[1,2], S. SECHI[2], R. COCCO[2], A. MURA[2];
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Abstracts: Microtubules (MTs), are heterodimers of α- and β-tubulin, generally involved in different cellular processes including mitosis, cell motility, intracellular transport, cell shape and polarization. In most eukaryotes tubulins, especially the α, are subjected to several post-translational modifications (PTMs) which include acetylation, tyrosination, detyrosination, Δ2 modification, polyglutamylation, that characterize different type of MTs and regulate the interactions between MTs and certain MAPs or motor proteins. Unlike neurons, in which presence and distributions of tubulin PTMs are most studied, in other neural cells little is known about the different tubulin PTMs amount, distribution and their functional role. So that, the purpose of the present work was to deepen the knowledge about the diverse tubulin PTMs in a commonly used immortalized Schwann cell line. Undifferentiated RT4-D6P2T rat schwannoma cells were cultured in standard conditions for 48 hours. Prior to reach complete confluence, cells were or fixed or collected and homogenized for underwent to immunofluorescence staining and Western blot procedures respectively. Our results, displayed some original data. Both Western blot analysis and immunofluorescence staining revealed significant levels of polyglutamylated and Δ2-modification α-tubulins, usually considered mainly expressed in neurons. In addition we found differences in amount and distribution of tubulin PTMs in Schwann cells. In details, Western blot analysis showed a higher amount of polyglutamylated and tyrosinated α-tubulin, whereas acetylated, Δ2 and detyrosinated α-tubulin were less expressed, in comparison with total α-tubulin. Immunofluorescence staining, highlighted the distribution of acetylated and detyrosinated α-tubulin along the Schwann cells prolongations, showing a pattern similar with neurons, were the detyrosinated and acetylated isoforms are usually most detectable in axons and dendrites. In contrast, polyglutamylated α-tubulin was more detectable close to the cell body of Schwann cells, whereas the Δ2-modification was mainly distributed round the nuclear profile, forming a characteristic and complete ring. The tyrosinated α-tubulin isoform, appeared uniformly distributed both in the cytoplasmic processes and to the cell body. Summing up, our investigation offers insight on several tubulin PTMs amount and distribution in Schwann cells. This could be a further contribution to better understand both the role played by different MTs in myelination processes and the possible microtubular involvment in the pathogenesis of certain Schwann cells disorders.

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Poster

589. Oligodendrocyte and Schwann Cell Biology

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 589.20/A31

Topic: B.11. Glial Mechanisms

Title: Loss of Pals1 in Schwann cells leads to radial sorting defects

Authors: *D. R. ZOLLINGER[1], K.-J. CHANG[2], T. BAUER[1], K. BAALMAN[1], S. KIM[3], M. N. RASBAND[1];
[1]Neurosci., [2]Program in Developmental Biol., Baylor Col. of Med., Houston, TX; [3]Dept. of Anat. and Cell Biol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstracts: Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS) surround axons with myelin to enable rapid and reliable action potential propagation by sequestering sodium channels at nodes of Ranvier. The developmental mechanisms regulating myelin formation and the radial sorting of axons in the PNS are only partially understood. Recently, highly conserved polarity proteins have been implicated in myelination and radial sorting. One polarity protein, Protein Associated with Lin7 (Pals1), which localizes to paranodes, Schmidt-Lanterman incisures, and the adaxonal domain in Schwann cells, has been proposed to regulate myelin thickness, length, and ultrastructure. To determine whether Pals1 is important in myelination, we generated conditional knockout (cKO) mice lacking Pals1 in Schwann cells and oligodendrocytes using the Cre-lox recombinase system under the control of the 2′,3′-cyclic nucleotide phosphodiesterase (CNP) promoter. As adults, CNP-Cre;Pals1 cKO mice demonstrate hind limb clasping and impaired motor coordination. Axons in the CNS and PNS of adult cKO mice are myelinated. Mature Schwann cells exhibit normal length and thickness and subcellular domains are present. However, some axon bundles in the sciatic nerve are aberrantly myelinated. Developmental analysis reveals a significant delay in PNS myelin formation up to postnatal day 21. Furthermore, transmission electron microscopy reveals that radial sorting of axons by non-myelinating Schwann cells is perturbed up to postnatal day 21. In contrast, CNS myelination is normal throughout development, suggesting divergent polarity mechanisms between Schwann cells and oligodendrocytes.

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Poster

589. Oligodendrocyte and Schwann Cell Biology

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Program#/Poster: 589.21/A32

Topic: B.11. Glial Mechanisms

Support: This study was partly supported by Grant-in-Aid for Scientific Research (25460399) in the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan.

Title: Ablation of NG2 glial cells induced neuronal cell death in the hippocampus through apoptotic pathway

Authors: *M. NAKANO[1,2], Y. TAMURA[1], A. EGUCHI[1], M. YAMATO[1], S. KUME[1], Y. MIYASHIGE[1], Y. KATAOKA[1,2];
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Abstracts: Glial progenitor cells expressing chondroitin sulfate proteoglycan 4 (NG2), are termed as NG2 glial cells (or oligodendrocyte precursor cells), and comprise the majority of proliferative cells in the adult central nervous system (CNS). Recently, it was reported that NG2 glial cells rapidly migrate and proliferate to restore their density in response to focal loss of the cells, such as acute CNS injury, ischemia, and demyelination. Moreover, NG2 glial cells are known to receive direct synaptic inputs from neurons, and are often located adjacent to neuronal somata, suggesting any interactions between NG2 glial cells and neurons, although substantive roles of the NG2 glial cells without cell reproduction have not been elucidated. Cell-specific ablation using transgenic animals is a powerful method to reveal cellular function. Thus, we produced transgenic rats expressing herpes simplex virus thymidine kinase (HSV-TK), known as a suicide gene, under control of the promoter for NG2 to explore the roles of NG2 glial cells. In the brain of transgenic rats, almost all NG2 glial cells expressed HSV-TK. We tried to selectively ablate proliferating NG2 glial cells by continuous administration of the anti-viral agent into the lateral ventricle, and succeeded in rapid ablation of NG2 glial cells in the tissue around the lateral ventricle including the hippocampus. In almost all of regions in the hippocampus, surprisingly, the number of hippocampal neurons was dramatically decreased depending on the reduction of NG2 glial cells. To investigate the mechanism of neuronal cell death, we applied microarray analysis to the hippocampal tissue following ablation of NG2 glial cells. The expression profile revealed that ablation of NG2 glial cells increased the expression of pro-apoptotic genes related to extrinsic pathway for apoptosis. Immunohistochemical staining demonstrated that the hippocampal neurons contained death receptors involved in the signaling pathway for apoptosis. Furthermore, TUNEL staining actually revealed that hippocampal neurons underwent apoptosis following the NG2 glial cell ablation. These results suggested that NG2 glial cells maintain neuronal survival by regulating anti-apoptotic pathways under the physiological condition.

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Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.01/A33

Topic: A.04. Stem Cells

Support: Charles and Mary Claire Phipps Family Foundation

University of Wisconsin-Madison Graduate School

NIH grant P30 HD03352

Title: Development of interneuron progenitors from human Down syndrome (Trisomy 21) induced pluripotent stem cells (iPSCs)

Authors: *A. BHATTACHARYYA, Y. LIU, M. MUSSER, A. PRASAD, R. REESE, S.-C. ZHANG;
Waisman Ctr., Univ. Wisconsin, MADISON, WI

Abstracts: Down syndrome (DS, trisomy 21, Ts21) is the most common genetic neurodevelopmental disorder and is characterized by mild to moderate intellectual disability and high risk for Alzheimer's disease. Abnormalities in DS brain suggest that developmental differences underlie neuroanatomic abnormalities and contribute to the specific cognitive deficits in individuals with DS. Reductions in both excitatory and inhibitory neurons appear to be present in many cortical regions in DS. Yet, our understanding of the subtype and cause of these deficits is largely unknown. Excitatory and inhibitory neurons that populate the cortex have different developmental origins. Excitatory projection neurons are derived from dorsal forebrain while inhibitory interneurons originate from ventral forebrain. Interneuron progenitors arise from the three neurogenic areas of the ventral telencephalon, with most developing from the medial ganglionic eminence (MGE). Analysis of the Ts65Dn DS mouse model shows an increased number of interneurons in the cortex. Although this data conflicts with the reductions in neuron number in human DS brain, the data highlight the fact that interneuron development is faulty in DS and warrant more studies in human. It is likely that mechanisms at play in mouse and human interneuron development differ. For example, the superficial cortical layers are considerably larger in human thus requiring generation of more interneurons and more complex developmental mechanisms. To investigate human interneuron development in DS, we differentiated human Ts21 induced pluripotent stem cells (iPSCs; Weick et al., PNAS, 2013) into NKX2.1-positive MGE-like progenitors (Liu et al., Nature Protocols, 2013) and compared them to isogenic euploid controls. Gene expression differences were observed in the Ts21 MGE

progenitors including misexpression of LHX6 and DLX genes that are known to be critical in interneuron migration. These results suggest that Ts21 may affect the migration of interneuron progenitors during cortical development. Further experiments will test whether Ts21 MGE progenitors have deficits in other steps of interneuron generation.

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Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.02/A34

Topic: A.04. Stem Cells

Title: Modeling the neuromuscular junction in C9ORF72-associated motor neuron disease using iPSCs

Authors: *E. SWARTZ[1], M. PRIBADI[2], J. BAEK[2], G. COPPOLA[2];
[1]Neurosci., UCLA, Los Angeles, CA; [2]Departments of Psychiatry & Neurol., Semel Inst. for Neurosci. and Human Behavior, Los Angeles, CA

Abstracts: A hexanucleotide expansion in the gene C9ORF72 is the most common cause of familial frontotemporal dementia (FTD), motor neuron disease (MND), and FTD/MND. Induced pluripotent stem cells (iPSCs) offer a way to explore the pathogenic mechanisms caused by specific genetic mutations in the tissues of interest. Because motor neuron dysfunction causes functional and structural abnormalities of the neuromuscular junction, we sought to model *in vitro* the neuromuscular junction by co-culturing motor neurons and muscle cells. We reprogrammed into iPSCs fibroblasts from (1) carriers of a C9ORF72 repeat expansion, (2) individuals diagnosed with sporadic FTD, and (3) healthy controls. iPSCs were differentiated into both motor neurons and skeletal muscle cells and seeded onto opposing sides of a microfluidic plate with two chambers connected by microgrooves. The microfluidic plate allows for the extension of motor neuron axons into the adjacent chamber which harbors skeletal muscle cells, and the establishment of neuromuscular junctions that can be monitored *in vitro* over many weeks. We use this platform to analyze the interactions between patient and control-derived cell types and explore hypothesized pathogenic mechanisms, such as the accumulation of RNA foci or dipeptide aggregates. Additionally, this system will allow for testing candidate compounds interfering with the hexanucleotide expansion.

Disclosures: **E. Swartz:** None. **M. Pribadi:** None. **J. Baek:** None. **G. Coppola:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Program#/Poster: 590.03/A35

Topic: A.04. Stem Cells

Title: Single transfection of a synthetic polycistronic self-replicative RNA yields human iPSCs that can be efficiently expanded & directed to neural progenitor cells

Authors: ***V. T. CHU**[1], M. LU[2], N. YOSHIOKA[3], S. DOWDY[4];
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Abstracts: Various methods utilizing DNA, RNA, miRNA and protein have been described to generate integration-free induced pluripotent stem cells (iPSCs). Some disadvantages to these methods include low reprogramming efficiency (i.e. DNA and protein), a requirement for negative selection and subsequent recloning steps to remove persistent traces of the virus (i.e. Sendai virus) or for daily transfections of four individual *in vitro* generated mRNAs over a 14 day period (i.e. mRNA based). Recently, an RNA species from non-infectious (non-packaging), self-replicating Venezuelan equine encephalitis (VEE) virus was engineered to encode all four reprogramming factors (RF; OCT4, KLF4, SOX2 and GLIS1) in a polycistronic RNA transcript that mimic cellular mRNA. Advantages to the RNA replicon system are that (1) the RNA can self-replicate for a limited number of cell division hence obviating the requirement for multiple transfections, and (2) the RNA only persists under positive selection and can be quickly cleared when the positive selective pressure (i.e. puromyocin) is released. A single transfection of the RNA replicon used in combination with a small molecule boost supplement efficiently reprogrammed two lines of human foreskin fibroblasts (HFFs). Reprogramming efficiencies using the RNA replicon ranged from 0.3% in the slower proliferating BJ fibroblasts to 1.1% in the faster proliferating HFF. The resulting human iPSCs displayed all the hallmarks of pluripotent cells and could be rapidly expanded in serum-free, feeder-free expansion systems and could also be readily directed to multipotent neural progenitor cells that retained capacity to differentiate into neurons and oligoglial cells.

Disclosures: **V.T. Chu:** A. Employment/Salary (full or part-time):; EMD Millipore. **M. Lu:** A. Employment/Salary (full or part-time):; EMD Millipore. **N. Yoshioka:** A. Employment/Salary

(full or part-time):; UCSD School of Medicine. **S. Dowdy:** A. Employment/Salary (full or part-time):; UCSD School of Medicine.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.04/A36

Topic: A.04. Stem Cells

Support: NIH Grant NS078338

Harvard Stem Cell Institute

Title: Characterization of iPSC-derived neuronal preparations and bioassays for use in transplantation in Parkinson's disease

Authors: *T. M. OSBORN, J. L. JANSSON, G. A. SMITH, J. A. BEAGAN, Z. E. SCHNEIDER-LYNCH, D. A. AHMADI, J. A. KORECKA, P. J. HALLETT, O. ISACSON; Neuroregeneration Res. Inst., McLean Hospital/Harvard Med. Sch., Belmont, MA

Abstracts: Patients with Parkinson's disease can gain improved motor function upon fetal ventral midbrain cell transplantations (Mendez et al. Nat Med. 2008 May;14(5):507-9). Fetal cell sources are however limited and patients receiving transplantations require immunosuppression. Induced pluripotent stem cells (iPSCs) present opportunities for autologous transplantations, provided that the cells are safely and effectively differentiated to midbrain dopaminergic neurons. We have derived iPSCs from cynomolgus monkeys for future and ongoing autologous transplantations. 6-OHDA lesioned hemi-parkinsonian rats have been transplanted with these iPSCs differentiated using previously published floor-plate-based protocols for midbrain dopaminergic fate (Cooper et al., 2010, Mol Cell Neurosci;45(3):258-66, Sundberg et al., 2013 Stem Cells;31(8):1548-62) in order to determine long-term (6 months) graft characteristics. Midbrain dopaminergic survival, presence or absence of proliferating cells and neural precursors, blood vessel growth in grafts, graft morphology and behavioral recovery have been documented in preparation for clinical IND applications. Furthermore, we have optimized our midbrain dopaminergic differentiation protocol to be feeder-free and xeno-free and to generate TH/FoxA2 expressing neurons at a similar level as previously described (Cooper et al., 2010, Mol Cell Neurosci;45(3):258-66, Sundberg et al., 2013 Stem Cells;31(8):1548-62). Cells generated using these protocols are being characterized using qPCR and ICC at the end of the protocol and after

long-term culturing *in vitro*. Together with the ongoing autologous transplantations into non-human primates, the long-term xenogeneic transplantations and *in vitro* characterizations evaluate the safety and efficacy of iPSC therapy for Parkinson's disease at a pre-clinical level. The collected data will be used when establishing GMP-grade midbrain dopaminergic neurons from patient-derived iPSCs for future clinical applications.

Disclosures: T.M. Osborn: None. **J.L. Jansson:** None. **G.A. Smith:** None. **J.A. Beagan:** None. **Z.E. Schneider-Lynch:** None. **D.A. Ahmadi:** None. **J.A. Korecka:** None. **P.J. Hallett:** None. **O. Isacson:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.05/A37

Topic: A.04. Stem Cells

Support: LeJeung Foundation

Title: Expression of oxidative stress genes in human stem cells in physiological versus atmospheric oxygen levels

Authors: *K. M. KLEMAN[1], R. REESE[2], S. HANSON[1], A. BHATTACHARYYA[2]; [1]Gilson, Middleton, WI; [2]Waisman Ctr., Univ. of Wisconsin-Madison, Madison, WI

Abstracts: Oxidative stress plays a role in the development as well as the degeneration of the brain. Oxygen levels in the developing embryo are low and proper regulation of oxygen levels is crucial for stem cells, neural progenitor cells and neurons. *In vitro* models enable examination of the mechanisms involved in neural development,neuronal function and neuronal responses to injury. However, most neuronal culture methods employ non-physiological oxygen conditions. Standard cell culture is performed at atmospheric (20%) oxygen levels. The ability to study oxidative stress in neurons in hyperoxic (atmospheric) conditions is particularly problematic when studying disorders that are characterized by oxidative stress. Therefore, it is crucial to investigate oxidative stress features of cells in a context that enables elucidation of the intrinsic features of the cells. Recently, it has become clear that culturing cells, particularly stem and neural progenitor cells, in low (physiological) oxygen makes the cells grow faster, healthier, less stressed, and with less DNA damage). We therefore investigated the role of oxidative stress in human induced pluripotent stem cells (iPS cells) by testing expression of several genes involved

in the cellular oxidative stress response when cells were maintained in different oxygen concentrations. iPS cells were cultured in 5% oxygen (physiological) or 20% oxygen (atmospheric) and analyzed for expression of specific chromosome 21 genes known to be involved in oxidative stress (SOD, APP, ETS2) and genes known to be upregulated in response to oxidative stress (CAT, CRYZ). Quantitative PCR (384-well) was performed using the PIPETMAX pipeting station with associated qPCR Assistant software and compared to manual pipetting. Automated plate setup enabled easy file export to ABI ViiA thermocycler. The results show that the expression of all of these genes is reduced when iPS cells are cultured in low oxygen. These results have implications for culturing and analyzing disease specific iPSC to model diseases that have an oxidative stress component.

Disclosures: K.M. Kleman: None. **R. Reese:** None. **S. Hanson:** None. **A. Bhattacharyya:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.06/A38

Topic: A.04. Stem Cells

Support: NIH Grant R01NS061983

NIH Grant R01ES015988

NMSS Grant

Shriners Grant

CIRM postdoc fellowship

Title: The role of Olig2 gene function in the production of interneurons in Down syndrome

Authors: *C. CHEN[1], P. JIANG[2], Y. LIU[3], W. DENG[2];
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Abstracts: Down syndrome (DS), caused by triplication of human chromosome 21 (HSA21), is the most frequent genetic cause of intellectual disability. Previous studies have reported a

widespread over-abundance of interneurons (INs) throughout the forebrain of Ts65Dn mice, the most widely used model of DS. The over-inhibition is one of the underlying causes of the cognitive deficits in Ts65Dn mice. However, it is largely unknown whether overproduction of INs and over-inhibition occur in human patients. Olig genes, including Olig1 and Olig2 are mapped to HSA21. Olig genes are highly involved in the production of INs during development. The early neural progenitor cells (NPCs) that express Olig2 give rise to GABAergic INs in the forebrain. In Ts65Dn mice, normalizing the over-dosage of Olig1 and Olig2 genes rescues the phenotype of over-abundance of GABAergic INs. Contradictorily, a recent study shows that Olig1 represses production of GABAergic neurons throughout the brain. To reconcile these contradictory observations, it is important to study the role of Olig2 in interneuron production during brain development, particularly by using human neural cells. In this study, we differentiated human Olig2-GFP human embryonic stem cell (hESC) reporter line to Olig2+/GFP+ NPCs with ventral forebrain identity and further purified them by FACS based on the expression of GFP fluorescence. Under neuronal differentiation condition, we found that these Olig2+/GFP+ NPCs gave rise only to GABAergic INs and astrocytes. Furthermore, human induced pluripotent stem cells (hiPSCs) reprogrammed from fibroblasts of DS patients (DS hiPSCs) generated significantly higher number of Olig2+ NPCs, compared to their isogenic control hiPSCs with identical genetic background and disomy 21 (Di-DS hiPSCs). Subsequently, the NPCs derived from DS iPSCs (DS NPCs) gave rise to more GABAergic INs under neuronal differentiation condition, compared to the NPCs derived from Di-DS hiPSCs (Di-DS NPCs). We then engrafted the DS NPCs and Di-DS NPCs into the brains of neonatal immune-deficient mice. Three months after cell transplantation, we found that both NPCs could largely repopulate the forebrain of the animals. Most interestingly, the DS NPCs generated more GABAergic neurons *in vivo* than Di-DS NPCs. Thus, the *in vitro* and *in vivo* hiPSC differentiation models provide us with a powerful tool to the study the role of Olig2 in the production of INs during human brain development in normal and diseased conditions. Findings from this study will also provide new insights into developing potential therapeutic applications for DS by regulating the expression of Olig genes to manipulate interneuron production.

Disclosures: **C. Chen:** None. **P. Jiang:** None. **Y. Liu:** None. **W. Deng:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.07/A39

Topic: A.04. Stem Cells

Support: EU AIMS

Title: The role of SHANK3 in ASD

Authors: *A. KATHURIA, G. COCKS, P. NOWOSIAD, V. WOOD, R. DIXON, W. LUCCHESI, D. SRIVASTAVA, J. PRICE;
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Abstracts: Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by persistent deficits in social interaction, restricted repetitive behavior and decreased cognitive abilities (Burbach et al. 2014). Recent genetic and genomic data have identified over 180 genes associated with ASD (Gauthier et al. 2009, 2010; Hamdan et al. 2011; Denayer et al. 2012; Boccuto et al. 2012). One of the most promising candidates is the SHANK3 gene, which encodes for a postsynaptic scaffolding protein PROSAP2. SHANK3 deletions have been identified in more than 600 patients affected with Phelan McDermid syndrome (22q13 deletion syndrome) in which SHANK3 is strongly implicated in autistic like behavior (Burbach et.al 2014). The various mouse models with mutant forms of SHANK3 exhibit abnormal behavior, synaptic dysfunction and deficits in learning. (Bozdagi et al. 2010, Wang et.al 2011, Peca.et al 2012). Pervious studies have also linked Shank3's role in spine morphology, striatal dendritic morphology and synapse formation (Peca.et al 2012,Durand et.al 2012).To study the role of Shank3 in ASD we have generated 4 iPS cell lines from two patients with ASD that have a terminal deletion of chromosome 22 including the genes SHANK3, ACR and RABL2. As controls, we used iPSCs from healthy individuals, as described previously (Cocks et al., 2013). In this study, we investigate the morphological properties of the control and Shank3 neurons. We use to two assays to assess the morphological features- the transient transfection via GFP and the ThermoScientific Cellinsight Personal Cell Imager. Morphological differences were found in the Shank3 neurons at day 30 (immature neurons) and day 45 (young neurons) of neuronal development. The cell soma diameter was lower in the patient neurons while the neurite length and the mean number of neurites per neuron was higher at both the stages. Thus, our data indicates that the SHANK3 gene has a role in the development of the neuronal structure.

Disclosures: **A. Kathuria:** None. **G. Cocks:** None. **P. Nowosiad:** None. **V. Wood:** None. **R. Dixon:** None. **W. Lucchesi:** None. **D. Srivastava:** None. **J. Price:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.08/A40

Topic: A.04. Stem Cells

Support: Sackler Scholar Programme in Psychobiology

NIGMS grant T32GM007753

NIA grant T32AG000222

NIA grant R21AG042776

NIMH grant R21MH096233

NIMH grant R00 MH085004

NIA grant R01AG06173

Title: Disease-relevant genomic DISC1 disruption has selective effects on DISC1 expression and Wnt signaling

Authors: *P. SRIKANTH[1,2], C. R. MURATORE[1,2], D. J. SELKOE[1,2], T. L. YOUNG-PEARSE[1,2];
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Abstracts: Schizophrenia is a debilitating psychiatric disorder that affects approximately 1% of the world's population. Genetic and clinical association studies have identified disrupted-in-schizophrenia 1 (DISC1) as a strong candidate risk gene for schizophrenia and other major mental illnesses. DISC1 was initially associated with mental illness upon the discovery that its coding sequence is interrupted by a balanced chr(1;11) translocation in a Scottish family, in which the translocation cosegregates with schizophrenia, bipolar disorder and major depression. DISC1 modulates many neuronal processes, including proliferation, Wnt signaling, synaptic maturation, neurite outgrowth, and neuronal migration. The relevance of a DISC1 loss-of-function versus gain-of-function model to the human disease state remains unclear. We are currently exploring the consequences of DISC1 interruption on human neurodevelopment using TALE nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas to disrupt the genome of human induced pluripotent stem cells (iPSCs) at the DISC1 locus. We have targeted DISC1 to disrupt all known transcripts or near the site of the Scottish translocation, resulting in isogenic wild-type, total-, and disease-relevant-DISC1 disrupted iPSCs. Genome-edited cells have been differentiated to neural progenitor cells (NPCs) and cortical neuronal fates and examined for altered gene expression, Wnt signaling, proliferation, and migration. We find that total DISC1 disruption dramatically reduces all DISC1 transcript levels, whereas disease-relevant DISC1-decreases expression of only longer DISC1

splice variants. In addition, we show diminished Wnt responsiveness in disease-relevant DISC1-targeted NPCs but not in iPSCs, implicating Wnt signaling in the pathophysiology of this disease-associated mutation. These data and future studies will help identify those functions of DISC1 which are likely to be perturbed in patients with the chr(1;11) translocation and which, when disrupted, contribute to the development of major mental illness.

Disclosures: P. Srikanth: None. **C.R. Muratore:** None. **D.J. Selkoe:** None. **T.L. Young-Pearse:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.09/A41

Topic: A.04. Stem Cells

Support: The John H. Tietze Stem Cell Scientist Award, University of Washington

Title: Modeling enteric nervous system function in children with Autism Spectrum Disorder

Authors: *A. L. WAGONER[1,2], D. L. MACK[3], E. E. MCKEE[2], S. J. WALKER[2];
[1]Wake Forest Univ. Hlth. Sci., Winston-Salem, NC; [2]Wake Forest Inst. for Regenerative Med., Winston-Salem, NC; [3]Inst. for Stem Cell and Regenerative Medicine, Univ. of Washington, Seattle, WA

Abstracts: Children with Autism Spectrum Disorders (ASDs) often have co-morbid medical conditions. One of the most debilitating of these is chronic gastrointestinal (GI) symptoms, which occur in as much as 70% of this population. The successful treatment of GI symptoms in ASD is often accompanied by improvements in behavior and cognition, suggesting that the gut and brain are interconnected. Numerous genes have been shown to harbor mutations or deletions that result in functional consequences in central nervous system (CNS) neurons of ASD individuals. Many of these genes, for example Shank3 and Neuroligin3, are also expressed in the enteric nervous system (ENS), making it likely that mutations causing CNS dysfunction are also operating at the level of the ENS. To explore this relationship we are developing an *in vitro* induced pluripotent stem cell (iPSC) model system for the characterization of ENS function in ASD children with GI symptoms. We have identified cell lines from two male siblings with ASD from the Autism Genetic Resource Exchange (AGRE) for our preliminary studies. These patients have chronic GI symptoms and mutations in Shank3, a mutation known to negatively impact

synaptic development and function. IPSCs, generated from Epstein-Barr virus (EBV) immortalized lymphoblastoid cell lines (LCLs), appeared Day16 post-transfection with Epi5 Episomal iPSC reprogramming plasmids. Four clonal iPSC lines (two from each parent LCL) were propagated out to passage 23. We have confirmed iPSC clones to be plasmid- and EBV-free. Three of four clones had expected expression of cell-autonomous pluripotency genes and normal karyotype. All clonal lines were allowed to spontaneously differentiate into embryoid bodies and were assayed for pluripotency markers and germline-specific transcripts using the Taqman® human pluripotent stem cell Scorecard™ Panel. Results indicated that all four iPSC lines are suitable for neuronal differentiation protocols. In parallel experiments, neural crest cells, differentiated from human embryonic stem cells, showed proper gene expression and cell morphology. Optimized protocols are currently being applied to ASD-iPSCs to generate enteric neurons. Function of these neurons, compared to neurons derived from individuals with unaffected synaptic proteins, will be fully characterized in a smooth muscle/enteric neuron co-culture system. ENS function in ASD children has not been studied nor have mature enteric neurons from ASD patient-derived cells been generated and functionally tested. This model will provide a useful tool to better understand neuropathogenesis of ASD in the gut and a screening mechanism for drug therapies.

Disclosures: A.L. Wagoner: None. **S.J. Walker:** None. **E.E. McKee:** None. **D.L. Mack:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.10/A42

Topic: A.04. Stem Cells

Support: The Richard Merkin Foundation

CIRM Grant TB1-01183

Title: Tissue responses and repair in cortical stroke after transplant of distinct iPS progenitor populations

Authors: *A. CRUZ[1], I. L. LLORENTE[3], J. CINKORNPUMIN[4], C. MALONE[2], W. E. LOWRY[4], S. T. CARMICHAEL[5];

[2]Biol., [1]California State University, Northridge, Northridge, CA; [4]Molecular, Cell and Developmental Biol., [5]Neurol., [3]UCLA, Los Angeles, CA

Abstracts: Stroke is the leading cause of disability in Americans. There is limited endogenous tissue repair in the brain after stroke. Stem/progenitor transplantation has emerged as a mechanism to enhance this endogenous repair. Induced pluripotent (iPS)-derived precursor cells provide a way to deliver a progenitor cell to areas of damage that may stimulate repair. With the wide range in differentiation potential of iPS cells, it is not clear what type of cell might be the most potent in this process. To study iPS precursor types in ischemic stroke, a photothrombotic cortical stroke was induced in NSG mice, causing cortical and a small amount of subcortical white matter damage. Two different types of induced pluripotent stem progenitor cells were utilized, IPS-Neural progenitor cells (iPS-NPCs) and iPS-Glial restricted progenitor cells (iPS-GRPCs). These iPS-NPCs or iPS-GRPCs were injected adjacent to the infarct area (100,000 cells/microL) 7 days after induced stroke to observe potential migration, differentiation of the transplanted cells and the effect of these cells on local tissue responses. Control stroke mice received no cell transplantation. Immunostains were used to identify microglia (IBA-1), astrocytes (GFAP), immature neurons (DCX), oligodendrocytes (Oligo2), myelin (MBP), and a transfected marker of the iPS cells (GFP). Endogenous neurogenesis defined by DCX migration to the stroke lesion, is observed 15 days after iPS-NPCs are transplanted. iPS-GRPC transplants have been completed and are under analysis. These experiments will provide further insight to the possibility of induced pluripotent stem cell treatment for ischemic stroke.

Disclosures: A. Cruz: None. **I.L. Llorente:** None. **J. Cinkornpumin:** None. **W.E. Lowry:** None. **S.T. Carmichael:** None. **C. Malone:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.11/A43

Topic: A.04. Stem Cells

Support: NIH Grant R01NS048271

NIH Grant R01HD069184

NIH Grant R33MH087874

NIH Grant R37NS047344

MSCRF

NARSAD

IMHRO

Title: Modeling of major mental disorders using GABAergic interneurons derived from patient iPSCs with a defined DISC1 mutation

Authors: *X. WANG[1,2], Z. WEN[2,3], H. NGUYEN[2,4], C. ZHANG[2], H. SONG[2,3,4], G.-L. MING[2,3];
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Abstracts: Schizophrenia is one of the most common mental disorders that affect about 1% of the population world-wide. Increasing clinical, pharmacological and genetic data suggest that the pathogenesis of schizophrenia is neurodevelopmental in nature with a prominent genetic basis. Disrupted-in-schizophrenia 1 (DISC1), has been suggested as one of the best supported susceptibility genes for major mental illness. For example, in an American family, many members carry a four-nucleotide deletion in the DISC1 gene and the majority of whom have been diagnosed with schizophrenia, schizoaffective disorders and major depression. How DISC1 dysfunction results in the clinical manifestation of a large set of severe psychological symptoms is largely unknown. Patient-derived induced pluripotent stem cells (iPSCs) provide a unique platform to study the contribution of the DISC1 locus to the etiology of schizophrenia and other major mental disorders. We have generated iPSCs from the American family with the DISC1 4-nucleotide deletion, as well as healthy family members without the mutation as controls. GABAergic interneurons are known to play a key role in psychiatric disease. By manipulating the Sonic Hedgehog pathway, we are able to generate a high-purity population of ventral forebrain NPCs which are NKX2.1+ and Lhx6+ from both patient and control iPSCs. These ventral forebrain NPCs can be further differentiated to cortical GABAergic interneurons including PV+, SST+, calretinin+, and calbindin+ interneurons. This system allows for the investigation of the molecular and cellular role of DISC1 in the development of interneurons and their potential contribution to the manifestation of major mental illness.

Disclosures: **X. Wang:** None. **Z. Wen:** None. **H. Nguyen:** None. **C. Zhang:** None. **H. Song:** None. **G. Ming:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.12/A44

Topic: A.04. Stem Cells

Support: NIH Grant NS078753

Autism Speaks

Angelman Syndrome Foundation

Title: Synaptic pathophysiology in stem cell-derived neurons from dup15q autism and Angelman syndrome patients

Authors: *J. J. FINK[1], T. M. ROBINSON[2], K. A. BOLDUC[2], E. S. LEVINE[2];
[1]Neurosci., [2]Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstracts: Individuals with a maternal deletion of chromosome 15q11-q13 suffer from Angelman syndrome (AS). Interestingly, individuals with a duplication of the same region suffer from a form of autism known as 15q duplication syndrome (dup15q), which is one of the most frequent chromosomal abnormalities associated with autism. Both of these syndromes are neurodevelopmental disorders that often present with common features, including intellectual disability, impairments in language, and seizures. The gene believed to be responsible for these phenotypes encodes the ubiquitin ligase UBE3A, although other genes may also contribute. In both syndromes, alterations in synaptic signaling and plasticity appear to play a critical role in the disease phenotype, but the relevant downstream targets of UBE3A and their functional consequences are unknown. The discovery of genomic reprogramming of human somatic cells into induced pluripotent stem cell (iPSC) lines provides a novel way to generate patient-specific neurons for *in vitro* studies. In this study, we are using electrophysiological approaches and calcium imaging to examine synaptic activity and plasticity of iPSC-derived neurons from AS, dup15q, and control subjects. Low levels of spontaneous excitatory synaptic activity were seen after six weeks *in vitro* in both patient-derived and control neurons. After twelve weeks, control neurons displayed a dramatic increase in the frequency of synaptic events, which was significantly greater than the frequency in both AS-derived neurons and dup15q-drived neurons, suggesting a genotypic difference in synapse number and/or release probability. There was no significant difference in mean amplitude across genotype. These iPSC-derived neurons also

show spontaneous action potential firing and synchronous activity, which can be enhanced by NMDA receptor activation and increased intracellular cAMP levels. We are currently exploring potential differences in these processes in AS and dup15q-derived neurons using single cell recordings and multi-cell calcium imaging. Overall, these approaches may prove useful for identifying novel targets for drug discovery and for screening potential therapeutics aimed at reversing the seizures, movement disorders, and language and cognitive impairments in Angelman syndrome and autism.

Disclosures: J.J. Fink: None. **T.M. Robinson:** None. **K.A. Bolduc:** None. **E.S. Levine:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.13/A45

Topic: A.04. Stem Cells

Support: Tom-Wahlig Foundation Advanced Fellowship

Federal Ministry of Education and Research (BMBF, 01GQ113)

Title: Axonal pathology in patient-derived neurons harboring spg11 mutations: An ipsc model for spatacsin-linked hereditary spastic paraplegia

Authors: H. K. MISHRA[1], F. PEREZ-BRANGULI[1], I. PROTS[1], S. HAVLICEK[1], Z. KOHL[2], D. SAUL[1], C. RUMMEL[1], J. DORCA-AREVALO[6], M. REGENSBURGER[2], D. GRAEF[1], E. SOCK[8], J. BLASI[7], T. W. GROEMER[3], U. SCHLÖTZER-SCHREHARDT[4], J. WINKLER[5], *B. WINNER[5];
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Abstracts: Hereditary spastic paraplegias (HSPs) are a heterogeneous group of inherited motor neuron diseases characterized by progressive spasticity and weakness of the lower limbs. They are classified genetically as autosomal dominant, autosomal recessive and X-linked HSP.

Mutations in the Spastic Paraplegia Gene11 (SPG11), encoding spatacsin, cause the most frequent form of autosomal recessive HSP. Partly due to lack of a relevant disease model, the underlying molecular mechanisms have not been studied in detail. To overcome this limitation we, for the first time, generated induced pluripotent stem cells (iPSCs) from two SPG11 patients, having heterozygous nonsense and/or splice site mutations, and two age matched controls. We differentiated these iPSCs into forebrain neurons and investigated the neuronal pathology associated with the disease. SPG11 patients' derived neurons exhibited severely impaired outgrowth and branching of axonal processes, implicating a compromised neuritic complexity compared to controls. Gene expression analysis further revealed down regulation of specific motor, synaptic and microtubule associated genes. A reduced expression of acetylated tubulin in the neuronal cells was indicative of the axonal instability, which was further corroborated by ultra structural analysis of these cells showing pathological accumulation of membranous bodies within axonal processes. Finally, time lapse assays performed in SPG11 patients' derived neurons highlighted a reduction in the anterograde vesicle trafficking indicative of impaired axonal transport. Altogether, our SPG11-iPSC model provides the first evidence that mutations in SPG11 have a detrimental effect on the homeostasis of neuronal cells and more importantly disturb the critical balance of transport activity in SPG11 patients' neurons. Furthermore, our human model offers an ideal platform to define new targets to intervene the course of this progressing motor neuron disease.

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Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.14/A46

Topic: A.04. Stem Cells

Title: The role of SLC25A12 on interneuron development

Authors: *J. CHEN[1,2], X. ORTIZ-GONZALEZ[2], A. RAVINDRAN[2], D. C. WALLACE[2], S. A. ANDERSON[2];

[1]Mahoney Inst. of Neurolog. Sci., Philadelphia, PA; [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstracts: SLC25A12 is a nuclear-encoded mitochondrial glutamate-aspartate carrier highly expressed in the brain. Genetic studies suggest that alterations of SLC25A12 may be associated with autism, and researchers have identified patients carrying homozygous loss of function SLC25A12 mutations. These patients suffer from developmental delay, autism-related symptoms, and intractable seizures, possibly resulting from an imbalance of excitation and inhibition (E/I) in the brain. To understand whether SLC25A12 loss of function results in altered E/I balance, we focused on interneuron development using homozygous SLC25A12 null mice (KO) and human induced pluripotent stem cells (hIPSCs) generated from an SLC25A12 patient. Similar to the human patients, and as previously reported, the KO mice are developmentally delayed and have abnormal brain electrical activity. Current studies are examining cortical histology and electrophysiology in the mouse model, in parallel with differentiations of the patient-derived hIPSCs. The goal is to identify the seizure-causing pathology in these samples, and use this knowledge to design and test novel treatments.

Disclosures: J. Chen: None. **X. Ortiz-Gonzalez:** None. **A. Ravindran:** None. **D.C. Wallace:** None. **S.A. Anderson:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Program#/Poster: 590.15/A47

Topic: A.04. Stem Cells

Support: NIMH Grant R33 MH087879-04

CT Innovations Grant 11SCB23 S01511

Title: Dissecting the role of FOXG1 in patients with autism using induced pluripotent stem cells

Authors: *J. MARIANI[1], G. COPPOLA[1], P. ZHANG[1], J. R. HOWE[2], F. M. VACCARINO[1,3]; [1]Child Study Ctr., [2]Dept. of Pharmacol., [3]Dept. of Neurobio., Yale Univ., New Haven, CT

Abstracts: We generated induced pluripotent stem cells (iPSCs) from individuals of 5 families each comprising a child with idiopathic ASD and macrocephaly. Large head circumference (HC) and brain volume (macrocephaly) in patients with ASD is associated with poorer outcome,

suggesting that it represents a core feature of the disorder. IPSCs from patients and unaffected family members were differentiated into cortical organoids containing pyramidal neurons as well as telencephalic GABAergic interneurons. RNA sequencing of 45 telencephalic organoids from patients and controls at three different stages of differentiation revealed alterations in cell signaling, Extracellular matrix organization, neural development and synaptic transmission, suggesting a significantly different developmental trajectory between patients and controls. Gene co-expression network analysis revealed various modules of genes differentially expressed between probands and controls. The top upregulated module was characterized by genes involved in neuronal development, particularly synaptic transmission and cell projection morphogenesis. At the immunocytochemical level we detected increased cell proliferation, enhanced GABAergic neuron fate and simplified neuronal architecture. Consistently, neuronal cells from patients showed increased expression of sodium channels with phenotypic properties similar to Nav1.1, an isoform preferentially expressed in inhibitory neurons. Transcription factor analysis on the up-regulated neuronal module showed enrichment for the Forkhead transcription factor family. FOXG1 was among the top upregulated genes in ASD-derived neurons. Using lentiviruses carrying short hairpin RNAs (shRNAs) targeting FOXG1, we then tested whether an attenuation of FOXG1 expression level in patients' neural cells was able to revert some of the neurobiological alterations, including cell proliferation, GABA/glutamate neuron fate, and architecture of neuronal connections. Patients' neuronal cells where FOXG1 was attenuated to a more physiological level showed restored balance between GABA/glutamate neuronal differentiation. Our results suggest a causal role for FOXG1 and its interacting genes in producing some of the neurobiological alterations found in cells of individuals with ASD. Establishing a direct relationship between the biological alterations found in patients' cells on the one hand, and FOXG1 and connected genes on the other, has concrete implications for finding target genes underlying the pathogenesis of idiopathic ADS with macrocephaly and new targets for therapeutic strategies.

Disclosures: J. Mariani: None. **G. Coppola:** None. **P. Zhang:** None. **J.R. Howe:** None. **F.M. Vaccarino:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.16/A48

Topic: A.04. Stem Cells

Support: Prechter Fund for Bipolar Research

Steven Schwartzberg Fund

Title: Interneuron alterations in ipsc models of bipolar disorder

Authors: M. G. MCINNIS[1], M. BAME[1], C. J. DELONG[2], A. WILLIAMS[1], Y.-C. TSAN[2], O. MABROUK[3], R. KENNEDY[3], *K. O'SHEA[2];
[1]Psychiatry, [2]Cell & Dev Biol, Univ. Michigan Sch. Med., ANN ARBOR, MI; [3]Chem., Univ. Michigan, ANN ARBOR, MI

Abstracts: Interneuron Alterations in iPSC Models of Bipolar Disorder Melvin G McInnis, Monica Bame, Cynthia J. DeLong, Aislinn Williams, Yao-chang Tsan, Omar Mabrouk, Robert Kennedy, K. Sue O'Shea Interneuron dysfunction has been suggested to underlie a number of neuropsychiatric conditions including bipolar disorder (BP), schizophrenia, and autism spectrum disorders. Alterations in the specification, tangential migration or function of cortical interneurons would be expected to disrupt the excitatory-inhibitory balance in the cortex, which may contribute to BP. We have derived iPSC from three individuals with BP and three healthy controls and differentiated them into telencephalic neurons. Microarray analysis of RNAs from undifferentiated and neuronally differentiated iPSC identified significantly increased transcripts that confer dorsal telencephalic fate in the control group, including: EMX2, GLI1,2,3, PAX6, SOX6, TBR2, TCF3,4, VGLUT, ZNF536. Neurons derived from BP iPSC expressed transcripts involved in the differentiation of ventral (MGE) brain regions, including: FOXP2, NKX2-1, and LHX8, which controls neuronal expression of SHH. Consistent with the expression of transcripts involved in interneuron cell fate, mass-spec analysis of the culture supernates indicated that GABA expression by BP neurons was elevated throughout the 7-week culture period and was significantly increased compared with controls following stimulation of an action potential ($p \leq 0.018$, Anova). To determine if the dorsaala/ventral phenotype could be altered, iPSC were exposed to ventralizing agents (purmorphamine) or dorsalizing agents (lithium). iPSC from both BP and controls were responsive to patterning factors; both groups increased expression of the dorsal marker EMX2 following lithium exposure, but only C iPSC respond to additional ventral cues by down-regulating EMX2. Although only 20% of the total number of cortical neurons, interneurons play a critical role in maintaining the normal balance in cortical activity by making local synapses on long-projecting excitatory pyramidal neurons. Alterations in neuronal fate allocation during early CNS differentiation, in their subsequent tangential cell migration or in the function of cortical interneurons could alter cortical lamination and cortical circuitry in BP.

Disclosures: **M.G. McInnis:** None. **M. Bame:** None. **C.J. Delong:** None. **A. Williams:** None. **Y. Tsan:** None. **O. Mabrouk:** None. **R. Kennedy:** None. **K. O'Shea:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.17/A49

Topic: A.04. Stem Cells

Support: NIH/NINDS HHSN271200800033C

Title: The NINDS Repository collection of patient-derived fibroblasts and induced pluripotent stem cells for neurodegenerative disease research

Authors: *C. A. PEREZ[1], S. HEIL[1], C. GRANDIZIO[2], S. GANDRE-BABBE[2], K. HODGES[3], M. SUTHERLAND[4], R. A. CORRIVEAU[4], C. TARN[1];
[1]NINDS Repository, [2]Stem Cell Biobank, [3]Cell Culture Lab., Coriell Inst., Camden, NJ;
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Abstracts: Induced pluripotent stem cells (iPSCs) reprogrammed from patient-derived primary fibroblasts have become an increasingly utilized and needed resource for the study of human disease and have proven especially valuable in studying neurodegenerative disorders for which disease models are difficult to establish. The National Institute of Neurological Disorders and Stroke (NINDS) Repository is a public resource established in 2002 aiming to provide a centralized and open collection of biological samples (DNA, lymphoblastoid cell lines, fibroblasts, iPSCs, biofluids such as plasma, serum, cerebrospinal fluids, and urine) and associated de-identified clinical data from a diverse population of patients and normal controls. Since 2011, the NINDS Repository has added to its web-based catalog (http://ccr.coriell.org/NINDS) close to 50 iPSC and 165 fibroblast lines. Most iPSC lines are contributed by Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) or Huntington's disease (HD) investigators from NINDS-sponsored Stem Cell Consortia. By making available to the research community neurodegenerative disease iPSCs and fibroblasts, the NINDS Repository continues to fulfill the NINDS mission of reducing the burden of neurological disease - a burden borne by every age group, by every segment of society, by people all over the world. To ensure the quality of these valuable resources, all iPSCs and fibroblasts submitted to the NINDS Repository by iPSC Consortia and other investigators undergo rigorous quality assessments (viability, pluripotency, karyotyping, differentiation status, gene expression analysis, sterility) prior to distribution by the NINDS repository. The results are summarized in a Certificate of Analysis and/or displayed on the web-based catalog along with the recommended culturing protocol. The NINDS Repository fibroblast and iPSC collections include mostly cell lines bearing specific genetic mutations associated with PD, ALS, HD, frontotemporal degeneration, or Alzheimer's disease, as well as samples derived from neurologically normal controls. For

certain affected individuals, the parental fibroblast, corresponding iPSC line, and whole blood DNA are available. The NINDS Repository serves as a unique and effective centralized resource where these iPSCs, fibroblasts and their de-identified phenotypic data, are available to basic and applied research investigators worldwide.

Disclosures: C.A. Perez: None. **S. Heil:** None. **C. Grandizio:** None. **S. Gandre-Babbe:** None. **K. Hodges:** None. **C. Tarn:** None. **M. Sutherland:** None. **R.A. Corriveau:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.18/A50

Topic: A.04. Stem Cells

Title: Extracellular vesicle-mediated spread of α-synuclein in the pathogenesis of multiple system atrophy

Authors: *J. FISCHER[1], R. GORRIS[1], A. LEINHAAS[1], K. DE MIROSCHEDJI[2], B. GIEBEL[2], M. KARUS[1], O. BRUESTLE[1];
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Abstracts: Intercellular communication and cargo delivery (e.g. proteins and nucleic acids) via small extracellular vesicles (EVs) under physiological and pathophysiological conditions has gained much attention throughout recent years. For the central nervous system it has been proposed that EV-mediated trafficking might contribute to the cellular spread of pathological agents, eventually culminating in severe neurodegeneration. Here, we address the question whether EV-mediated spread of α-synuclein (SNCA) might contribute to the pathogenesis of Multiple System Atrophy (MSA), a spontaneous neurodegenerative disorder characterized by neuronal and oligodendroglial cytoplasmic SNCA inclusions. To this end, we initially generated hiPSCs from MSA patients and further differentiated these cells into a radial glia-like neural stem cell population (RGL-NSCs). RGL-NSCs readily give rise to neurons, astrocytes, and, most importantly, oligodendrocytes. RT-PCR and immunocytochemical analyses revealed that SNCA is strongly expressed by neurons in our culture system. In contrast, oligodendrocytes (OLs) do not express the SNCA gene. However, they exhibit strong SNCA immunoreactivity in mixed neural cultures differentiated from RGL-NSCs, suggesting a yet unknown uptake-mechanism of SNCA by oligodendrocytes. Upon employing Western-blot analyses of neuronal cell culture

supernatants we detected SNCA and CD63, a tetraspanin typically used as EV marker protein. We next harvested EVs from neuronal cell culture supernatants. Biochemical and biophysical characterization demonstrated that SNCA was present in vesicles with diameters of 120-170 nm. These data imply that SNCA being expressed in neurons is secreted as an EV cargo protein and taken up by oligodendrocytes via EV trafficking. Interestingly, pathological aggregation of SNCA can be induced in both neurons and oligodendroytes via inhibition of mitochondrial or proteasomal function. We expect this iPSC-based culture model to provide novel insights into the pathomechanisms underlying MSA-associated SNCA spread and aggregation.

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Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.19/A51

Topic: A.04. Stem Cells

Support: MSCRFF-0120-00.

Title: Glucocerebrosidase deficiency results in lysosomal depletion and autophagy block in neuropathic Gaucher disease ipsc-neurons

Authors: *O. AWAD[1], C. SARKAR[2], M. LIPINSKI[2], D. MILLER[1], X. ZENG[3], L. M. PANICKER[1], J. A. SGAMBATO[1], R. A. FELDMAN[1];
[1]Dept. of Microbiology and Immunol., [2]Departments of Anesthesiology, Anat. and Neurobio., Univ. of Maryland, Baltimore, MD; [3]Buck Inst. for Age Res., Novato, CA

Abstracts: Gaucher's disease (GD) is the most common lysosomal storage disease (LSD). GD is caused by mutations in the GBA1 gene, which encodes the lysosomal enzyme glucocerebrosidase (GCase). Neuropathic form of GD is associated with neurodegeneration of the central nervous system with either rapid (type 2), or slow progression (type 3). To investigate the mechanisms by which GCase deficiency leads to neurodegeneration, we used iPSC-derived neurons from patients with types 1, 2 and 3 GD as a model system. We found that types 2 and 3 but not non- neuropathic type 1 GD neurons exhibited widespread depletion and defective clustering of LAMP1-labeled lysosomes. Neuropathic GD neurons also showed increase in the basal levels of both the autophagosome-associated protein, LC3 and the ubiquitin-binding

protein, p62/SQSTM1. Autophagy induction by treatment with the mTOR inhibitor, Rapamycin resulted in accumulation of GFP-LC3-labeled autophagosomes and p62/SQSTM1 protein in GD-neurons, indicative of an autophagy block. Prolonged Rapamycin treatment resulted in decreased survival of neuropathic but not non-neuropathic GD neurons, suggesting that the defective autophagic clearance can lead to neuronal death. To determine whether the reduction in lysosomal number in GD neurons is caused by decreased lysosomal biogenesis, we examined the expression of transcription factor EB (TFEB), the master regulator of autophagy and lysosomal genes. TFEB levels were significantly decreased in types 2 and 3 GD neurons suggesting altered lysosomal biogenesis. Treatment of neuropathic GD-neurons with recombinant GCase enzyme reverted lysosomal depletion and the autophagy block. These results suggest for the first time, that normal GCase activity is required for maintaining a functioning pool of intraneuronal lysosomes, and that this action is likely mediated through TFEB. Our results provide new insights into the mechanisms of GD- associated neurodegeneration and illustrate the value of GD-iPSC to study disease mechanisms and as a platform for drug discovery.

Disclosures: O. Awad: None. **C. Sarkar:** None. **M. Lipinski:** None. **D. Miller:** None. **X. Zeng:** None. **L.M. Panicker:** None. **J.A. Sgambato:** None. **R.A. Feldman:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.20/A52

Topic: A.04. Stem Cells

Title: Dendritic spines in cortical neurons derived from human pluripotent stem cells

Authors: L. GOUDER[1], H. GOUBRAN-BOTROS[1], A. POULET[2], A. BENCHOUA[2], S. PONS[3], U. MASKOS[3], M. J. SCHMEISSER[4], T. M. BOECKERS[4], T. BOURGERON[1], *I. CLOËZ-TAYARANI[1];
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Abstracts: Neurons derived from human pluripotent stem cells represent new cellular tools for investigation of human cortical physiology and psychiatric disorders. In cortical circuits, dendritic spines play a major role in the establishment of excitatory synapses. However, their quantitative analysis in reprogrammed neurons remains poorly documented. For this study,

fibroblasts from two control individuals were reprogrammed to iPSCs. These pluripotent cells were then derived in pyramidal glutamatergic neurons of superficial cortical layers (II-IV), according to published protocol (Boissart C et al, Transl. Psychiatry, 2013, 3, e294). Spinogenesis was analyzed in pyramidal glutamatergic cortical neurons by using a three-dimensional quantitative approach. We labelled neurons using a GFP lentiviral vector coupled to double immunofluorescence. Fluorescent signals were detected by confocal microscopy, and spine quantification was performed on deconvolved images after three dimensional reconstructions, using Huygens Pro and Imaris software. Under our experimental conditions, pyramidal glutamatergic cells accounted for 80 % of total cells. The remaining cells were mostly GABAergic and sparse astrocytes. Nestin and beta III tubulin labelling were used to follow neuronal differentiation. Spinogenesis was followed on glutamatergic neurons over a period of 70 days post differentiation. Our preliminary results show the presence of detectable spines with variable morphologies. Only filopodia and thin spines could be detected at early stages of differentiation of neural stem cells (< 20 days). At 40 days post differentiation, spine shapes were diverse with a high proportion of cup-shaped / branched spines. Stubby spines were predominantly represented at later stages (60-70 days post differentiation). Our ongoing studies include the analysis of pre- and postsynaptic compartments, with a specific focus on Shank proteins. Indeed, Shank proteins are known to promote spinogenesis. In addition, morphological spine defects have been observed in neurons transfected with SHANK mutations identified in patients with autism spectrum disorders (ASD). Synaptic and spine morphologies will be analyzed in reprogrammed cortical neurons from patients with ASD carrying SHANK3 mutations.

Disclosures: **L. Gouder:** None. **I. Cloëz-Tayarani:** None. **H. Goubran-Botros:** None. **T. Bourgeron:** None. **A. Poulet:** None. **A. Benchoua:** None. **S. Pons:** None. **U. Maskos:** None. **M.J. Schmeisser:** None. **T.M. Boeckers:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Topic: A.04. Stem Cells

Support: The New York Stem Cell Foundation

Cure Alzheimer's Fund

NIH Grant R21AG042965

NIH Grant 1U01AG046170-01

Title: Psen-1 mutant and isogenic control familial Alzheimer's disease ipsc models

Authors: *A. SPROUL[1], S. JACOB[1], D. PAQUET[2], M. ORTIZ-VIRUMBRALES[3], D. PAULL[1], B. A. CAMPOS[1], M. E. EHRLICH[3], S. GANDY[3], M. TESSIER-LAVIGNE[2], S. A. NOGGLE[1];
[1]The New York Stem Cell Fndn. Res. Institu, New York, NY; [2]Lab. for Brain Develop. and Repair, Rockefeller Univ., New York, NY; [3]Neurol. and Psychiatry and the Alzheimer's Dis. Res. Ctr., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstracts: The lack of effective treatments for Alzheimer's disease (AD) underscores the importance of developing better human-specific models of AD to further understand disease mechanism and provide better platforms for therapeutic screening. Human induced pluripotent stem cells (iPSCs) and their differentiated progeny provide a powerful new approach to study the relationship of genotype to molecular function for disease-related mutations, where affected genes are expressed under endogenous transcriptional and posttranslational control in non-transformed human cell types affected by the disease in question. We recently published a study in which we created a familial Alzheimer's disease (FAD) iPSC model analyzing neural progenitors from *presenilin*-1 (PSEN1) mutant carriers and their unaffected relatives. PSEN1 is the most common gene mutated in FAD, and forms the catalytic core of the gamma-secretase complex that cleaves amyloid precursor protein among other targets. In order to reduce inherent variability between iPSCs derived from different subjects, we are developing an allelic series, in which PSEN1 mutations can be introduced into an unaffected control line via TALEN-mediated gene editing. We have successfully knocked in heterozygous and homozygous PSEN1 M146L mutations, as well as generated cell lines with homozygous PSEN1 knockout. We are now studying FAD phenotypes in the context of a more specific disease-relevant cell type, namely basal forebrain cholinergic neurons (BFCNs). BFCNs are vulnerable in AD, and are the target of cholinesterase inhibitors such as Donepezil (Aricept), which are one of the few classes of drugs that can ameliorate AD symptoms in some patients. We have developed a method of isolating an iPSC-derived BFCN neural progenitor by FACS, which can then be propagated as a neuronal embryoid body (NEB). NEBs can be subsequently dissociated into a monolayer for terminal differentiation and phenotypic analysis. We are currently optimizing our differentiation protocol and translating it to an automated platform. Automated BFCN differentiation will allow investigation of a large cohort of late-onset AD iPSCs, which is critical for understanding and further dissecting the far more common sporadic forms of the disease. Recent phenotypic analysis of FAD BFCNs will be presented at the meeting.

Disclosures: **A. Sproul:** None. **S. Jacob:** None. **D. Paull:** None. **D. Paquet:** None. **B.A. Campos:** None. **M. Ortiz-Virumbrales:** None. **M. Tessier-Lavigne:** None. **M.E. Ehrlich:** None. **S.A. Noggle:** None. **S. Gandy:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.22/A54

Topic: A.04. Stem Cells

Support: CIRM RFA 10-01 0316020

Title: Targeting aspartoacylase-deficiency in a mouse model of canavan's disease using human induced pluripotent stem cell-derived neural cells

Authors: ***V. C. KAPS**[1], M. MIZHOROVA[1], J. FISCHER[1], M. REISENHOFER[2], M. BLOSCHIES[1], J. SASS[3], T. QUANDEL[1], M. KARUS[1], O. BRUESTLE[1];
[1]Reconstructive Neurobio., Bonn, Germany; [2]Paul-Flechsig-Institute, Leipzig, Germany; [3]Clin. Chem. & Biochemistry, Univ. Children's Hosp. Zuerich, Zuerich, Switzerland

Abstracts: Canavan's Disease (CD) is an early onset, autosomal-recessive neurodegenerative disorder characterized by massive demyelination and spongiform appearance of the brain parenchyma. Such severe histopathological alterations are accompanied by elevated systemic levels of the neural metabolite N-acetylaspartate (NAA), which is usually hydrolysed by the oligododendroglial enzyme aspartoacylase (ASPA) to aspartate and acetate. Mutations in the ASPA gene have been identified as the main genetic course of CD. We reasoned that stem cell-mediated administration of functional ASPA might represent a beneficial strategy to tackle CD-associated alterations in a mouse model of CD. For that purpose, we initially reprogrammed fibroblasts from 3 CD patients with mutations in the ASPA gene into human induced pluripotent stem cells (hiPSCs) using Sendai viral vectors. The resulting CD-hiPSCs were fully validated for virus inactivation, pluripotency marker expression (Tra1-60, Tra1-81, and SSEA4) and their ability to differentiate into all three germ layers *in vitro* and *in vivo*. CD-hiPSCs were then differentiated into proliferative radial glia-like neural stem cells (RGL-NSCs) according to a recently established protocol. CD-RGL-NSCs expressed typical radial glia markers including BLBP, CD133, Nestin, SOX2, SOX9 and PAX6. Moreover, under defined conditions CD-RGL-NSCs could be successfully differentiated into βIII-tubulin-/MAP2-positive neurons, GFAP-/NFIA-positive astrocytes and OLIG2-/O4-/MBP-positive oligodendrocytes. Based on the initial

differentiation capacity two lead CD-RGL-NSC lines were next chosen for lentiviral overexpression of the wild-type human ASPA gene. The successful overexpression in the resulting ASPA-RGL-NSCs could be verified both on transcript and protein level. Overexpression did neither affect marker expression nor genomic integrity of the cells. A potential therapeutic value of the ASPA-RGL-NSCs in comparison to CD-RGL-NSCs will be assessed using transplantation into an immundeficient ASPA-mutant mouse strain. We expect these studies to provide important information about the applicability of stem cell-based enzyme replacement as a potential therapeutic strategy to treat CD.

Disclosures: V.C. Kaps: None. **M. Mizhorova:** None. **J. Fischer:** None. **M. Bloschies:** None. **M. Reisenhofer:** None. **M. Karus:** None. **T. Quandel:** None. **O. Bruestle:** None. **J. Sass:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Topic: A.04. Stem Cells

Support: NIA Grant R01AG042776

Harvard NeuroDiscovery Center

Title: Aβ promotes astrocyte activation in iPSC-derived neural cultures from familial Alzheimer's disease lines

Authors: *C. R. MURATORE[1,2], T. SHIN[1,2], D. G. CALLAHAN[1,2], C. ZHOU[1,2], T. L. YOUNG-PEARSE[1,2];
[1]Neurosci., Harvard Med. Sch., Boston, MA; [2]Neurol., Brigham and Women's Hosp., Boston, MA

Abstracts: Alzheimer's disease (AD) is a complex neurodegenerative disorder, characterized by extracellular plaques consisting primarily of amyloid beta (Aβ), and intracellular tangles consisting primarily of tau. Additionally, inflammation appears to be a strong component in the pathophysiology of AD. Many studies have revealed evidence of inflammation in the AD brain, including 1) an increase in cytokines and chemokines and 2) an increase in activated astrocytes and microglia. Activated astrocytes and microglia have been shown to surround Aβ plaques, and

clear and degrade Aβ, and it is likely that astrocytes and microglia play a central role in the disease process. While these glial cells aid in clearance of Aβ, gliosis may also stimulate and accelerate the progression of AD through the induction of neuronal damage following the release of cytokines, chemokines and ROS by activated astrocytes and microglia. Here, we aim to interrogate whether altered Aβ levels secreted from familial AD (fAD) neurons are sufficient to induce activation of astrocytes. We have previously shown that fAD iPSC-derived neuronal cells secrete an elevated Aβ42/40 ratio, as well as express increased levels of total and phosphorylated tau, compared to control iPSC-derived neurons. Here we show that astrocytes generated in fAD neural cultures express increased levels of GFAP by immunostaining, Western blot and NanoString technologies, without an increase in overall astrocyte numbers. Additionally, fAD neural cultures show significantly higher levels of secreted IL-6, indicating an activated immune response. Co-culture of fAD neurons, but not control neurons, with WT astrocytes induced astrocyte activation. Likewise, treatment of WT astrocytes with fAD neuronal conditioned media (CM), but not control CM, induced astrocyte activation. Astrocyte activation induced by fAD CM is attenuated through the inhibition of Aβ secretion from fAD neurons. Immunodepletion of fAD Aβ also prevented the increase in astrocyte activation, when compared to mock-immunodepleted media. These results indicate that the elevated Aβ42/40 ratio observed in the fAD lines may largely contribute to the gliosis phenotype observed *in vitro*. Interestingly, the gliosis phenotype is present in our neural cultures prior to any Aβ plaque or tangle formation (60-120 days of differentiation). Taken together, these results suggest that human iPSC-derived neural cultures provide a well-controlled and biochemically amenable system to model the role of gliosis in AD pathophysiology, and suggest that astrocyte activation and altered cytokine secretion may emerge early on in the disease process.

Disclosures: C.R. Muratore: None. **T. Shin:** None. **D.G. Callahan:** None. **C. Zhou:** None. **T.L. Young-Pearse:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Topic: A.04. Stem Cells

Support: NIH/NINDS NS064578

NIH/NINDS NS045926

The Bleser Family Foundation

The Busta Foundation

NICHD (P30 HD03352)

Title: Motor neurons from spinal muscular atrophy patients exhibit hyperexcitability

Authors: *H. LIU, J. LU, H. CHEN, Z. DU, S.-C. ZHANG;
Physiol., Waisman Ctr., MADISON, WI

Abstracts: Motor Neurons from Spinal Muscular Atrophy Patients Exhibit Hyperexcitability Huisheng Liu[1], Jianfeng Lu[1], Hong Chen[1], Zhongwei Du[1], and Su-Chun Zhang[1, 2] [1]Waisman center, University of Wisconsin, Madison, WI, 53705, USA [2]Department of Neuroscience and Department of Neurology, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53705, USA Spinal Muscular Atrophy (SMA) is caused by mutations in the survival motor neuron (*SMN)* gene, resulting in diminished production of the full length (FL) SMN protein. It is characterized by selective spinal motor neuron (MN) impairment, muscle weakness/atrophy, and eventual MN degeneration. However, animal models of SMA show severe muscle weakness, but with limited MN loss, at an early stage, suggesting potential functional alteration in MNs that contributes to SMA symptom presentation. Induced pluripotent stem cells (iPSCs) derived from SMA patients offer an alternative model to dissect the relationship between SMN and MN function. By comparing MNs that are derived from SMA and non-SMA iPSCs, we observed that SMA MNs were efficiently generated and survived for several weeks in a similar manner as non-SMA MNs. However, by electrophysiological recording, we found that SMA MNs exhibited increased membrane input resistance and action potential size, and hyperpolarized threshold, thus displaying hyperexcitability. Furthermore, we discovered that enhanced sodium channel activity (increased current size and faster recovery) in SMA MNs is associated with the hyperexcitability. Interestingly, non-MNs differentiated from SMA iPSCs did not exhibit hyperexicitability. We propose MN hyperexcitability as a compensatory mechanism for reduced neurotransmission, which exacerbates neuromuscular transmission via a negative feedback loop, thus contributing to severe symptoms in early stage SMA.

Disclosures: H. Liu: None. **J. Lu:** None. **H. Chen:** None. **Z. Du:** None. **S. Zhang:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.25/A57

Topic: A.04. Stem Cells

Support: Swedish State Support for Clinical Research (ALFGBG-136991)

Swedish Research Council (K2013-62X-12600-16-4)

Title: Electrophysiological properties and secretion of Abeta, sAPP and Tau during development of cortical neurons differentiated from human iPS cells of chondrocytic origin

Authors: *T. OLSSON BONTELL[1,2], J. STRANDBERG[2], L. AGHOLME[3], F. NAZIR[3], E. HANSE[2], K. BLENNOW[3], H. ZETTERBERG[3], P. BERGSTRÖM[3];
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Abstracts: The method of differentiating human iPS cells toward different kinds of tissue is an attractive model to use in the study of pathological processes and pharmacological substances on human tissue. In the present study we have differentiated human iPS cells of chondrocytic origin towards cortical neurons and characterized them using electrophysiology, immunocytochemistry and biochemistry. We used patch-clamp recordings to investigate action potential firing and spontaneous synaptic activity at different holding potentials. Culture media was collected during neuronal development, up until 90 days of differentiation. Secreted levels of sAPPα, sAPPβ and Aβ 38, 40 and 42 within the media were measured using Mesoscale. The levels of total tau and P-tau were measured using ELISA. The maximum rate of rise of the action potentials more than doubles between day 60 and 90, indicating clear maturation of the neurons. AMPA and NMDA synaptic activity was detected by application of specific antagonists. At 0 mV we also detected spontaneous synaptic activity that was blocked by picrotoxin, indicating the presence of GABA synaptic activity. Immunocytochemical staining indicated the presence of astrocytes, as well as excitatory cortical neurons. Secreted Aβ 40 and 42, sAPPα and both total tau and P-tau in the media increased markedly at day 60 of differentiation, while the secretion of Aβ 38 started later, at day 70. Between days 75 to 85 we observed an increase in sAPPβ. These results show that human iPS cells of chondrocytic origin differentiated toward cortical neurons as demonstrated by the presence of action potentials, excitatory and inhibitory synaptic inputs. The maturation of electrophysiological properties was paralleled by a distinct pattern of secreted Aβ-species, sAPPα/β, total tau and P-tau.

Disclosures: **T. Olsson Bontell:** None. **J. Strandberg:** None. **L. Agholme:** None. **E. Hanse:** None. **K. Blennow:** None. **H. Zetterberg:** None. **F. Nazir:** None. **P. Bergström:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.26/A58

Topic: A.04. Stem Cells

Title: Lineage priming in pluripotent human stem cells

Authors: S.-K. KIM, A. JAISHANKAR, Y. WANG, G. STEIN O'BRIEN, S. SEO, C. COLANTUONI, J. SHIN, J. G. CHENOWETH, *D. HOEPPNER, R. D. MCKAY;
Lieber Inst. For Brain Develop., Baltimore, MD

Abstracts: Significant investments are being made worldwide in basic and translational research on stem cell biology in an effort to reveal the next generation of molecular targets for neurologic and psychiatric disorders. These *in vitro* models have yielded novel insight into signaling pathways that restore CNS function *in vivo* and are strongly informing novel regenerative therapeutic strategies. The powerful new idea revealed by these studies is that the molecular pathways that regulate stem cell function in development also play key roles throughout life in both homeostasis and disease. Factors associated with risk for neurodevelopmental disorders such as AKT1, ERBB family members, and NRG1 are critical regulators of stem cell survival, growth, and proliferation. Here we show lineage bias and reversible differences in susceptibility to differentiation exist in distinct spatial regions of human pluripotent stem cell colonies. The expression of core pluripotency regulators OCT4, NANOG and SOX2 are regulated in a spatially constrained way. OTX2 and SOX21, transcription factors essential for brain development, are dynamically regulated in pluripotent cells playing a crucial role in the early specification of neurectoderm. Neuregulin 1 -ERBB2/3 signaling suppresses neurectoderm specification through AKT activation. This work shows that NRG1-AKT signaling regulates the balance between spatial domains biased towards mesendodermal or neurectodermal fates under self-renewing conditions. Genome specific regulation of these early mechanisms opens the way to a systematic definition of the developmental landscape of genetic risk for brain disorders.

Disclosures: S. Kim: None. **A. Jaishankar:** None. **Y. Wang:** None. **G. Stein O'Brien:** None. **S. Seo:** None. **C. Colantuoni:** None. **J. Shin:** None. **J.G. Chenoweth:** None. **D. Hoeppner:** None. **R.D. McKay:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.27/A59

Topic: A.04. Stem Cells

Support: The Wellcome Trust

Medical Research Council

Title: The *in vitro* development of oligodendrocytes from oligodendrocyte precursor cells derived from human pluripotent stem cells

Authors: *M. R. LIVESEY[1], D. MAGNANI[2], B. T. SELVARAJ[2], K. BURR[2], N. A. VASISTHA[2], D. STORY[2], G. E. HARDINGHAM[1], S. CHANDRAN[2], D. J. A. WYLLIE[1]; [1]Ctr. for Integrative Physiol., [2]Ctr. for Clin. Brain Sci., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstracts: Generation of regionally defined cellular populations from human pluripotent stem cells (hPSCs) provides an important experimental resource for the study (and potentially treatment) of neurodevelopmental and neurodegenerative disorders. We developed a protocol that generates highly enriched PDGFRα-positive oligodendrocyte (OL) precursor cells (OPCs), derived from multiple hPSC lines, that differentiate into O4, GalC and myelin basic protein expressing OLs. Co-expression of PDGFRα and O4 was evident in <10 % of OPCs whilst the majority of O4-positive OLs also expressed the mature OL-marker myelin basic protein (>85%). We observed that OL differentiation coincided with an increase in cell size (OPCs: 8.4 ± 0.1 pF, n = 22; OLs: 25.8 ± 0.3 pF, n = 36) and OL development was associated with an increase in the number and length of OL processes, as quantified by Sholl analysis. Using pharmacological and biophysical approaches we have examined the profile of passive membrane properties of hPSC-derived OPCs through to their maturation into OLs. Depolarization of OPCs induced large, sustained outwardly rectifying currents. Tetrodotoxin-sensitive spiking activity was also observed in OPCs. OL differentiation was associated with a loss of spiking activity and a reduced rectification of outward currents that indicated a change in the ion channel expression profile of OLs compared to those expressed in OPCs. Specifically, the decreases in ion channel current densities as cells differentiated from OPCs to OLs were: tetrodotoxin-senstive voltage-gated Na^+-channels (OPCs: 29.1 ± 1.1 pA/pF, n = 18; OLs: 0 pA/pF, n = 5), TEA-sensitive delayed-outwardly rectifying K^+-channels (OPCs: 26.4 ± 0.4 pA/pF, n = 15; OLs: 0.5 ± 0.1 pA/pF, n = 9) and transient A-type K^+-channels (OPCs: 105.6 ± 4.5 pA/pF, n = 12; OLs: 8.1 ± 0.8 pA/pF, n = 6). Conversely, immunohistochemistry revealed prominent expression of inwardly-rectifying K^+-channel 4.1 subunits in OLs, whereas expression of this channel subunit was only negligibly detected in OPCs. Our data are in good agreement with ion channel

expression profiles exhibited by native (rodent) OL-lineage cells and indicate many aspects of rodent OL-lineage cell development are recapitulated in OPCs and OLs derived *in vitro* from hPSCs. This data provides a platform for the assessment of whether the maturation profile of channel expression and density are similar in OLs and OPCs derived from hPSC lines obtained from patients suffering from neurological diseases.

Disclosures: **M.R. Livesey:** None. **D. Magnani:** None. **B.T. Selvaraj:** None. **K. Burr:** None. **N.A. Vasistha:** None. **D. Story:** None. **G.E. Hardingham:** None. **S. Chandran:** None. **D.J.A. Wyllie:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.28/A60

Topic: A.04. Stem Cells

Title: Efficient generation of NKX2.2 and OLIG2 positive progenitor cells from human pluripotent stem cells in defined serum free culture

Authors: ***Y. CHEN**, N. ASBROCK, V. CHU;
EMD Millipore, Temecula, CA

Abstracts: During embryonic development, NKX2.2 and OLIG2 positive progenitors reside in the ventral neural tube and later give rise to oligodendrocyte in the spinal cord. To identify conditions that would override intrinsic cell line lineage propensities and specify cells toward an oligodendrocyte lineage, extrinsic factors such as sonic hedgehog, retinoic acid, PDGF and thyroid hormone were tested using a modified neural induction platform called NIM. At day 4, when the neuroectodermal lineage would be specified, a potent sonic hedgehog agonist was introduced to initiate ventral patterning. A pulse treatment of retinoic acid was added at a specified timepoint and found to upregulate NKX2.2 and OLIG2 expression. Highly pure populations of NKX2.2 and OLIG2 positive progenitors were efficiently generated in as little as 8 days from 3 separate lines of human pluripotent stem cells, two of which possessed known lineage propensity. H9 and H7 human ES cell lines are widely known to have neuroectodermal and mesodermal potential, respectively. A third human iPS cell line was also evaluated. Expandable NKX2.2 and OLIG2 positive progenitors are useful tools to screen for small molecules that can overcome intrinsic cell line differences and to specify a majority of the cells towards a mature oligodendrocyte lineage.

Disclosures: **N. Asbrock:** A. Employment/Salary (full or part-time):; EMD Millipore. **Y. Chen:** A. Employment/Salary (full or part-time):; EMD Millipore. **V. Chu:** A. Employment/Salary (full or part-time):; EMD Millipore.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.29/A61

Topic: A.04. Stem Cells

Support: NIH grant EB00790

NIH grant S10RR029050

CIRM grant TR2-01814

NIH grant 1-DP2-OD006495-01

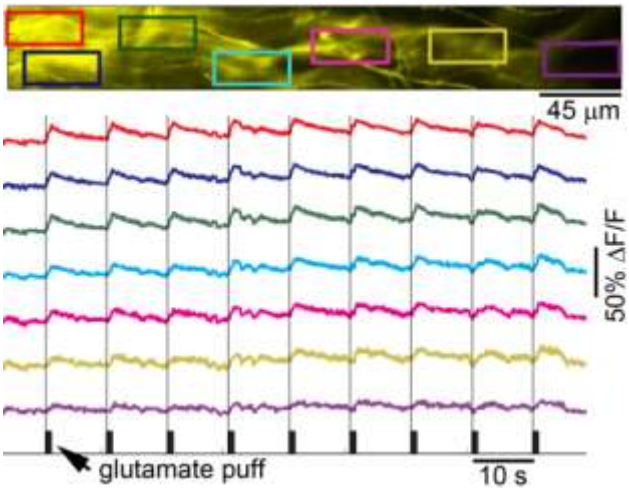
International Headache Society Postdoctoral Fellowship

Title: Functional imaging of human iPSCs-derived neurons integrated in mouse cortex using 2-photon microscopy *in vivo*

Authors: C. A. TRUJILLO[1], H. UHLIROVA[2], K. KILIC[3], P. A. SAISAN[3], Q. CHENG[3], K. L. WELDY[3], A. M. DALE[4], *A. R. MUOTRI[1], A. DEVOR[4,5];
[1]Pediatrics/Cellular Mol. Med., [2]Radiology, [3]Neurosciences, [4]Neurosciences and Radiology, UCSD, La Jolla, CA; [5]Martinos Ctr. for Biomed. Imaging, MGH/Harvard Med. Sch., Charlestown, MA

Abstracts: Recent advances in stem cell technology have enabled generation of neuronal cell lines from induced pluripotent stem cells (iPSCs) derived from human peripheral tissues. This opens unprecedented opportunities for investigation of human brain disease linked to known genetic variations. However, the lack of the natural brain microenvironment in a dish can influence the phenotype and maturation. One potential strategy to overcome these limitations is transplantation of iPSC-derived neuronal precursor cells (NPCs) into the mouse brain. Previous studies have proved the feasibility of this “chimeric” approach and demonstrated that the transplanted neurons form synaptic connections using electrophysiological recordings in brain slices (1, 2). Ideally, however, activity of human neurons should be measured in the intact mouse

brain. In the present study, we addressed this challenge by using *in vivo* 2-photon calcium imaging. Human iPSC-derived NPCs were injected into the brains of newborn mice. In adult mice, the cell bodies of human neurons were typically found in clusters along the injection track. Calcium indicator OGB1 was loaded using the standard procedures at the location of a cluster and was uptaken by both mouse and human neurons. Human dendrites, however, extended outside the volume of loaded mouse tissue allowing selective measurements of their activity. Stimulation was delivered using a puff of glutamate through a glass micropipette inserted into the tissue within ~100 microns from the imaging field of view (FOV). Glutamate produced clear increases in OGB1 fluorescence in the human dendrites. Figure 1 illustrates the response to 9 consecutive stimulus trials; the regions of interest are color-coded on the FOV image. Thus, human iPSC-derived neurons *in vivo* have functional glutamate receptors and calcium channels. The chimeric approach may become a translational tool for studying disease mechanisms and evaluating candidate drugs on adult human neurons. 1.Muotri AR, et al. (2005) PNAS 102(51):18644-18648. 2.Espuny-Camacho I, et al. (2013) Neuron 77(3):440-456.



Disclosures: **C.A. Trujillo:** None. **H. Uhlirova:** None. **A.R. Muotri:** None. **K. Kilic:** None. **P.A. Saisan:** None. **Q. Cheng:** None. **K.L. Weldy:** None. **A.M. Dale:** None. **A. Devor:** None.

Poster

590. Disease Modeling Using Pluripotent Stem Cells II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 590.30/A62

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant 1K02AG044473

NIH Grant 1R01NS072395

Title: Model of non-cell autonomous effects of presenilin-2 mutations on neurons using human induced pluripotent stem cells

Authors: *P. AMOS[1], B. L. SOPHER[1], A. CASE[1], J. C. GILLESPIE[1], J. M. SULLIVAN[2], H. NEUMANN[3], G. A. GARDEN[1], S. JAYADEV[1];
[1]Neurol., [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [3]Inst. of Reconstructive Neurobio., Univ. Bonn, Bonn, Germany

Abstracts: Mutations in the gene encoding presenilin 2 (PSEN2), expressed by neurons and microglia and involved in the cleavage of amyloid precursor protein, cause familial Alzheimer's disease (fAD). The clinical and pathological parallels between fAD and sporadic AD suggest that identification of pathogenic mechanisms in PSEN2 fAD may provide mechanistic clues relevant to the development of effective therapeutics in AD. *In vivo* experiments using transgenic animals are limited with respect to imaging live interactions between microglia and neurons, and cells isolated from transgenic animals retain caveats with regard to species-specific genetic regulation. Therefore, the aim of this work was to establish an *in vitro* model of neuron-microglia interaction using cells derived from human induced pluripotent stem cells (iPSCs) for the purpose of evaluating neuron function and survival in the context of two AD associated PSEN2 mutations. iPSCs harboring either a single N141I mutation or a recently reported 2 base pair deletion in the PSEN2 gene were differentiated toward neurons via embryoid body formation followed by dual smad inhibition and treatment with brain-derived neurotrophic factor and neurotrophin-3. The iPSCs were also differentiated toward microglia via embryoid body formation, dual smad inhibition, and treatment with growth factors. Cell phenotypes were validated by immunocytochemistry, flow cytometry, and FM4-64 synaptic vesicle dye incorporation, and mutant PSEN2 genes were confirmed with PCR and sequencing. In addition, mutant iPSC lines were “reverted” to wild type genotypes using clustered regularly interspaced short palindromic repeat/Cas9 nuclease genome editing (CRISPR/Cas9) and were differentiated toward microglial and neuronal phenotypes. Mutant and “reverted” iPSC-microglia and -neurons were co-cultured with one another or with microglia and neurons derived from wild type human iPSCs, and neurons were assessed for viability and function via caspase 3 staining, gamma-secretase activity, and FM4-64 dye incorporation. Assessment of spontaneous miniature excitatory postsynaptic currents was performed over 10 second intervals through whole-cell voltage clamping of neurons at a constant voltage of -70mV. This model offers a useful platform for future investigations into the mechanisms of PS2-mediated neurodegeneration and for pharmaceutical investigations into the amelioration of dysfunctional PS2 production by microglia.

Disclosures: **P. Amos:** None. **B.L. Sopher:** None. **A. Case:** None. **J.C. Gillespie:** None. **J.M. Sullivan:** None. **H. Neumann:** None. **G.A. Garden:** None. **S. Jayadev:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.01/A63

Topic: A.05. Axon and Dendrite Development

Support: Shriners Grant 490304

NIH Grant NS060784

Shriners Grant 84050

Shriners Grant 85200

Title: Promotion of axonal regeneration by manipulating microtubule posttranslational modification

Authors: ***S. LIN**[1], Y.-P. LIU[1], X. TANG[1], P. W. BAAS[3], G. M. SMITH[2];
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Abstracts: Axonal regeneration in the CNS remains an intractable problem. One way to augment regeneration could be through reorganization of the cytoskeleton to rejuvenate damaged axons and promote growth cone formation. Microtubule dynamics are regulated by posttranslational modifications and play a key role in maintaining axonal integrity and growth. Microtubule acetylation, which is found at higher levels on stable microtubules along the axon shaft is a reversible process controlled by two enzyme families, the Histone Deacetylases (HDACs) and the Histone Acetyl Transferases (HATs). Recent studies show that HDAC5 and HDAC6, upregulated in response to injury, may play a role in axonal regeneration. Studies also show that overexpression of alpha tubulin acetyl transferase (aTAT1), which also regulates microtubule dynamics independently of catalytic activity, can increase stability. We hypothesize that modulating HDAC5, and aTAT1 expression can alter microtubule stability, increasing the rate of axonal growth. In this study, a lentivirus was generated to express GFP-HDAC5 (Lenti-

HDAC5) and a novel lentivirus construct containing GFP-aTAT1 (Lenti-aTAT1) was cloned. Microfluidic chambers have also been fabricated to investigate the effects of overexpressing HDAC5 and aTAT1 on axon growth *in vitro*. Adult rat DRG neurons are plated onto laminin and into the cell body compartments of microfluidic chambers where they can extend axons uniformly along parallel microchannels towards distal compartments. To investigate both axonal outgrowth and behavior of regenerating axons encountering an inhibitory border, chondroitin sulfate proteoglycans (CSPG) stripes are placed perpendicular to the microgrooves in between the cell body and distal compartments. Using this model, we are testing the effects of Lenti-HDAC5 and Lenti-aTAT1 expression on axonal outgrowth and on the CSPG crossing assay. Furthermore, we plan to injected Lenti-HDAC5 and Lenti-aTAT1 into adult rat lumbar (L4/L5) dorsal root ganglia after dorsal root rhizotomy and we will assess the ability of axons to re-grow beyond the DREZ. By using lentiviruses to deliver both HDAC5 and aTAT1 to damaged neurons, we will better understand the effects of microtubule acetylation and microtubule stability on axonal regeneration in adult neurons after injury.

Disclosures: S. Lin: None. **Y. Liu:** None. **X. Tang:** None. **P.W. Baas:** None. **G.M. Smith:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.02/A64

Topic: A.05. Axon and Dendrite Development

Support: NORTE-07-0124-FEDER-000001 - Neurodegenerative disorders

FCT - PTDC/SAU-ORG/118863/2010

Title: Cytoskeleton remodeling as a target for TTR-induced neurodegeneration

Authors: J. EIRA[1], M. SOUSA[2], *M. LIZ[1];
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Abstracts: Mutations in transthyretin (TTR) are associated with familial amyloid polyneuropathy (FAP), a neurodegenerative disorder characterized by the deposition of insoluble TTR aggregates, particularly in the peripheral nervous system. However the molecular mechanism that underlies TTR toxicity in peripheral nerves is unclear. Abnormalities in

cytoskeletal organization are a common feature of many neurodegenerative disorders. Amyloidogenic proteins such as beta-amyloid peptide (Aβ) have been shown to mediate neurotoxicity by inducing major alterations in both microtubules and actin filaments. In this work we aimed to examine the effect of TTR on the cytoskeleton organization of dorsal root ganglion neurons (DRG). We observed that TTR oligomers promoted a complete reorganization of both actin and microtubules in the growth cones of DRG neurons, whereas soluble TTR has no significant effect. TTR oligomers led to a marked reduction in the growth cone area with the phalloidin staining showing a complete depletion of the typical filopodial and lamelipodial actin structures of neuronal growth cones. Regarding microtubule organization, in control neurons we observed the usual organization of neurons growing in permissive substrates with tightly bundled axonal microtubules entering the growth cone in half of the growth cones, whereas in the other half, microtubules splayed out as they entered the growth cone. In contrast, in neurons treated with TTR oligomers while growth cones with splayed microtubules were still observed, the remaining half of the growth cones were dystrophic with microtubules confined to the central zone with little extension into the periphery. Based on these observations, our study proposes growth cone cytoskeletal components as a target for TTR-induced neurodegeneration in FAP.

Disclosures: J. Eira: None. **M. Sousa:** None. **M. Liz:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.03/A65

Topic: A.05. Axon and Dendrite Development

Title: Live STED nanoscopy of the actin organization in the axon initial segment

Authors: E. D'ESTE, *D. KAMIN, A. EL HADY, S. W. HELL;
NanoBiophotonics, Max Planck Inst. for Biophysical Chem., Göttingen, Germany

Abstracts: The axon initial segment (AIS) is thought to be the site of action potential initiation and the border between the somatodendritic compartment and the axon. Recent studies performed mainly with electron microscopy reveal a peculiar organization of the cytoskeleton in the AIS but led to results that are discordant from the ones obtained with optical imaging techniques. The main reason is the technical difficulty in preparing samples in which the actin structures are preserved. Here we use SiR-Actin, a new actin labelling probe, in combination with two-color Stimulated Emission Depletion (STED) optical nanoscopy. SiR-Actin is

membrane permeable and fluorogenic, meaning that it fluoresces only when it is bound to its target, providing an excellent signal-to-noise ratio. These features make it highly suitable for live-cell fluorescence nanoscopy. Thus, possible artifacts due to sample preparation or transfection become of lesser concern. Our study of the AIS in living cultured hippocampal neurons using SiR-Actin reveals the presence of an actin lattice with ~180 nm of spacing in both the AIS and the distal axon, in agreement with a previous study using STORM on fixed samples. We demonstrate that this peculiar structure arranges already after two days *in vitro* (DIV), before the AIS is formed. After five DIV, virtually all axons exhibit actin rings but lack any indication for the presence of an actin filter barrier. These results have been confirmed by conventional phalloidin staining, indicating that no significant alteration of the actin structure is caused by SiR-Actin. In agreement with what has been reported by electron microscopy studies, live STED imaging shows the presence of some actin patches along the axon. Immunolabelling with the synaptic markers Bassoon and Synaptotagmin-1 reveals that these actin patches represent synaptic sites in mature cultures. In conclusion, STED nanoscopy reveals the presence of an actin lattice in the axons of living hippocampal neurons, which spans all over the length of the axons. We were not able to prove the existence of an actin barrier in the AIS. Furthermore, we speculate that there may be interplays between actin patches that can be observed along the axon and synaptic sites.

Disclosures: **E. D'Este:** None. **D. Kamin:** None. **A. El Hady:** None. **S.W. Hell:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.04/A66

Topic: A.05. Axon and Dendrite Development

Title: APC and alpha-catenin binding regions for beta-catenin differentially and cooperatively modulate growth cones, arborization and targeting of optic axons *in vivo*

Authors: *T. M. ELUL[1], E. WU[2], G. PENG[2];
[1]Touro Univ., Vallejo, CA; [2]Touro Univ. California, Vallejo, CA

Abstracts: Formation of neuronal circuits involves axons navigating specific paths to their targets in the brain, where they then elaborate terminal arbors that form synaptic connections. β-catenin is a target of Wnt and Cadherin ligands that regulates axon pathfinding and branching, but the mechanisms of its' associated factors are not well studied. Here, we examined how

domains in APC and α-catenin that bind β-catenin differentially sculpt optic axons, their growth cones and their arbors in tectal midbrains of intact, living Xenopus tadpoles. Overexpression of the APCβ-cat binding domain both dispersed and disorganized optic axons in the optic tract, whereas the α-catNTER domain that binds β-catenin dispersed optic axons in the optic tract but did not perturb their organization. Optic axons expressing APCβ-cat also formed narrow growth cones with no or a single long filopodia while many α-catNTER expressing growth cones were wider with no filopodia. At a later stage, APCβ-cat axons developed arbors with very short branches that targeted to the appropriate region in optic tecta of intact tadpoles. In contrast, axons that expressed α-catNTER developed arbors that had fewer branches and were frequently mistargeted within the tectum. These data suggest that the APC and α-catenin binding domains for β-catenin differentially and cooperatively regulate growth cones, arbors and target locations of optic axons *in vivo*.

Disclosures: **T.M. Elul:** A. Employment/Salary (full or part-time):; Touro University California. **E. Wu:** None. **G. Peng:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.05/A67

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01NS041963

Title: Actin and myosin-dependent localization of mrna to dendrites

Authors: ***V. BALASANYAN**, D. ARNOLD;
USC, Los Angeles, CA

Abstracts: The localization of mRNAs within axons and dendrites allows neurons to manipulate protein levels in a time and location dependent manner and is essential for processes such as synaptic plasticity and axon guidance. However, an essential step in the process of mRNA localization, the decision to traffic to dendrites and/or axons, remains poorly understood. Here we show that Myosin Va and actin filaments are necessary for the dendritic localization of the mRNA binding protein Staufen 1 and of mRNA encoding the microtubule binding protein Map2. Blocking the function or expression of Myosin Va or depolymerizing actin filaments leads to localization of Staufen 1 and of Map2 mRNA in both axons and dendrites. Furthermore,

interaction with Myosin Va plays an instructive role in the dendritic localization of Hermes 1, an RNA binding protein. Wild-type Hermes 1 localizes to both axons and dendrites, whereas Hermes 1 fused with a Myosin Va binding peptide, localizes specifically to dendrites. Thus, our results suggest that targeting of mRNAs to the dendrites is mediated by a mechanism that is dependent on actin and Myosin Va.

Disclosures: V. Balasanyan: None. **D. Arnold:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.06/A68

Topic: A.05. Axon and Dendrite Development

Support: NNSFC 31330046

Title: MEC-17 regulates α-tubulin acetylation and modulates axon growth in hippocampal neurons

Authors: *L. BAO, D. WEI, N. GAO;
Inst. of Biochem. and Cell Biology, Chinese Acad. of Sci., Shanghai, China

Abstracts: Objective Neuronal morphogenesis is a fundamental process during the brain development. This process is regulated by cytoskeleton components. Microtubule dynamics can be modulated by posttranslational modifications to tubulin subunits. Acetylation of α-tubulin at lysine 40 is important in regulating microtubule properties, and it is controlled by acetyltransferase and deacetylase. MEC-17 is an α-tubulin acetyltransferase that has been found to play major roles in the acetylation of α-tubulin in different species *in vivo*. However, the physiological function and mechanism of MEC-17 during neuronal morphogenesis are largely unknown. The present study is to explore the roles of MEC-17 in neuronal morphogenesis. **Methods** The MEC-17 truncation and mutation plasmids were constructed. The level and distribution patterns of α-tubulin acetylation were detected by immunoblotting and immunohisto/cytochemistry. The MEC-17 expression was regulated by knockdown using shRNA electroporation and knockout. The culture of hippocampal neurons from rats and mice has been used for studying the effect of MEC-17 on neuronal morphogenesis. **Results** (1) MEC-17 acetylated α-tubulin via its N-terminus sequence and displayed strong interaction with α-tubulin through its C-terminus sequence. (2) MEC-17 knockdown promoted axon elongation and

axonal branching of cultured rat hippocampal neurons. Hippocampal neurons cultured from MEC-17 knockout mice displayed longer axons and more axonal branches compared with those from wildtype mice. (3) Both α-tubulin acetylation and strong tubulin interaction of MEC-17 is necessary for regulation of neuronal morphogenesis. (4) *In situ* upregulation of α-tubulin acetylation reduced axon elongation and axonal branching of cultured rat hippocampal neurons. **Conclusion** MEC-17 and α-tubulin acetylation are important for neuronal morphogenesis.

Disclosures: L. Bao: None. **D. Wei:** None. **N. Gao:** None.

Poster

WITHDRAWN

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.07/B1

Topic: A.05. Axon and Dendrite Development

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.08/B2

Topic: A.05. Axon and Dendrite Development

Title: Pftaire kinase (eip63e/cdk14) regulates axogenesis via rhoa activation in *Drosophila* melanogaster and mice during embryonic cns development

Authors: *Y. RODRIGUEZ GONZALEZ, F. KAMKAR, P. JAFARNEJAD, S. WANG, L. SANCHEZ ALVAREZ, P. ALBERT, R. S. SLACK, M. SONNENFELD, D. S. PARK; Neurosci., Univ. of Ottawa, Ottawa, ON, Canada

Abstracts: Characterizing the processes that mediate the development of the Central Nervous System (CNS) is fundamental to our ability to develop successful therapies that modifies them. PFTAIRE kinase 1 and 2 (Pftk1/ Pftk2) are members of the family of Cyclin Dependent Kinases (Cdks), a group of kinases with diverse functions important to the CNS beyond cell cycle regulation. We hypothesized that, analogous to Cdk5 - the best characterized of neuronal Cdks - Pftks play a role CNS development. Importantly, Pftk1 is highly expressed in neurons in Cdk5-like patterns; Pftk1 and Cdk5 share the highest amino acid sequence similarity within the cdk family (~50-52%) and the *Drosophila* melanogaster Pftk (Eip63E) is required for development, since Eip63E deficient flies die at early larval stages. Our unpublished data shows that Eip63E is required in neurons to regulate axogenesis, since Eip63E deficiency or neuronal downregulation (using RNAi) leads to premature axon outgrowth, axon misguidance and defasciculation *in vivo*. We also show that Eip63E and Rho1 functionally interact in fly-embryos, since neuronal co-expression of Eip63E-RNAi and dominant-negative (DN) or constitutively active (CA) Rho1 leads to modification of the axon defects. Equally, using primary murine cortical neurons, we found that overexpression of DN-Pftk1 leads to faster-growing processes when compared to controls. This was recapitulated by the discovery that cultured E13 Pftk1-deficient cortical neurons elongate their axons significantly more rapidly than their wildtype littermates. We have also determined that this correlates with a lack of detectable RhoA activity in Pftk1-deficient neurons, in spite of an upregulation of RhoA protein levels when compared to wildtype neurons. Additionally, we show that Pftk1 directly phosphorylates RhoA *in vitro*. Our work represents the first steps in the characterization of Pftks neuronal functions and points to a novel mechanism of RhoA activation to regulate axogenesis.

Disclosures: Y. Rodriguez Gonzalez: None. **F. Kamkar:** None. **P. JafarNejad:** None. **S. Wang:** None. **L. Sanchez Alvarez:** None. **R.S. Slack:** None. **M. Sonnenfeld:** None. **P. Albert:** None. **D.S. Park:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.09/B3

Topic: A.05. Axon and Dendrite Development

Support: TWU

Title: Formins in neuronal cells

Authors: *A. HLEIHEL, D. HYNDS;
TWU, Denton, TX

Abstracts: Formins are actin-nucleating proteins that assist in the formation of unbranched actin structures like those seen in filopodia. Filopodia are the finger-like shaped structures observed in several neuronal areas including the growth cones that are formed at the tips of the neurites during neuronal development and regeneration. Dynamic rearrangement of the actin cytoskeleton through polymerization and depolymerization enables filopodia to explore their environment, leading to extending, retracting, and steering behaviors. Formins, including isoforms of the diaphanous subfamily (Dia1, Dia2, and Dia3), are major actin nucleators in filopodia. However, their distribution and interplay is not completely defined in several neuronal models, including B35 rat neuroblastoma cells. . Using Immunocytochemistry (ICC) and Co-Immunoprecipitation (C0-IP), we found that the diaphanous proteins are expressed in B35 neuroblastoma cells and they co-localize in growth cone filopodia. Their expression and co-localization suggest potential interaction between formin isotypes. Therefore, we are currently assessing their physical interactions and identifying their specific roles through gain-of-function and loss-of-function approaches. Collecting these data will improve our understanding the process of neuronal development and regeneration.

Disclosures: **A. Hleihel:** None. **D. Hynds:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.10/B4

Topic: A.05. Axon and Dendrite Development

Title: Analysis of correlation between second messenger dynamics and structural changes of the growth cone

Authors: *F. NAGASE, T. KOBAYASHI, K. HOTTA, K. OKA;
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Abstracts: Axon growth directed by the growth cone plays an important role during neural circuit formation. The growth cone is a sensory and motile part at the tip of the axon, and its main function is path finding. It receives chemical guidance cues from circumstance via specific receptors. A variety of attractive and repulsive guidance cues and following cascades inside the

growth cone have been revealed by previous reports (Tojima et al. 2011). Moreover, spatiotemporal crosstalk between second messengers such as cAMP, cGMP, and Ca2+ had been revealed (Kobayashi et al. 2013). These whole cascades, finally, triggers the structural changes of actin filaments and microtubules. However, the spatiotemporal relationship between the second messenger dynamics and following cell structure change has been poorly understood. Previous our study (Kobayashi et al. 2013) was mainly focused on the movement of the growth cone but not detailed structural change of actin and tubulin. Here, we show the correlation in these second messenger cascades in dorsal root ganglia neurons from rats. To reveal these mechanisms, we used FRET sensors to monitor concentration of second messengers and fluorescent-labeled actin and tubulin, simultaneously. We visualized second messenger dynamics without stimulation in specific sub-domains in the growth cone: peripheral and central domain. We found that cAMP is dominant at the peripheral domain of the growth cone during the axon outgrowth. Ca2+ concentration at the peripheral domain also increases with fluctuation during the outgrowth. To reveal the growth cone structure reconstruction, we visualized actin at the peripheral domain and checked the relationship to the outgrowth. We found that actin concentration increases gradually during the outgrowth. These results, demonstrate that the dominant increase of cAMP and Ca2+ at the peripheral domain of the growth cone causes the actin concentration increase and the following outgrowth of the axon.

Disclosures: **F. Nagase:** None. **K. Hotta:** None. **K. Oka:** None. **T. Kobayashi:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.11/B5

Topic: A.05. Axon and Dendrite Development

Title: Activation of Sonic Hedgehog signaling in dendrites accelerates axon outgrowth in hippocampal neurons

Authors: P. J. YAO, F[1], *R. S. PETRALIA[2], M. P. MATTSON[1,3];
[1]Lab. of Neurosciences, NIA/NIH, Baltimore, MD; [2]Advanced Imaging Core, NIDCD/NIH, BETHESDA, MD; [3]Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstracts: The Sonic hedgehog (Shh) signaling pathway is known to play important roles in development of the nervous system where it functions as a morphogen that controls progenitor cell proliferation and induction of the floor plate, and regulation of cell fate. In addition, recent

findings suggest that Shh signaling can enhance axon growth, although the underlying mechanisms are unknown. We previously showed that the cell surface Shh receptor Patched, and the Shh signal transducer Smoothened, are preferentially localized to the dendrites of hippocampal neurons. Here we provide evidence that activation of Shh receptors in dendrites enhances axon outgrowth in embryonic hippocampal neurons. Using microfluidic chambers in which neurons are plated in one chamber, and their axons grow through microgrooves into another chamber, we show that application of Shh to the dendritic compartment accelerates axon outgrowth, whereas application of Shh directly to the axons does not affect their outgrowth. Shh has no observable direct or indirect effect on dendrite outgrowth. The axon outgrowth induced by dendritic Shh signaling requires a Gli transcription factor. Shh-treated neurons expressed elevated levels of the actin modifier protein profilin, while the level of activated cofilin, another actin modifier was reduced. Our findings suggest a scenario in which Shh released by one neural cell (presumably a neuron or glial cell) onto the dendrites of a neuron can stimulate the growth of the axon of the same neuron. We are currently working to better understand the molecular sequence of events underlying this intriguing polarized subcellular signaling pathway, and its physiological roles in developmental and adult neuroplasticity.

Disclosures: P.J. Yao: None. **R.S. Petralia:** None. **M.P. Mattson:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

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Program#/Poster: 591.12/B6

Topic: A.05. Axon and Dendrite Development

Support: JSPS KAKENHI Grant 24790097

JSPS KAKENHI Grant 21590082

JSPS KAKENHI Grant 24590104

Title: Ezrin regulates neuritogenesis through down-regulation of RhoA signaling pathway

Authors: Y. MATSUMOTO[1], *M. INDEN[2], A. TAMURA[3], R. HATANO[1], S. TSUKITA[3], S. ASANO[1];
[1]Ritsumeikan Univ., Kusatsu, Japan; [2]Gifu Parmaceutical Univ., Gifu, Japan; [3]Osaka Univ., Suita, Japan

Abstracts: Establishment of neural circuits in the central nerve system requires generation and development of multiple dendrites and single axon. Cultured neurons that showed sequence of morphological changes are well studied for neuronal development. Ezrin/Radixin/Moesin (ERM) proteins link between membrane proteins and actin cytoskeleton, contribute to maintenance of cellular function and morphology. In cultured hippocampal neurons, suppression of both radixin and moesin showed deficits in growth cone morphology and neurite extensions. On the other hand, down-regulation of ezrin using siRNA caused impairment of netrin-1-induced axon outgrowth in cultured cortical neurons. However, roles of ezrin in the neuronal morphogenesis of the cultured neurons have been poorly understood. In this report, we performed detailed studies on the roles of ezrin in the cultured cortical neurons prepared from the ezrin knockdown (Ezrin KD) mice embryo that showed a very small amount of ezrin expression compared with the wild-type neurons. First, we confirmed that ezrin was mainly expressed in cell body in the cultured cortical neurons as repoted in other types of cultured neurons. We demonstrated that the cultured cortical neurons prepared from the Ezrin KD mice embryo exhibited impairment of neuritogenesis, not neurite and axon outgrowth. We next examined whether ezrin knockdown affects RhoA activity in the cultured cortical neurons using Rho GTPase pull-down assay. GTP-bound RhoA in the Ezrin KD neurons was increased more than three-folds compared with the wild-type neurons. In contrast to RhoA, other Rho family members, Rac1 and Cdc42 were not affected by ezrin knockdown. Moreover, we observed increased phosphorylation of myosin light chain 2 (MLC2), as a downstream effector of RhoA in the Ezrin KD neurons. In addition, treatment of Y-27632, an inhibitor specific for Rho kinase, rescued the abnormalities in neuritogenesis in the Ezrin KD neurons. These data altogether suggest a novel role of ezrin in the neuritogenesis of the cultured cortical neurons via down-regulation of RhoA/Rho kinase/MLC2 pathway.

Disclosures: **Y. Matsumoto:** None. **M. Inden:** None. **A. Tamura:** None. **R. Hatano:** None. **S. Tsukita:** None. **S. Asano:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.13/B7

Topic: A.05. Axon and Dendrite Development

Support: CIHR Grant

Title: Mical is required for retinal ganglion cell axon repulsion

Authors: *K. P. ATKINSON-LEADBEATER[1], C. L. HEHR[2], J. JOHNSTON[2], G. BERTOLESI[2], S. MCFARLANE[2];
[1]Psychology, Mount Royal Univ., Calgary, AB, Canada; [2]Cell Biol. and Anat., Univ. of Calgary, Calgary, AB, Canada

Abstracts: Axons are guided in their development through the actions of extracellular cues on the growth cone, a sensory-motile structure found at the tip of the growing axon. In order to guide developing axons, these cues must have the ability to effect changes in the intracellular structure of the growth cone. Sema3a, a secreted Semaphorin, is a guidance molecule known to repel developing axons in many systems. In *Drosophila* the binding of Sema3a to a Plexin/Neuropilin holoreceptor is thought to recruit a Molecule Interacting with CasL (Mical). Once recruited, Mical triggers F-actin disassembly and actin reorganization, thereby linking Sema3a signaling with cytoskeletal rearrangement. It is currently unknown whether Mical is important in the guidance of vertebrate axons. Xenopus laevis retinal ganglion cell (RGC) axons rely on repulsive signals from Sema3a and Slit1 to repel them through a caudal turn in the mid-diencephalon, which directs these axons to their midbrain target. We used RGC axon navigation of this caudal turn to ask whether Mical also mediates the repulsive actions of Sema3a in vertebrate axon development. Importantly, mical3a mRNA is expressed in Xenopus RGCs during the period of axon outgrowth. We exposed developing RGC axons *in vivo* to the inhibitor of Mical epigallocatechin gallate (EGCG). In the presence of EGCG the majority of axons failed to make the mid-diencephalic turn and stalled in the dorsal diencephalon (control 3% stalls, n=33; 25 uM EGCG 81% stalls, n=27). A similar phenotype was observed as we showed previously, when the expression of both Sema3a and Slit1 was inhibited. Furthermore, in the presence of jasplakinolide, a compound that promotes actin polymerization, RGC axons also fail to navigate the mid-diencephalic turn (control 3% stalls n=36; 75 uM Jas 56% n=17). These data are consistent with a model proposing that RGC axon repulsion requires actin depolymerization, and that Mical function is critical for repulsive cues to exert their effects on developing axons.
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Disclosures: K.P. Atkinson-Leadbeater: None. **C.L. Hehr:** None. **J. Johnston:** None. **G. Bertolesi:** None. **S. McFarlane:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

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Program#/Poster: 591.14/B8

Topic: A.05. Axon and Dendrite Development

Support: JSPS Grant-in-Aid for Young Scientists (B) Grant #24791624

Title: Neurotropin suppresses lidocaine-induced inhibition of neurite growth in cultured rat spinal neurons

Authors: *R. ISONAKA[1], T. TAKENAMI[2], T. KATAKURA[1], T. KAWAKAMI[1];
[1]Physiol., [2]Anesthesiol., Kitasato Univ. Sch. of Med., Sagamihara, Japan

Abstracts: Neurotropin is an analgesic agent, and commonly used for the treatment of chronic pains in the fields of orthopedics, neurology and anesthesia. It is a non-protein extract from inflamed skin of rabbits inoculated with vaccinia virus. Lidocaine is one of the typical local anesthetics and it relieves a variety of neuropathic pain, while sometimes it has neurotoxicity. In clinical practice, neurotropin is used for the treatment in addition to lidocaine to prevent neurotoxicity. However, the mechanism of these reactions remains unclear. In this study, we investigated that the effects of lidocaine and neurotropin on neurite growth in cultured rat spinal neurons. Spinal neurons are stained with mouse anti-phosphorylated neurofilaments (SMI-31) antibody. The length of the individual neurites of SMI-31-immunoreactive neurons was measured using NIH Image software on the captured images. Neurotropin alone had no effect on neurite growth, whereas it protected against the growth inhibitory effects of lidocaine in spinal neurons. These results suggested that neurotropin may lead to rescue lidocaine-induced neurotoxicity.

Disclosures: R. Isonaka: None. **T. Takenami:** None. **T. Katakura:** None. **T. Kawakami:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

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Topic: A.05. Axon and Dendrite Development

Support: ANR Grant ANR-09-MNPS-004-01

Title: Cannabinoid-induced actomyosin contractility shapes neuronal morphology and growth

Authors: *Z. LENKEI[1,2], A. ROLAND[2], A. RICOBARAZA[3], D. CARREL[3], F. RICO[4], A. SIMON[3], M. HUMBERT-CLAUDE[3], B. F. JORDAN[5], S. SCHEURING[4];
[1]ESPCI-CNRS UMR 7637, Paris cedex 05, France; [2]Brain Plasticity Unit, [3]ESPCI-ParisTech, Paris, France; [4]Inst. Curie, Paris, France; [5]FAS Ctr. for Systems Biol., Harvard Univ., Cambridge, MA

Abstracts: Endocannabinoids are recently recognized regulators of brain development, but molecular effectors downstream of neuronal type-1 cannabinoid receptor (CB1R) activation remain incompletely understood. We report atypical coupling of neuronal CB1Rs, after activation by endo- or exocannabinoids such as the marijuana component Δ9-tetrahydrocannabinol, to heterotrimeric G12/G13 proteins that triggers rapid and reversible non-muscle myosin II (NM II) dependent contraction of the actomyosin cytoskeleton, through Rho-associated kinase (ROCK). This induces dramatic rapid neuronal remodeling, such as neurite retraction, elevated neuronal rigidity, reshaping of somatodendritic morphology and retraction of axonal growth cones. Chronic pharmacological inhibition of NM II prevents cannabinoid-induced reduction of dendritic development *in vitro* and leads, similarly to blockade of endocannabinoid action, to excessive growth of CB1R-expressing corticofugal axons into the subventricular zone *in vivo*. Our results suggest that CB1R can rapidly transform the neuronal cytoskeleton through actomyosin contractility, resulting in cellular remodeling events ultimately able to affect neuronal architecture and brain wiring.

Disclosures: Z. Lenkei: None. **A. Roland:** None. **A. Ricobaraza:** None. **D. Carrel:** None. **F. Rico:** None. **A. Simon:** None. **M. Humbert-Claude:** None. **S. Scheuring:** None. **B.F. Jordan:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.16/B10

Topic: A.05. Axon and Dendrite Development

Support: NIH R00 MH095768

Title: TACC3 is a microtubule plus-end tracking protein that promotes axon elongation and microtubule polymerization in growth cones

Authors: *L. A. LOWERY, B. NWAGBARA, A. FARIS, B. ERDOGAN, E. BEARCE, P. EBBERT, M. EVANS, C. BAKER, T. ENZENBACHER;
Boston Col., Chestnut Hill, MA

Abstracts: Proper neural connections, essential to nervous system function, depend upon precise navigation by the growth cone during development. A fundamental problem in growth cone biology is how guidance pathways are integrated to coordinate cytoskeletal dynamics and drive accurate axonal navigation. To address this question, we focus on the plus-ends of microtubules (MTs), which explore the growth cone and play a key role in growth cone steering. MT plus-end dynamics are regulated by a conserved family of proteins called 'plus-end-tracking proteins' (+TIPs). Yet, it is unclear how +TIPs interact with each other and with plus-ends to control MT behavior, and how signaling mechanisms downstream of extracellular cues coordinate +TIPs to guide the growth cone in the right direction. We previously determined that the microtubule minus-end regulator, TACC3, interacts with the +TIPs, CLASP and XMAP215, in multiple developmental contexts, including during eye development. However, TACC3 has not yet been defined as a +TIP, its possible function during neurite outgrowth has not been explored, and the detailed mechanism by which it interacts with other +TIPs has not been elucidated. Here, by using automated analysis of live-imaging data of cultured Xenopus laevis growth cones, we show that TACC3 promotes axon outgrowth and regulates microtubule dynamics by increasing microtubule polymerization rates *in vivo*. We also demonstrate that TACC3 performs this function by acting as a +TIP in vertebrate growth cones. Using high-resolution live-imaging data of tagged +TIPs, we reveal that TACC3 localizes to the distal-most tip of MTs in growth cones, where it is directly in front of the microtubule polymerization marker, EB1, and specifically co-localizes with the microtubule polymerase XMAP215. As we previously highlighted that XMAP215 displays multiple functions within growth cones, including both a plus-end-dependent polymerization function as well as a microtubule-lattice binding-dependent microtubule translocation function, we hypothesize that TACC3 specifically enhances the ability of XMAP215 to drive microtubule polymerization downstream of axon guidance cue signaling. We are currently testing this model, to further decipher the regulatory mechanisms that control the growth cone cytoskeletal machine.

Disclosures: L.A. Lowery: None. **B. Nwagbara:** None. **A. Faris:** None. **E. Bearce:** None. **B. Erdogan:** None. **P. Ebbert:** None. **M. Evans:** None. **C. Baker:** None. **T. Enzenbacher:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.17/B11

Topic: A.05. Axon and Dendrite Development

Support: NIH grant NS14428

Title: The role of tau phosphorylation in regulating cytoskeletal dynamics in cortical growth cones during axon outgrowth

Authors: *S. BISWAS, Q. GAN, K. KALIL;
Dept. of Neurosci., Univ. of Wisconsin, Madison, WI

Abstracts: Tau is a highly phosphorylated microtubule associated protein (MAP) that binds directly to microtubules (MTs) and regulates their dynamics, which is essential for growth cone function during axon growth and guidance. Tau has also been implicated in association with actin filaments, suggesting that tau might modulate actin/microtubule interactions. The state of tau phosphorylation is known to be important in regulating MT dynamics by changing the ability of tau to bind to MTs. Previously we found that inhibiting phosphorylation at a single site on tau, Serine 262, prevented the dynamic reorganization of MTs in cortical growth cones required for axon outgrowth and guidance in response to the morphogen Wnt5a. Phosphorylation at different sites on tau has been shown to regulate MT dynamics in cell free systems. However, the influence of tau phosphorylation on cytoskeletal dynamics has been little studied in living neurons. To address this question we transfected cultured hamster cortical neurons with various forms of tau mutated at different residues to increase or reduce tau phosphorylation. We then applied Wnt5a to increase axon outgrowth. Using immunocytochemistry and biochemical analysis, we found that Wnt5a increases phosphorylation at tau S262 at the leading edge of cortical growth cones, suggesting that tau phosphorylation at S262 increases its association with actin filaments prevalent at the leading edge. We are currently performing live cell imaging with TIRF microscopy on axonal growth cones of neurons transfected with various phospho mimetic and phospho deficient mutations in tau. We are labeling actin filaments with tdTomato tractin and the dynamic plus ends of MTs with EB3-EGFP in neurons transfected with tau constructs with mutations on different phosphorylation sites. We are imaging cytoskeletal dynamics in the growth cones of these neurons during axon outgrowth stimulated by Wnt5a. We are particularly interested in whether changes occur in the association of tau with actin filaments versus MTs as a consequence of phospho mutations in tau. Further, we are investigating whether such changes in tau associations with the cytoskeleton regulate the ability of actin/MT interactions to promote growth cone extension during axon outgrowth.

Disclosures: **S. Biswas:** None. **Q. Gan:** None. **K. Kalil:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

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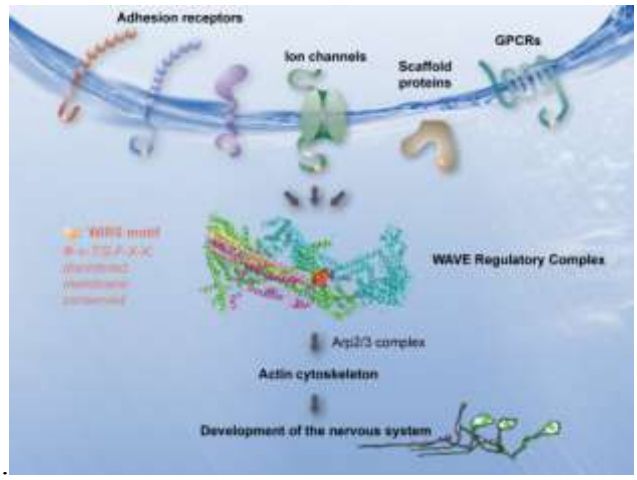
Howard Hughes Medical Institute

Grant from Deutsche Forschungsgemeinschaft

Title: The WAVE regulatory complex links diverse neuronal receptors to the actin cytoskeleton

Authors: *B. CHEN[1], P. CHIA[2], K. BRINKMANN[3], Z. CHEN[1,4], C. W. PAK[1], Y. LIAO[1], P. LI[2], S. SHI[1], L. HENRY[1], N. V. GRISHIN[1], S. BOGDAN[3], K. SHEN[2], M. K. ROSEN[1]; [1]Dept. of Biophysics, UT Southwestern Med. Center, HHMI, Dallas, TX; [2]Dept. of Biol., Stanford University, HHMI, Stanford, CA; [3]Inst. für Neurobiologie, Univ. Münster, Münster, Germany; [4]Tsinghua Univ. - Current Address, Beijing, China

Abstracts: WAVE proteins play an essential role in actin cytoskeletal dynamics throughout the development of the nervous system. In cells, WAVE exists in a heteropentameric protein assembly, named the WAVE Regulatory Complex (WRC), which stimulates the actin-nucleating activity of the Arp2/3 complex at distinct membrane sites. However, the factors that recruit the WRC to specific locations remain poorly understood. Here, we have identified a large family of potential WRC ligands, consisting of ~120 diverse membrane proteins, mainly enriched in the nervous system, including protocadherins, ROBOs, netrin receptors, neuroligins, GPCRs, and ion channels. Structural, biochemical, and cellular studies reveal that a consensus sequence motif (WRC interacting receptor sequence, or WIRS) that defines these ligands binds to a highly conserved interaction surface of the WRC formed by the Sra and Abi subunits. In *Drosophila*, mutating this binding surface disrupted oogenesis and retinal neuron targeting. In *C. elegans*, mutating the WIRS motif in the synaptic cell adhesion protein SYG-1 leads to loss of local F-actin, synaptic vesicles and active zone proteins, and axonal branches. Our findings directly link diverse neuronal membrane proteins to the WRC and actin cytoskeleton and have broad

physiological and pathological ramifications in metazoans. 

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Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.19/B13

Topic: A.05. Axon and Dendrite Development

Title: Protein arginine methylation of CRMP and tubulin in neurons - a role in axonal development?

Authors: ***N. K. JWAD**[1], L. HEJAZI[2], R. GAMSJAEGER[1], N. J. SUCHER[4], S. C. PILLER[3]; [2]Sch. of Med., [3]Sch. of Sci. and Hlth., [1]Univ. of Western Sydney, Campbelltown, Australia; [4]Roxbury Community Col., Boston, MA

Abstracts: Collapsin response mediator proteins (CRMPs) are a family of five cytosolic phosphoproteins that are expressed during different stages of the development of the nervous system. Originally, CRMPs were identified to play a role in the growth cone collapse pathway. Later studies showed that CRMPs have multiple functions in the developing brain, mainly in axonnal formation, extension, elongation and branching. CRMPs are homologous proteins, sharing 50-75% of their sequence and exist as homo- and heterotetramers. In the process of

axonal growth, CRMP-2 acts as an escort protein for tubulin heterodimers along the growing axon. The binding of CRMP-2 to tubulin heterodimers is regulated by CRMP-2 phosphorylation. Tubulin itself is highly modified by multiple post-translational modifications such as acetylation and glutamylation. Post-translational modifications of tubulin also seem to regulate its binding affinity to motor neurons and microtubule-associated proteins. In this study, arginine methylation of α-tubulin, β-tubulin and CRMP-2 was investigated. Asymmetrical di-methylation of CRMP-2, α-tubulin and β-tubulin was detected using immunoprecipitation and Western blotting. The site of methylation was confirmed with mass spectrometry, revealing two methylated arginine residues on α-tubulin (R79 and R390), two arginines on the β-subunit (R62 and R282, and various arginine residues on of members of the CRMP family proteins (R226 in CRMP-1 and R173 in CRMP-2, and R238 and R270 in CRMP-3). We also found, using Cco-immunoprecipitation experiments indicated, that the inhibition of methylation in mouse primary cortical neurons seems appeared to results in a change in the binding pattern of CRMP-2 and tubulin. The location of methylated arginine on α-tubulin and β-tubulin suggests a potential role in microtubule stability as well as protein-protein interactions with microtubule-associated proteins such as CRMP-2. The location of methylated residues in CRMPs suggests points to a possible association with the regulation of tetramer formation. In summary, our results indicate that protein arginine methylation plays an important role in the regulation of CRMP-tubulin interaction and in axonal development in primary cortical mouse neurons.

Disclosures: **N.K. Jwad:** None. **L. Hejazi:** None. **R. Gamsjaeger:** None. **N.J. Sucher:** None. **S.C. Piller:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

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Program#/Poster: 591.20/B14

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS062047

Title: Identification of a novel developmental mechanism that promotes sensory axon collateral formation via the regulation of microtubule bundling

Authors: S. TYMANSKYJ, *L. MA;
Zilkha Neurogenetic Inst., USC, LOS ANGELES, CA

Abstracts: In complex neural circuits, neurons often make contacts with multiple targets by sprouting collateral branches from single axonal shafts. Development of these collaterals is highly regulated by intrinsic genetic programs and extracellular environments. Using sensory neurons in the dorsal root ganglia (DRG) as a model, we have identified a novel intrinsic branching mechansim mediated by a microtubule associated protein, MAP7 (also known as ensconsin or E-MAP-115). First, MAP7 expression in the DRG is developmentally regulated, appearing in neurons only at the onset of collateral initiation and reaching to all neurons at the peak of collateral formation. Second, perturbation of MAP7 expression in isolated DRG neurons demonstrates its branch promoting function: MAP7 over-expression stimulated precocious branch formation in young DRG neurons that have no collaterals formed yet and normally do not branch in culture, whereas shRNA knockdown in old neurons with collaterals already formed *in vivo* reduced their ability of promoting branch formation in culture. Further molecular characterization of MAP7 reveals that this branching function requires both the amino-terminal microtubule binding (N) domain and the central phosphorylation (P) domain. In addition, the two domains (NP) together can reorganize microtubules into long and curly bundles when overexpressed in COS cells. Such functional correlation suggests a critical role of microtubule bundles in branch formation. This conclusion is further supported by the study of a spontaneous mouse mutant, as it expresses a truncated NP-equivalent protein and exhibits excessive entries of DRG axons in the spinal cord, a phenotype that is consistent with an increase in collateral branches. Finally, both microtubule bundling and axon branching depend on a specific phosphorylation site in the P domain, suggesting that these activities can be regulated by extracellular cues. Thus, our study uncovers a novel intrinsic program for collateral branch development and demonstrates an importance role of microtubule bundles in branching regulation.

Disclosures: S. Tymanskyj: None. **L. Ma:** None.

Poster

591. Axon Growth and Guidance: Cytoskeletal Dynamics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 591.21/B15

Topic: A.05. Axon and Dendrite Development

Support: Human Frontier Science Program

Title: Fully automated quantification of growth cone dynamics at high resolution reveals differential regimes in RhoGTPase function

Authors: *M. M. BAGONIS[1,2], L. FUSCO[2], G. AZARIAS[2], O. PERTZ[2], G. DANUSER[1]; [1]Cell Biol., Harvard Univ., Brookline, MA; [2]Univ. of Basel, Basel, Switzerland

Abstracts: Elucidating the signaling mechanisms of neurite outgrowth is essential for understanding, and eventually manipulating, larger-scale biological processes associated with neuronal development and the re-establishment of functional neuronal connections after injury. How the geometrically distinct actin sub-networks of the growth cone integrate to elicit larger-scale growth cone functional behavior, such as growth cone advance and turning, is still not completely clear. This is due to the complexity of the growth cone cytoskeletal architecture making it difficult to quickly and robustly quantify the totality of growth cone morphological changes during a specific larger-scale functional event. Here, using a combination of live-cell fluorescent imaging and computer vision, we have developed a method for the automated quantification of growth cone structural and dynamic parameters. Using this assay we methodically compared the growth cone dynamic profiles corresponding to several RhoGTPase activity perturbation conditions leading to different neurite outgrowth phenotypes. We found that very diverse growth cone structural/dynamic profiles can lead to enhanced neurite outgrowth. Furthermore, we quantitatively compared the effects of direct, global perturbation of RhoGTPase activity to indirect, potentially spatially/temporally localized, perturbation of RhoGTPase activity via the knockdown of several putative upstream RhoGTPase activity regulators of Rac1 activity. We found that while knockdown of the presumed RhoGTPase activators mimicked some aspects of the corresponding RhoGTPase knockdown, consistent with a role for these proteins in RhoGTPase regulation, knockdown of these proteins likewise elicited distinct growth cone architectures and dynamics. Therefore, we show that this technique is capable of deciphering highly nuanced growth cone phenotypes and therefore is ideal for studying how regulation of smaller scale local dynamic changes in growth cone cytoskeletal architecture elicit different types of global functional neurite behavior. While here we focus on the outgrowth responses elicited in N1E-115 neuroblastoma cells in the presence of the extracellular matrix protein laminin, our method for measurement of growth cone structure and dynamics is generic and provides a means to quickly and unbiasedly quantify functionally relevant growth cone signaling outputs in response to an extracellular cue - a vital first step in future studies aiming at the reconstruction of signaling pathways regulating neurite outgrowth

Disclosures: **M.M. Bagonis:** None. **L. Fusco:** None. **G. Azarias:** None. **O. Pertz:** None. **G. Danuser:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.01/B16

Topic: A.05. Axon and Dendrite Development

Support: NIH RO1

Title: Regulation of mouse cochlear spiral ganglion neuron innervation by EphA7

Authors: *Y. KIM[1], L. A. IBRAHIM[1], S.-Z. WANG[2], H. W. TAO[1], L. I. ZHANG[1];
[1]USC, Los Angeles, CA; [2]Neurol., Univ. of California, San Francisco, San Franscisco, CA

Abstracts: During the cochlear development, different types of spiral ganglion neuron (SGN) fibers are precisely wired to hair cells (HCs) in the organ of Corti, giving rise to a fine tonotopic organization of SGN-HC connectivity. This requires carefully orchestrated series of events including axon outgrowth and guidance. In the current project, we sought to determine signaling pathways influencing SGN development by generating gene expression profiles in mouse cochlea with RNA-sequencing. We found significant EphA7 expression in the cochlea, which was restricted to SGNs and the greater epithelial ridge (GER). In EphA7-/- mutant mice, we found a significant reduction in SGN fibers innervating hair cells. This reduction was caused by diminished outgrowth of type I afferent fibers, which resulted in reduced number of ribbon synapses on inner hair cells. *In vitro* analysis indicated that both forward and reverse signaling of EphA7 could have the same effect of promoting SGN neurite outgrowth. Furthermore, we identified extracellular signal-regulated kinase 1&2 as possible downstream targets of EphA7 signaling pathway. Our results suggest that EphA7 regulates the outgrowth of type I SGN fibers to ensure optimal connectivity between SGNs and inner hair cells.

Disclosures: Y. Kim: None. **S. Wang:** None. **L.A. Ibrahim:** None. **H.W. Tao:** None. **L.I. Zhang:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.02/B17

Topic: A.05. Axon and Dendrite Development

Title: CLAC-P/collagen type XXV in muscle, but not in motor neurons, is required for intramuscular development of motor axons

Authors: *H. OIZUMI[1], T. TANAKA[1], S. NISHIO[1], H. MUNEZANE[2], T. HASHIMOTO[2], A. HARADA[3], T. WAKABAYASHI[2], T. IWATSUBO[2];
[1]Grad. Sch. of Pharmaceut. Sci., [2]Grad. Sch. of Med., Univ. of Tokyo, Tokyo, Japan; [3]Grad. Sch. of Med., Osaka Univ., Osaka, Japan

Abstracts: CLAC-P/collagen type XXV was identified as a component of amyloid plaques in Alzheimer's disease brains. To reveal the *in vivo* function of CLAC-P, we previously generated Col25a1 knock-out (KO) mice, of which the motor axons failed to arborize within the target muscles during embryonic development, followed by massive cell death of motor neurons. Because Col25a1 mRNA was expressed both in motor neurons and in myotubes during neuromuscular development, we examined whether CLAC-P expressed in motor neurons or that in myotubes is required for the intramuscular development of motor axons. To address this question, we generated Col25a1 conditional KO mice by crossing Col25a1flox mice with Cre-expressing mice. In this study, we used two Cre-expressing mouse lines, i.e., Hb9-Cre mice and HSA-Cre mice to abolish Col25a1 gene specifically in motor neurons and myotubes, respectively. When we disrupted the Col25a1 gene in motor neurons just after their differentiation using Hb9-Cre mice, the motor axons arborized normally within the target muscle and formed neuromuscular junctions by E16.5. In contrast, the motor axons of muscle-specific Col25a1 KO embryos lacked intramuscular arborization at E12.5, which completely disappeared from the diaphragm muscles by E15.5. Consistently, the number of motor neurons of muscle-specific Col25a1 KO fetuses was dramatically decreased to an extent similar to that of conventional Col25a1 KO fetuses. These results clearly demonstrate that CLAC-P expressed in skeletal muscles, but not in motor neurons, is essential for the axonal development and survival of the cell bodies of motor neurons. Currently, investigations into the molecular mechanism of CLAC-P function in neuromuscular development are underway by searching for its receptor that may presumably be expressed on the motor axon side.

Disclosures: H. Oizumi: None. **T. Tanaka:** None. **S. Nishio:** None. **H. Munezane:** None. **T. Hashimoto:** None. **A. Harada:** None. **T. Wakabayashi:** None. **T. Iwatsubo:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.03/B18

Topic: A.05. Axon and Dendrite Development

Title: The role of α6β4 integrin receptor in laminin-mediated axon outgrowth

Authors: J. WETHERELL, A. BUONACCORSI, N. DOPPLER, *M. I. JAREB;
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Abstracts: Previous data suggest that a laminin receptor is localized in the axons of chick forebrain neurons mediating the axon-growth promoting properties of laminin. Many heterodimers from the integrin family of proteins have previously been identified as laminin receptors, including the α6β4 heterodimer. We tested the role of β4, β1, and α6 integrins in laminin-induced increases in axonal growth using function blocking antibodies. Axons of neurons grown on laminin treated with β4 integrin function blocking antibodies or α6 integrin function blocking antibodies were significantly shorter compared to untreated cultures or cultures treated with a β1 integrin function-blocking antibody. These data are consistent with the hypothesis that the α6β4 heterodimer acts as the axonal laminin receptor in embryonic chick forebrain neurons and is important in axonal development and growth. To directly test whether α6 or β4 integrin was localized specifically to axons, we transfected cultured forebrain neurons from embryonic chick with DNA constructs encoding both α6 and β4 integrin genes. Preliminary results show α6 and β4 integrin expression in axons.

Disclosures: J. Wetherell: None. **M.I. Jareb:** None. **A. Buonaccorsi:** None. **N. Doppler:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.04/B19

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 1R15NS070172-01A1

Title: Investigating the role of integrins in Caenorhabditis elegans axon patterning

Authors: D. OLIVER[1], M. M. FRANCIS[2], *M. L. LEMONS[1];
[1]Dept. of Natural Sci., Assumption Col., WORCESTER, MA; [2]Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstracts: During development, billions of neurons extend axons to their appropriate targets. Growth cones at the tips of extending axons integrate extracellular cues and guide axons to their correct destinations. Although this navigational ability is essential for proper wiring of the nervous system, the molecular mechanisms of neurons' GPS systems are not yet well understood. Here we show that members of the integrin family of transmembrane receptors regulate axon patterning in a cell type-specific manner in *Caenorhabditis elegans*. This genetically powerful model organism provides a well-defined landscape to examine integrin function *in vivo* due to: 1) the transparency of the animal, 2) the abundance of axon markers, 3) the known spatial and temporal aspects of axonal projections and 4) the limited number of integrin subunits (three compared to ~twenty four vertebrate subunits). In addition, viable integrin hypomorphic worm strains are readily available. We initially focused our studies on INA-1, a laminin-binding integrin subunit that shares sequence homology with vertebrate alpha subunits 3, 6 and 7. We evaluated a strain carrying a missense mutation in a region of *ina-1* that is thought to be critical for integrin activation (*i.e.* a conformational change to a high ligand-affinity state). We found that decreased *ina-1* function produced: 1) striking defects in the patterning of commissural axons from GABA neurons, 2) strong, but less robust effects on commissural axons from ACh neurons, and 3) no obvious effects on longitudinal axons projecting from the tail to the head *(e.g* from the interneuron DVA). Using a transgenic strain expressing GFP-tagged INA-1, we found that INA-1 was expressed in both GABA and ACh motor neurons, suggesting that *ina-1* may act cell autonomously in these neurons. Interestingly, errors in GABA and ACh axon patterning were most pronounced at distinct times during development. For example, axon patterning defects in GABA neurons were most pronounced at the third larval stage (L3), whereas defects in ACh neurons were most pronounced at L4. This evidence suggests that the role of integrins is not equal in all neurons, nor is the functional contribution of integrins equal over time. Future studies will elucidate mechanisms by which integrin signaling achieves cell-type specific effects on axon guidance as well as synapse development.

Disclosures: D. Oliver: None. **M.L. Lemons:** None. **M.M. Francis:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.05/B20

Topic: A.05. Axon and Dendrite Development

Title: Proteolytic cleavage of IgLON adhesion proteins by MMPs promotes neurite outgrowth

Authors: *R. L. SANZ, A. FOURNIER;
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Abstracts: Matrix metalloproteinases (MMPs) are a family of zinc endopeptidases capable of cleaving extracellular matrix and cell surface proteins resulting in degradation or release of biologically active fragments. Processing of surface proteins through MMPs affects developmental processes including axon guidance, survival and synaptogenesis. Moreover, MMPs process ligands and receptors that regulate neuronal plasticity and neurite growth following injury in the Central Nervous System. In the present study, we evaluated the role of MMPs in regulating neurite growth. We find that pan-MMP inhibitors inhibit outgrowth of cortical neurons and dorsal root ganglion neurons (DRGs) and that this effect is dependent on the stage of neuronal maturity. Outgrowth assays with the endogenous MMP inhibitors (TIMPs) confirmed our previous result with the pan-MMP inhibitors. Moreover, through the TIMP inhibitory profile we find that a member of the ADAM family of MMPs is likely responsible for promoting neurite growth. Through tandem mass spectrometry we identified the IgLON family of glycosyl-phosphatidyl inositol (GPI)-anchored neural cell adhesion molecules as proteins that are shed in an MMP-dependent manner. IgLONs are the earliest and most abundant GPI-anchored proteins expressed in the nervous system and are implicated in the process of neuronal outgrowth and cell adhesion. Using reverse-transcription PCR and cell surface biotinylations, we observed a correlation between the expression of IgLON family members *in vitro* and our outgrowth phenotype with MMP inhibitors. Our findings suggest that a near full length fragment of the IgLON ectodomain is cleaved from the surface of cortical neurons in an MMP-dependent manner. Outgrowth experiments on immobilized full-length IgLON proteins identified Neurotrimin and LSAMP as IgLON family members that promote neurite extension in cortical neurons. Together our findings support a role for MMP-dependent shedding of IgLON family members in regulating neurite extension.

Disclosures: R.L. Sanz: None. **A. Fournier:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.06/B21

Topic: A.05. Axon and Dendrite Development

Support: Cell Dynamics Research Center Grant NRF (2007-0056157)

National Leading Research Laboratory

Title: Vstm5, a putative cell adhesion membrane protein, regulates dendritic protrusion and synapse formation in hippocampal neurons

Authors: A.-R. LEE[1,2], H. LEE[1,2], M.-R. SONG[1,2], *C.-S. PARK[1,2,3];
[1]Sch. of Life Sci., GIST, Gwangju, Korea, Republic of; [2]Cell Dynamics Res. Ctr., Gwangju, Korea, Republic of; [3]Natl. Leading Res. Lab., Gwangju, Korea, Republic of

Abstracts: Dendritic protrusions are highly dynamic structures in neurons, and morphological change from dendritic filopodia to spines is closely related with the stabilization and strengthening of synapse during neuronal development. Here, we report that Vstm5 (V-set and transmembrane domain containing protein 5), a cell adhesion molecule belonging to the Ig superfamily, is found in various regions of mouse brain. Using cultured hippocampal neurons, we examined the functions of Vstm5 in neuronal development and synaptogenesis. To elucidate the function of Vstm5 in neuronal morphology, we analyzed the dendrites and dendritic protrusions in Vstm5-overexpressing and Vstm5-deficient neurons. Overexpression of Vstm5 caused a dramatic increase in both dendrite complexity and the density of dendritic filopodia. Conversely, Vstm5-deficient neurons showed a decreased density of dendrites and filopodia. At late stage, Vstm5 expressing neurons showed increased in total spine density especially thin-type spine and the number of clusters of the presynaptic vesicle-associated protein synaptophysin. In Vstm5-knockdown neurons, the density of spines and synapses are significantly decreased. These results demonstrate that Vstm5 regulates neuronal morphology by promoting the dendritic protrusion and dendrite formation and it caused synapse formation. In addition, we found that Vstm5 regulate neuronal morphology changes *in vivo*, it caused delayed neuronal migration. Taken together, these results indicate that Vstm5 may be a new member of cell adhesion protein critically involved in dendrite morphology and synapse formation through promotion of neuronal membrane dynamics.

Disclosures: **A. Lee:** None. **H. Lee:** None. **M. Song:** None. **C. Park:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

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Program#/Poster: 592.07/B22

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS074732

NIH Grant NS041564

NIH Grant GM007507

Title: Calcium/Calpain dependent cleavage of Talin and FAK regulates axon pathfinding

Authors: *P. C. KERSTEIN, K. PATEL, T. M. GOMEZ;
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Abstracts: Intracellular calcium (Ca^{2+}) is an essential second messenger of many signaling events within neuronal growth cones. Remarkably, Ca^{2+} can mediate opposing affects on growth cone motility downstream of axon guidance cues. The diversity of Ca^{2+} signaling arises from precise localization of Ca^{2+} influx into microdomains containing specific Ca^{2+} effectors. Previously, we showed that changes in mechanical and chemical composition of the underlying substrata elicit local Ca^{2+} signals within growth cone filopodia that regulate axon outgrowth through activation of the protease calpain. We hypothesize that calpain influences axon guidance through local modulation of adhesion dynamics. Here, we investigated the role calpain mediated proteolysis of specific adhesion proteins in *Xenopus* neuronal growth cones. Our results suggest that Talin and Focal Adhesion Kinase (FAK) are cleaved by calpain in spinal neurons *in vitro* and *in vivo*. Inhibition of calpain increases the localization of endogenous adhesion signaling to growth cone filopodia. Further, pharmacological and molecular inhibition of calpain increased adhesion turnover through increased formation and decreased duration of paxillin-mediated adhesions. Using specific calpain-resistant point-mutants of talin and FAK (Talin L432G, FAK V744G), we demonstrate that calpain specific cleavage events are necessary for proper adhesion formation and turnover *in vitro*. Finally, inhibition of calpain proteolysis *in vivo* leads to increased Rohan-Beard peripheral axon outgrowth into the skin. These data suggest that calpain serves an important role in regulating adhesion dynamics and growth cone motility in axon pathfinding.

Disclosures: P.C. Kerstein: None. **T.M. Gomez:** None. **K. Patel:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

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Program#/Poster: 592.08/B23

Topic: A.05. Axon and Dendrite Development

Support: Spanish Ministry of Economy SAF2010, 21723

EMBO, EUI-EURYIP-2011-4312

Title: The role of focal adhesion kinase in axonal remodeling

Authors: A. I. NAVARRO, *B. RICO;
Inst. de Neurociencias,UMH-CSIC, Sant Joan Alacant, Spain

Abstracts: During the last phases of brain development, once neurons reach their final destination, they extend axons that arborize through the extension of collaterals that bifurcate again to form terminal fields. Neurons typically produce more collaterals and axon terminals that will eventually endure in the adult brain, since many of them are pruned. It has been described two different types of pruning: large-scale pruning with an elimination of long collaterals, and small-scale pruning with a local refinement of axons within the termination zone. This remodeling of the axonal arbor allows the refinement of the network and generates a precise adult connectivity. Over the past few years our group has explored the function of Focal Adhesion Kinase (FAK) in neural circuits development. We have described that FAK controls axonal arborization of hippocampal neurons *in vitro*. Mutant neurons form bigger axonal arbors due to both: increase in branch formation and reduction in branch retraction. Here we have combined mouse genetics with a set of different experimental approaches to show that FAK is required for the proper remodeling of axonal arbors *in vivo*. The absence of FAK causes specifically an impaired small-scale remodeling but does not affect the long-scale pruning. In particular, FAK-deletion in pyramidal cells leads to an abnormal refinement of the axons in the terminal field. Remodeling of the axons could be mediated by local and transcriptional changes. To understand the molecular mechanisms by which the kinase commands this process *in vivo*, we focused in a possible implication in pathways that promote transcriptional changes. FAK has been shown to be upstream of ERK-signaling cascades. Also, FAK can physically associate to regulatory regions of the targeting genes, regulating gene expression. Therefore, to test its role in transcription we have carried out a high throughput screening of genes that are transcriptionally regulated by FAK, using microarray analysis.

Disclosures: A.I. Navarro: None. **B. Rico:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.09/B24

Topic: A.05. Axon and Dendrite Development

Support: National Natural Science Foundation of China (81330026, 31271259, 30990261, 30871425)

the National Basic Research Program, Ministry of Science and Technology of China (2013CB945604)

Title: Loss of neural recognition molecule NB-3 delays the normal projection and terminal branching of developing corticospinal tract axons

Authors: *Y. LIU;
Inst. of Neuroscience, Soochow Univ., Jiang Su, China

Abstracts: Neural recognition molecule NB-3 is involved in neural development and synapse formation. However, its role in axon tract formation is unclear. In this study, we found that the temporal expression of NB-3 in the deep layers of the motor cortex in mice was coincident with the development of the corticospinal tract (CST). Clear NB-3 immunoreactivity in the CST trajectory strongly suggested that NB-3 was expressed specifically in projecting CST axons. By tracing CST axons in NB-$3^{-/-}$ mice at different developmental stages, we found that these axons were capable of projecting and forming a normal trajectory. However, the projection was greatly delayed in NB-$3^{-/-}$ mice compared to wild-type (WT) mice from the embryonic to postnatal stages, a period that is coincident with the completion of the CST projection in mice. Subsequently, although their projection was delayed, CST axons in NB-$3^{-/-}$ mice gradually completed a normal projection. By stage P21, the characteristics of CST projections in NB-$3^{-/-}$ mice were not statistically different from those in WT mice. In addition, we found that the branching of CST axons into spinal gray matter also was delayed in NB-$3^{-/-}$ mice. The CST innervation area in the spinal gray matter of NB-$3^{-/-}$ mice was greatly reduced in comparison to WT mice until P30 and gradually became normal by P45. These data suggest that NB-3 is involved in the normal projection and terminal branching of developing CST axons.

Disclosures: Y. Liu: None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.10/B25

Topic: A.05. Axon and Dendrite Development

Title: Spatiotemporally coordinated Tetraspanin-3 proteins provide a functional microdomain for Nogo-A-D20-induced spreading and neurite outgrowth inhibition

Authors: *N. K. THIEDE-STAN[1], D. ALBRECHT[2], B. TEWS[3], Z. RISTIC[1], H. EWERS[2], M. SCHWAB[1];
[1]Brain Res. Inst. UZH and Dept. of Hlth. & Technol. ETH, Zuerich, Switzerland; [2]Randall Div. of Cell and Mol. Biophysics, King's Col. London, London, United Kingdom; [3]Div. of Mol. Mechanisms of Tumor Invasion, German Cancer Res. Ctr., Univ. of Heidelberg and DKFZ, Heidelberg, Germany

Abstracts: Neurite outgrowth and axonal regeneration are restricted by myelin-associated inhibitors, in particular Nogo-A. Nogo-A-D20 (rat aa544-725) is besides Nogo-66 the most potent neurite growth inhibitory fragment of the membrane-spanning protein Nogo-A, which additionally restricts adhesion and spreading of non-neuronal cells. Recently, the G-protein-coupled receptor Sphingosine-1-phosphate receptor 2 (S1PR2) has been identified in a yeast two-hybrid system as specific Nogo-A-D20 signal-transducing receptor. The same screen revealed the 4-transmembrane-spanning protein Tetraspanin-3 (Tspan-3) as an additional high-affinity binding partner for Nogo-A-D20. Based on the described minor relevance of Tetraspanin proteins in direct signal transduction, but their pronounced feature as microdomain organizers, we hypothesized a coordinative co-receptor function for Tspan-3 in a potential multi-subunit Nogo-A-D20 receptor complex. Direct binding assays showed Tspan-3 as high-affinity binding partner for Nogo-A-D20. siRNA or lentiviral shRNA-mediated knock-down of Tspan-3 leads to a significant counteraction of Nogo-A-D20-mediated cell spreading inhibition, revealing a functional relevance of Tspan-3 in Nogo-A-D20 signaling. Tspan-3 accompanies Nogo-A-D20 along its multi-step route from cell surface binding to early endocytosis and noticeably affects Nogo-A-D20 cell surface binding at a very early stage of initial contact with the cell surface. Live-imaging single-molecule-localization microscopy revealed that Nogo-A-D20 induces a spatiotemporally coordinated Tspan-3 re-organization. Tspan-3 co-localizes minimally with S1PR2 in absence of Nogo-D20; addition of Nogo-D20 induces an increased dynamically regulated complex formation of Tspan-3 and S1PR2. Palmitoylations (post-translational S-acylation with palmitate of juxtamembrane protein cysteine residues) are known to stabilize assemblies of Tetraspanins and Tetraspanin-associated proteins in Tetraspanin-enriched microdomains. Interference with palmitoylations with 2-Bromopalmitate (2-Bp) counteracts Nogo-A-D20-induced spreading inhibition. Furthermore, 2-Bp treatment or Tspan-3 knock-down decrease Nogo-A-D20-induced RhoA activation. These results point to a relevant modulatory role of a Tetraspanin-3 microdomain in the Nogo-A-D20-induced G protein 13 - LARG - RhoA

signaling axis. Our findings provide new insights into the succession and coordination of major cell biological mechanisms in Nogo-A-D20-mediated growth inhibition.

Disclosures: **N.K. Thiede-Stan:** None. **D. Albrecht:** None. **B. Tews:** None. **H. Ewers:** None. **M. Schwab:** None. **Z. Ristic:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.11/B26

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant RX2240006

Title: Determining the influence of confinement on growth cone response to inhibitory biochemical borders

Authors: ***M. S. SMIRNOV**[1], H. M. GELLER[3], J. S. URBACH[2];
[1]IPN, [2]Physics, Georgetown Univ., Washington, DC; [3]Natl. Heart, Lung, and Blood Inst., NIH, Bethesda, MD

Abstracts: Developing and regenerating axons *in vivo* encounter many chemical and topographic guidance cues. The response to these cues is mediated by the growth cone, a structure at the axonal tip. The morphology of the growth cone is often correlated with growth rate, e.g., growth cones increase in complexity and size and also slow down at decision regions. The details of this mechanism are not well-understood. We hypothesize that confinement of growth cones increases axonal extension and makes them less sensitive to guidance cues. To approach this problem, we have created a model system for evaluating growth cone behavior at the interface between molecular and structural cues under direct microscopic observation. In this system, we use two-photon laser ablation of polyvinyl alcohol to create laminin-coated, micron-scale adhesive channels (Smirnov, et al., Biomaterials, 2014). We then create local depositions of inhibitory chondroitin sulfate proteoglycans on the pattern, forming a distinct permissive/inhibitory boundary within each channel. Mouse postnatal cerebellar granule neurons are plated onto the substrate and the behavior of growth cones is observed with time-lapse microscopy. This system permits the study of growth cone sensitivity to guidance cues, as well as their cytoskeletal mechanisms, based on changes in channel width.

Disclosures: **M.S. Smirnov:** None. **J.S. Urbach:** None. **H.M. Geller:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.12/B27

Topic: A.05. Axon and Dendrite Development

Title: Synaptic activity regulates vGAT and vGlut1 expression in developing cortical neurons

Authors: *V. L. SAVCHENKO[1,2];
[1]Geriatrics, Univ. of Arkansas, Little Rock, AR; [2]Anesthesiol., Univ. of Florida, Gainesville, FL

Abstracts: The maintenance of excitatory and inhibitory neurons and their axonal projection are determined by different factors during development. An imbalance of glutamatergic and GABAergic neurons leads to abnormal brain development. The aim of this study was to assess the regulation of excitatory and inhibitory synapses in developing cortical neurons during the modulation of synaptic activity. Vesicular glutamate transporter 1 (vGlut1) and vesicular GABA transporter (vGAT) were used as markers for glutamatergic and GABAergic synapses, respectively, and their expression was established on MAP-2-labeled soma and dendrites of cortical neurons using immunofluorescent methods and quantitative analysis. The concentration of neurotransmitter in synaptic vesicles depends on the density and activity of vesicle transporters. The expression of vesicle transporters was evaluated as an average intensity of fluorescent signals during the modulation of neuronal activity. APV, an antagonist of NMDA receptors, inhibited spontaneous activity. APV regulated the distribution of vGlut1- and vGAT-labeled synapses. On the other hand, NMDA increased vGlut1 expression and decreased the number of vGlut1 labeled synapses. APV given prior to NMDA treatment prevented the reduction of glutamatergic synapses. The activation of NMDA receptors induced c-Fos expression in cortical neurons, and APV attenuated this effect. APV also enhanced the adhesive properties of cortical neurons and their synapses. This suggests that the inhibition of NMDA receptor activity induces Neuroligin and Neurexin adhesive trans-synaptic interaction in cortical neurons. An alteration of synaptic activity is significant for the regulation of vGlut1 and vGAT expression during axonal growth and synaptogenesis.

Disclosures: **V.L. Savchenko:** None.

Poster

592. Axon Growth and Guidance: Adhesion Molecules

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 592.13/B28

Topic: A.05. Axon and Dendrite Development

Support: 3DNeuroN project in the European Union's Seventh Framework Programme, Future and Emerging Technologies, grant agreement no. 296590

Title: Electrically driven axonal outgrowth on multi-electrode arrays

Authors: *S. A. WEYDERT, C. FORRO, J. CHAABAN, H. DERMUTZ, L. DEMKO, J. VOROS;
Lab. of Biosensors and Bioelectronics, ETH Zurich, Zurich, Switzerland

Abstracts: One of the grand challenges of this century is to learn how our brain functions. Despite of the vast amount of technical possibilities we still have very little insight (and especially consensus) into e.g. how memory is stored. One reason for this might be that our current knowledge in neurosciences mostly derives from single cell technologies such as patch clamping, or system level studies (e.g. MRI). Multi-electrode arrays (MEA) try to fill in this gap by providing information about neural slices and cultures, however, even though several attempts have been made to build more controlled neuron networks (microcontact printing, microfluidics, etc.) there is still no reliable technology that could provide well-defined *in vitro* networks with oriented connections on MEA chips. Due to the lack of such "study networks", the field is forced to perform experiments on highly complex systems which makes a complete understanding of the fundamental aspects very difficult. In this work we describe a comprehensive method to realize small neuronal networks on MEA chips in a controlled and reproducible manner. The type, number, position of the neurons, as well as the connections and their directionalities can be defined and kept under control by combining PDMS sheets and electrically driven axonal outgrowth. The role of the PDMS sheets are multifold: first they provide control over the seeding concentration and cell positions, then they confine neurite outgrowth into the predefined directions and make closed compartments for improved electrical recordings. We demonstrate the applicability of the method with a self-designed MEA chip as a proof of concept, by realizing directed small neuronal loops of rat hippocampal neurons and measuring their activity. Here the specially arranged electrodes also have multiple roles: they can induce electrochemically controlled surface chemistry changes, can be used as "electric fences" to control neurite outgrowth, and of course can also fulfill their primary duty of recording extracellular activities and applying electrical stimuli.

Disclosures: **S.A. Weydert:** None. **C. Forro:** None. **J. Chaaban:** None. **H. Dermutz:** None. **L. Demko:** None. **J. Voros:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.01/B29

Topic: A.05. Axon and Dendrite Development

Support: CIHR Grant MOP-130282

Title: The proinflammatory cytokine, interleukin-17A, augments axonal plasticity and mitochondrial function of cultured adult sensory neurons

Authors: ***T. HABASH**[1,2], A. SALEH[1], S. ROY CHOWDHURY[1], P. FERNYHOUGH[1,2]; [1]St-Boniface Hosp. Res. Ctr., Winnipge, MB, Canada; [2]Pharmacol. and therapeutics, Univ. of Manitoba, Winnipeg, MB, Canada

Abstracts: Rationale and hypothesis: Diabetic neuropathy involves dying back of nerve endings that reflects impairment in axonal plasticity and regenerative nerve growth. Metabolic changes in diabetes can lead to a dysregulation of hormonal mediators, such as cytokines. Thus we studied the effect of interleukin-17A (IL-17A), a proinflammatory cytokine produced by T-cells, on the phenotype of sensory neurons derived from control or diabetic rats. We hypothesized that diabetes induces suboptimal cytokine levels in neurons that reduces axonal growth and this may underlie impaired axon regeneration and plasticity within the skin leading to sensory loss. Objectives: Determine the ability of IL-17A to enhance neurite outgrowth in cultured sensory neurons. Investigate the signalling pathways activated by IL-17A and mechanistically link to neurite outgrowth. Study the ability of IL-17A to improve mitochondrial function of sensory neurons (since axon outgrowth consumes high levels of ATP). Methodology: Cultured adult dorsal root ganglia (DRG) sensory neurons derived from age matched control or streptozotocin (STZ)-induced type 1 diabetic rats were fixed and stained for fluorescent imaging to determine total neurite outgrowth. Western blotting determined the levels of MAPK and PI-3K activation by IL-17A and for measuring levels of proteins of mitochondrial oxidative phosphorylation pathway. Mitochondrial bioenergetics function was tested in cultured DRG neurons using the Seahorse XF Analyzer. Results: We found that IL-17A (10 ng/ml; $P<0.05$) significantly increased total neurite outgrowth in cultures derived from both control and STZ-diabetic rat models. This enhancement was mediated by IL-17A-dependent activation of MAPK and PI-3K

pathways with maximal effect at 15 minutes ($P<0.05$). Pharmacological blockade of one of these activated pathways led to total inhibition of neurite outgrowth. IL-17A improved mitochondrial bioenergetics function of sensory neurons. Bioenergetics function was associated with augmented expression of proteins of mitochondrial oxidative phosphorylation. Conclusion: IL-17A enhanced axonal plasticity through activation of MAPK and PI-3K pathways and was associated with augmented mitochondrial bioenergetics function in sensory neurons.

Disclosures: T. Habash: None. **A. Saleh:** None. **S. Roy Chowdhury:** None. **P. Fernyhough:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.02/B30

Topic: A.05. Axon and Dendrite Development

Support: Marie Curie Actions (IEF), Project 253502

Title: Imaging axonal varicosities of developing neurons by cryo-electron tomography

Authors: N. SCHROD[1], D. VANHECKE[2], W. BAUMEISTER[1], *V. LUCIC[1];
[1]Dept. of Mol. Structural Biol., Max Planck Inst. of Biochem., Martinsried, Germany; [2]Adolphe Merkle Inst., Univ. of Fribourg, Fribourg, Switzerland

Abstracts: Axons of developing neurons are known to contain packets of vesicles and other membranous structures required for synaptogenesis. Here, we used cryo-electron tomography to image developing hippocampal neurons grown in culture. As a result, we obtained three-dimensional images of fully hydrated, vitrified neuronal processes. Our analysis focused on axonal varicosities, in particular on those that contained synaptic vesicle packets or other membranous structures. In addition to describing morphology of vesiclular and tubulovesicular structures, we analyzed the vesicle organization and their inter-connectivity in a quantitative manner. Our results indicate that synaptic vesicles are interconnected by filamental structures, as is the case in presynaptic terminals of mature synapses that we analyzed in the past, but are significantly less tethered to the plasma membrane then the vesicles of mature synapses. In addition, we used automated segmentation methods to detect and visualize actin in axonal varicosities and filopodia.

Disclosures: **N. Schrod:** None. **D. Vanhecke:** None. **W. Baumeister:** None. **V. Lucic:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.03/B31

Topic: A.05. Axon and Dendrite Development

Support: CIHR Grant MOP. 130282

Title: Sirtuin 2 is a sensor of energy status and inducer of neurite outgrowth in adult sensory neurons

Authors: ***E. D. SCHARTNER**[1,2], A. SALEH[1], R. VIEIRA DA SILVA[1], S. CHOWDHURY[1], D. SMITH[1], P. FERNYHOUGH[1,2];
[1]St. Boniface Hosptial Res., Winnipeg, MB, Canada; [2]Pharmacol. and Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada

Abstracts: Introduction: Diabetic sensory neuropathy is a nerve disorder that results in distal dying back of nerve fibers. Neuronal mitochondrial function is impaired in diabetes and Sirtuin 2 (SIRT2) is a key sensor of redox state that regulates cellular bioenergetics. We hypothesized that defective SIRT2 signaling contributed to deficiencies in energy supply and nerve regeneration in diabetic neuropathy. Objectives: We studied the different mechanisms of SIRT2 signaling within adult sensory neurons that drive axon regeneration and how these pathways were impaired under diabetic conditions. Methods: Type 1 diabetes was induced in rodents by intraperitoneal injection of streptozotocin (STZ). Dorsal root ganglia (DRG) were isolated from age matched and 5 month STZ-diabetic mice and SIRT2 protein levels analyzed. DRG sensory neurons derived from control and STZ-diabetic rats were cultured in defined media with varying concentrations of neurotrophic factors and D-glucose. Quantitative Western blotting was performed to determine protein levels and immunocytochemistry utilized to quantify neurite outgrowth. Lentiviral transduction and plasmid transfection were instigated for shRNA knockdown of SIRT2 and overexpression of SIRT2 constructs. Results: SIRT2 isoforms 2.1 and 2.2 were reduced by 20-30% in DRG of diabetic mice ($P<0.05$). In cultured adult sensory neurons derived from age matched control or STZ-diabetic rats over-expression of wild-type SIRT2 significantly enhanced total neurite outgrowth. Overexpression of the dominant negative mutant (SIRT2-H150) or shRNA to SIRT2 blocked neurite outgrowth. Application of varying doses of AGK2, a selective inhibitor of SIRT2, significantly reduced total neurite outgrowth ($P<0.05$). Treatment with

increasing doses of NAD+, an endogenous activator of SIRT2, significantly enhanced neurite outgrowth. After 72hrs in media with high D-glucose (25mM), cultured sensory neurons showed a significant 2-fold ($P<0.05$) decrease in the protein levels of SIRT2 isoforms. Conclusions: SIRT2 is a key component driving axon regeneration and this pathway was impaired in diabetic sensory neuropathy.

Disclosures: E.D. Schartner: None. **A. Saleh:** None. **R. Vieira Da Silva:** None. **S. Chowdhury:** None. **D. Smith:** None. **P. Fernyhough:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.04/B32

Topic: A.05. Axon and Dendrite Development

Support: NSF Grant DBI-0852081

NSF Grant IBN-1355045

Title: Process retraction requires the calcium-activated protease calpain in homolog avoidance leading to tiling by motor neurons

Authors: L. SOLANO[1], E. R. MACAGNO[1], *M. W. BAKER[2];
[1]Div. of Biol. Sci., UCSD, La Jolla, CA; [2]Div. of Biol. Sci., Unive California, San Diego, La Jolla, CA

Abstracts: Oppositely directed projections of some homologous neurons in the developing CNS of the medicinal leech, such as the AP cells, undergo a form of contact-dependent homolog avoidance, a form of cellular tiling behavior. Embryonic APs extend axons within the connective nerve toward adjacent ganglia, in which they (1) meet and form gap junctions (GJs) with the oppositely directed axons of their segmental homologs, (2) stop growing, and are (3) later permanently retracted (Wolszon et al., 1994, J. Neurosci. 14). However, early deletion of an AP neuron (Gao and Macagno, 1987, J. Neurobiol. 18) or selective knockdown of the gap junction gene Inx1 (Baker at al., 2013, J. Neurosci. 33), leads to resumed growth and permanent maintenance of the projections of neighboring APs. Continued growth was also observed when a closed-channel mutant of Inx1 was expressed by the AP neuron indicating the likely exchange of a soluble signal between the cells. Here we explore the nature of the signal that causes the AP's

connective processes to retract. Following the formation of the transient GJ, the distal processes of the AP neuron were observed to undergo Wallerian fragmentation, suggesting the possible activation of an intracellular protease. Calcium imaging has also revealed that these distal GJs permit calcium exchange between the contacting processes suggesting that one potential candidate for mediation may be the calcium-activated protease calpain which has been shown to function downstream of calcium transients. Accordingly, we employed RNAi to knockdown a neuronal leech calpain (CalI) in individual AP neurons in the developing embryo and found that homolog avoidance was significantly impaired. AP neurons were observed to extend processes into and beyond their adjacent ganglia, mimicking the effects of cellular ablation or Inx1 knockdown. Ongoing studies will explore the possible link between GJ signaling and CalI activation by targeting the source of compartmental calcium transients by examining the role-played by voltage-gated calcium channels and mechanisms for the release of calcium from internal stores.

Disclosures: **L. Solano:** None. **E.R. Macagno:** None. **M.W. Baker:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.05/B33

Topic: A.05. Axon and Dendrite Development

Title: The quantitative proteomes and transcriptomes of projection-specific growth cones from mouse brain

Authors: *A. POULOPOULOS, A. J. MURPHY, P. F. DAVIS, J. D. MACKLIS;
Stem Cell and Regenerative Biol., Harvard Univ., Cambridge, MA

Abstracts: The development of wiring diagrams in the nervous system is driven by the cellular machineries of growth cones, the leading edges of extending axons. These specialized cellular compartments contain select sets of macromolecules that enable growth cones to determine the paths, and ultimate target fields of their dedicated axon projections. Though we lack an understanding of the integrated molecular mechanisms, and layers of complex orchestration, neuronal circuit development may be viewed as an emergent property of the coordinated interactions between the molecular machinery of individual growth cones, and the sequence of molecular surroundings they encounter. This hierarchical organization from growth cone to brain circuits is made possible by the high degree of molecular specialization, compartment autonomy,

and acute responsiveness to stimuli that growth cones display. Functioning at great distances from the cell soma, growth cones contain specific and functionally integrated sets of proteins, RNA, and lipids, endowing each class of growth cones with a distinct molecular complement so as to contribute its part to the wiring diagram. This makes growth cones highly heterogeneous, likely mirroring the heterogeneity of neurons and axon projections themselves. As such, growth cone heterogeneity is an important feature of nervous system development, but also a major experimental obstacle to the comprehensive, analytical study of the physiological molecular machineries that drive circuit development. In order to have direct experimental access to the molecular compositions of individual classes of growth cones in their native context, we have developed a new approach, combining *in vivo* hodological and genetic labeling techniques, biochemical fractionation, and FACS to obtain the endogenous growth cone proteomes and transcriptomes of individual projection classes in the mouse brain. Applying this approach to projections that cross the corpus callosum, we have acquired quantitative datasets that provide a comprehensive view of the growth cone molecular machinery, its compartmentalization, and heterogeneity.

Disclosures: **A. Poulopoulos:** None. **A.J. Murphy:** None. **P.F. Davis:** None. **J.D. Macklis:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.06/B34

Topic: A.05. Axon and Dendrite Development

Support: NSC102-2321-B-010-026

Title: Functions of Cdk12 in axonal elongation and neural development

Authors: ***M.-J. FANN**, H.-R. CHEN, C.-K. HUANG;
Dept of Life Sci., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstracts: Cdk12 is a Cdc2-related protein and expresses at high levels in the developing nervous system. We explored the roles of Cdk12 in neuronal differentiation in the P19 neuronal differentiation model. Upon knockdown of Cdk12, there is no effect on numbers of differentiated cells, but it generates a substantial decrease of numbers of neurons with long neurites. Similarly, knockdown of Cdk12 in cultured cortical neurons shortens the averaged axonal length. A

microarray analysis was used to examine changes in gene expression after knockdown or overexpression of Cdk12 and we identified Cdk5 as a molecule potentially involved in mediating the effect of Cdk12. Depletion of Cdk12 in P19 cells significantly reduces Cdk5 expression at both the mRNA and protein levels. Furthermore, overexpression of Cdk5 protein in P19 cells partially rescues the neurite outgrowth defect observed when Cdk12 is depleted. To define the role of Cdk12 during neural development *in vivo*, we conditionally targeted Cdk12, using a mouse line expressing Cre recombinase driven by a nestin promoter. Cdk12 cKO mice die around P1 and exhibit a significant size reduction of the brain, especially in the cortex, but are indistinguishable in body sizes. Cdk12 ablation results in reduced thickness of the neuronal layers and delayed migration of late-born neurons in the Cdk12-ablated E14.5 dorsal telencephalon. In addition, expression of Cdk5 protein in developing mouse brain is reduced in Cdk12 cKO mice proportionally to the amounts of residual Cdk12 protein. Deficiency of Cdk12 also markedly attenuates phosphorylation of Erk in neural tissues. Taken together, function of Cdk12 is crucial for several aspects of neuronal development, including axonal growth and cell migration, by modulating Cdk5 expression.

Disclosures: **M. Fann:** None. **H. Chen:** None. **C. Huang:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.07/B35

Topic: A.05. Axon and Dendrite Development

Support: Stowers Insitute for Medical Research

Title: A developmental switch of the axon guidance in the continues regeneration mouse olfactory system

Authors: ***Y. WU**[1,2], L. MA[1], Q. QIU[1], H. SCHEERER[1], A. MORAN[1], C. R. YU[1,3];
[1]Stowers Insitute For Med. Res., Kansas City, MO; [2]the Open Univ., Milton Keynes, United Kingdom; [3]Dept. of Anat. and Cell Biol., Univ. of Kansas Med. Ctr., Kansas city, MO

Abstracts: The mammalian olfactory sensory neurons are regenerated through the life of an animal. Each sensory neuron specifically expresses only one type of olfactory receptor out of a ~1000 repertoire. The axons of the neurons expressing the same receptor converge into the same stereotypic position in the brain, thus forming a topographic map, critical for odorant perception.

This precise connection between olfactory sensory neuron and the brain is maintained through life. Regenerated sensory neurons must project properly to the target glomeruli. We have identified a critical period during the postnatal development of the olfactory sensory neuron. After the critical period, the olfactory sensory neuron adopts a different axon guidance mechanism and follows existing axons of the same receptor type. This observation suggests a mechanism for the maintenance of the odor map. Furthermore, we have taken a RNA-seq approach to study the genetic program and to identify a group of potential genes that may be involved in the control of the critical period.

Disclosures: Y. Wu: None. **L. Ma:** None. **Q. Qiu:** None. **H. Scheerer:** None. **A. Moran:** None. **C.R. Yu:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.08/B36

Topic: A.05. Axon and Dendrite Development

Support: University of Otago

Gravida: National Centre for Growth and Development PhD scholarship

Title: Understanding the roles of intrinsic and extrinsic influences on fetal arcuate nucleus neuron development in gestational obesity

Authors: T. R. SANDERS, *C. JASONI;
Ctr. for Neuroendocrinology and Dept. of Anat., Univ. of Otago, Dunedin, New Zealand

Abstracts: An association has been clearly shown in humans and rodent models between maternal obesity during pregnancy, and subsequent risk for later life disease, including obesity and the metabolic syndrome, in the offspring. Furthermore, we and others have shown that the ability of body weight regulating neurons in the arcuate nucleus of the hypothalamus (ARC) to innervate their targets is disrupted in the prenatal and postnatal offspring of obese mothers. However, the mechanism behind this is unknown. It has recently emerged that maternal obesity is associated with increases in certain inflammatory cytokines, including interleukin-6 (IL-6), in both the maternal and fetal circulation. Further to this, we have used *in vitro* cultured explants from gestational day 17.5 (GD17.5) mouse ARC, to show that IL-6 dose dependently retards

neurite outgrowth and is able to significantly alter the expression of DCC and Unc5d, receptors for the axon growth regulator Netrin-1. These developmental gene expression changes as well as retarded growth of ARC NPY neurites to one of their target structures, the paraventricular nucleus of the hypothalamus, were also observed *in vivo* in the fetuses of pregnancies complicated by maternal obesity. Although it appears that IL6 causes NPY neurons to alter their responsiveness to Netrin-1, whether this effect is direct or indirect, and/or whether an environment of maternal obesity alters the intrinsic ability of NPY neurons to develop normally is unknown. To address this further, we have established a novel *in vitro* culture system in which ARC neurons, in particular the weight regulating NPY (Neuropeptide Y) and POMC (Pro-opiomelanocortin) neurons, from fetuses developing in either obese or normal weight mothers are assessed for their intrinsic neurite growth properties. In these cultures, the relative proportions of surviving NPY and POMC neurons mirror the normal proportions observed *in vivo* across ARC development, indicating that this novel system adequately represents normal ARC development. In summary, the cytokine IL-6 has the ability to perturb the normal growth of developing ARC neurites, a mechanism which could explain maternal obesity-induced decreases in the ability of offspring ARC neurons to innervate their targets. Establishment of a novel cell culture system, which mirrors normal development, gives us the ability to identify both intrinsic differences in ARC neuronal growth characteristics, and extrinsic modulators of normal development. Data from these experiments is critical to understanding the underlying brain-level changes that elevate offspring health risk in pregnancies complicated by maternal obesity.

Disclosures: T.R. Sanders: None. **C. Jasoni:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.09/B37

Topic: A.05. Axon and Dendrite Development

Support: NIH-NIA R01AG031524

NIH-NINDS F32NS080464

Title: Presynaptic mitochondrial capture is required for proper axon branching *in vitro* and *in vivo*

Authors: *T. LEWIS, JR, J. COURCHET, F. POLLEUX;
Dept. of Neurosci., Columbia Univ. Med. Ctr., New York, NY

Abstracts: The mechanisms underlying axonal development are inadequately understood but are critical for the proper formation of neural circuits. We have uncovered a new role for LKB1 and its downstream kinase NUAK1 in regulating axon branching both *in vitro* and *in vivo* by controlling mitochondria immobilization along axons (Courchet, Lewis et al., Cell 2013). Using manipulation of Syntaphilin, a protein necessary and sufficient to arrest mitochondrial transport specifically in the axon, we demonstrate that the LKB1-NUAK1 kinase pathway regulates axon branching by promoting mitochondria immobilization. Finally, we show in dissociated neuronal cultures that LKB1 and NUAK1 are necessary and sufficient to immobilize mitochondria specifically at nascent presynaptic sites. To determine if mitochondrial dynamics and capture are similar *in vivo*, we are using sparse labeling of layer 2/3 pyramidal neurons followed by live time-lapse microscopy in brain slices and head-fixed mice. Our results reveal a link between presynaptic mitochondrial capture and axon branching.

Disclosures: T. Lewis: None. **J. Courchet:** None. **F. Polleux:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.10/B38

Topic: A.05. Axon and Dendrite Development

Support: HHMI

Title: Hyperactivating mutations in alpha2-chimaerin alter motor neuron development to cause Duane Retraction Syndrome

Authors: *A. A. NUGENT, Y. WEI, J. G. PARK, M. M. DELISLE, E. C. ENGLE;
Boston Children's Hosp., Boston, MA

Abstracts: In Duane Retraction Syndrome (DRS), the abducens cranial nerve does not appropriately innervate the lateral rectus (LR) eye muscle and the oculomotor nerve may aberrantly innervate the LR, resulting in limited outward gaze and globe retraction upon inward gaze. People with DRS can carry heterozygous, gain-of-function missense mutations in *CHN1*, which hyperactivate the encoded protein α2-chimaerin. We have introduced one of the human

DRS mutations into mouse, creating a *Chn1* L20F mutant knock-in (*Chn1*^{KI}). *Chn1*$^{WT/KI}$ and *Chn1*$^{KI/KI}$ mice are viable and fertile, but have an external eye retraction phenotype, with varying penetrance. In developing *Chn1*$^{WT/KI}$ and *Chn1*$^{KI/KI}$ mice, mutant α2-chimaerin causes aberrant axon growth and guidance of VI and subsequent elimination of Hb9-positive abducens motor neurons in the hindbrain nucleus. Inhibiting apoptosis does not prevent abducens nerve growth and guidance defects, suggesting DRS is primarily an axon growth or guidance disorder. The oculomotor nerve aberrantly branches toward the LR, suggesting *Chn1*^{KI} mice recapitulate human DRS. Additional motor neuron populations appear abnormal in *Chn1*$^{KI/KI}$ mice, as the trochlear nerve, facial nerve, and first cervical segment of the spinal cord also have developmental defects. While patients can exhibit oculomotor and trochlear nerve dysinnervation and/or misinnervation, it remains to be determined whether human DRS patients have facial or spinal nerve abnormalities. This work establishes and characterizes a mouse model for DRS, and provides the basis for further studies to investigate the etiology of DRS.

Disclosures: A.A. Nugent: None. **Y. Wei:** None. **J.G. Park:** None. **E.C. Engle:** None. **M.M. Delisle:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.11/B39

Topic: A.05. Axon and Dendrite Development

Support: R01 CA95060

Title: Competing aPKC isoforms regulate neuronal polarity

Authors: S. S. PARKER[1], S. M. HAPAK[2], E. K. MANDELL[3], *S. GHOSH[3];
[1]Univ. of Arizona, Tucson, AZ; [2]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [3]Yale Univ. Sch. of Med., New Haven, CT

Abstracts: Atypical Protein Kinase C (aPKC) isoforms zeta and iota interact with polarity complex protein Par3 and are evolutionarily conserved regulators of cell polarity. The Prkcz gene encodes the proteins aPKCzeta and PKMzeta, a truncated, neuron-specific alternative product, while Prkci gene encodes aPKCiota protein. Here we show that, in embryonic hippocampal neurons, two aPKC isoforms - aPKCiota and PKMzeta are expressed. The localization of these isoforms is spatially distinct in a polarized neuron. aPKCiota, as well as

Par3, localizes at the presumptive axon, while PKMzeta and Par3 are distributed at non-axon forming neurites. PKMzeta competes with aPKCiota for binding to Par3 and disrupts the aPKCiota-Par3 complex. Silencing of PKMzeta, genetic ablation of Prkcz locus or overexpression of aPKCiota in hippocampal neurons alters neuronal polarity, resulting in neurons with supernumerary axons. In contrast, the overexpression of PKMzeta prevents axon specification. Our studies suggest a molecular model wherein mutually antagonistic intermolecular competition between aPKC isoforms directs symmetry breaking and allows for the establishment of neuronal polarity.

Disclosures: **S.S. Parker:** None. **S. Ghosh:** None. **S.M. Hapak:** None. **E.K. Mandell:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.12/B40

Topic: A.05. Axon and Dendrite Development

Title: Sildenafil promotes axonal growth of dorsal root ganglia neurons under high glucose conditions

Authors: *L. JIA;
Neurol. Res., Henry Ford Hosp., Detroit, MI

Abstracts: Objective: Diabetic peripheral neuropathy impairs the sciatic nerve. Sildenafil, a phosphodiesterase type 5 inhibitor, ameliorates neurological function in diabetic peripheral neuropathy. However, the direct effect of sildenafil on axonal growth has not been investigated. Using rat primary dorsal root ganglia (DRG) neurons cultured in a microfluidic chamber, we investigated the effect of sildenafil on axonal growth under a high glucose conditions. **Methods and Results:** DRG neurons were harvested from embryonic day 18 Wistar rats and cultured in a microfluidic chamber that separates axons from neuronal cell bodies. The neurons were cultured under regular glucose (25mmol/L, RG), high glucose (45mmol/L, HG), or high glucose with sildenafil (300ng/ml). Axonal length was measured on day 3 *in vitro*. The HG condition significantly reduced the axonal growth **(**838±189mm) compared to the RG condition **(**1073±203mm, n=45/group, $P<0.01$**).** However, sildenafil suppressed HG-reduced axonal growth **(**1096±198mm, n=45, $P<0.01$). Real-time RT-PCR and immunocytochemistry analysis of DRG neurons revealed that HG downregulated miR-146a levels (0.54 ± 0.12 vs. 1±0.02 in RG, n=6, $P<0.01$) and substantially ($p<0.01$) increased miR-146a target gene, IRAK1 (32±6% vs.18.9% in RG, n=6/group) and TRAF6 (34±6% vs.17.5% in RG, n=6/group) positive neurons. In contrast, sildenafil significantly ($p<0.01$) reversed HG-reduced miR-146a levels (0.93 ± 0.18, n=6) and HG-increased IRAK1 (16±3.1%) and TRAF6 (17±2.3%) positive cells. Elevation of miR-146a in DRG neurons by transfection of miR-146a mimics significantly increased axonal growth (362±68mm vs.313±56mm, n=6) under HG condition, while decrease of miR-146a by miR-146a inhibitor reduced axonal growth (196±23mm vs.305±43mm, n=6). **Conclusion:** Our data suggest that sildenafil suppresses the inhibitory effect of high glucose on axonal growth of DRG neurons and miR-146a and its target genes, IRAK1 and TRAF6 may mediate this process.

Disclosures: **L. Jia:** A. Employment/Salary (full or part-time):; Henry Ford Hospital. 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.; RO1 NS075084.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.13/B41

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS30255

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

UNM Lane scholarship (JS)

Title: Post-transcriptional control of axonal growth by binding of HuD and KSRP to common neuronal targets

Authors: A. S. GARDINER[1], C. GOMES[2], J. SAAVEDRA[1], J. L. TWISS[2], *N. PERRONE-BIZZOZERO[1];
[1]Neurosciences, Univ. of New Mexico HSC, ALBUQUERQUE, NM; [2]Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstracts: Post-transcriptional mechanisms are known to play important roles in neuronal development as they allow for faster responses to environmental cues and provide spatially restricted compartments for local control of protein expression in both axons and dendrites. Among these mechanisms, the control of mRNA stability by the interaction of RNA-binding proteins (RBPs) with AU-rich instability elements (ARE) in the 3' UTR is one of the least understood. We have recently shown that two ARE-BPs, HuD and KSRP, compete for binding to the ARE in GAP-43 mRNA (Bird et al, PLoS One 2013). HuD binding stabilizes and localizes GAP-43 mRNA, promoting axonal outgrowth, while KSRP binding destabilizes GAP-43 mRNA and stunts outgrowth. These results suggest that these proteins compete for the control of target expression and process outgrowth during neuronal development. Supporting this idea, we recently found that KSRP and HuD show a different temporal pattern of expression both in culture and *in vivo*, with HuD preceding KSRP expression. Moreover, HuD and KSRP localize

to different RNA granules both in the cell body and processes. To initially identify the repertoire of neuronal transcripts regulated by these ARE-BPs, we used UV-crosslinking and RNA immunoprecipitation followed by microarray assays (CLIP-chip). Briefly, E18 C57BL/6 mouse neocortical tissue was processed for CLIP using antibodies to HuD, KSRP or control IgG and RNAs from the IPs analyzed on Affymetrix GeneChip 430 2.0 arrays. Results from these assays were validated using qRT-PCR and KSRP KO mice. We found that KSRP and HuD bound both distinct and shared sets of target mRNAs. Besides GAP-43, other common targets included β-actin *(Actb),* catenin β1 *(Cttnb1),* eukaryotic initiation factor 4G2 *(Eif4g2),* fasciculin 1 *(Fscn1),* heat shock cognate protein 70kDa *(Hspa8),* myristoylated alanine-rich protein kinase C substrate *(Marcks*), Stathmin 1 *(Stmn1),* transthyretin *(Ttr)*, and Tubulin β2b *(Tubb2b).* Interestingly, many of these targets are localized to axons and upregulated in KSRP KO mice, which express normal levels of HuD protein. Localized mRNAs binding selectively to either HuD or KSRP were also identified, including *Atxn10*, *Eif5, Pebp1* and *Tuba1b* (KSRP) and *Calr, Tpm3, Tubb5*, and *Vim* (HuD). These results suggest that the opposing roles of HuD and KSRP in axonal outgrowth are due in part to the differential regulation of common and unique targets, with HuD stabilizing and KSRP destabilizing localized transcripts.

Disclosures: A.S. Gardiner: None. **C. Gomes:** None. **J. Saavedra:** None. **J.L. Twiss:** None. **N. Perrone-Bizzozero:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.14/B42

Topic: A.05. Axon and Dendrite Development

Support: Grants-in-Aid for Scientific Research (C) (23500439)

Grants-in-Aid for Scientific Research for Young Scientists (B) (23700376) from Ministry of Education, Culture, Sports, Science and Technology of Japan. Natural Medicine and Biotechnology Research, Toyama Prefecture, Japan.

Title: Extracellular vimentin promotes axonal growth via IGF1R

Authors: *M. SHIGYO, T. KUBOYAMA, C. TOHDA;
Div. of Neuromedical Sci., Inst. of Natural Medicine, Univ. of Toyama, Toyama, Japan

Abstracts: Vimentin, an intermediate filament protein, is generally known as an intracellular protein related to cell adhesion and cell migration. Recently, we reported that vimentin secreted from astrocytes promoted axonal growth in cultured mouse cortical neurons. The effect of extracellular vimentin in neurons was a new finding, but its signal pathway was unrevealed. In this study, we aimed to clarify the signaling mechanism of extracellular vimentin that facilitates axonal growth. Primary cultured rat cortical neurons (SD, E17) were treated with human recombinant vimentin (1.75 μM) for 10 min, and then cell lysates from vehicle treated or vimentin-treated neurons were analysed with phosphoprotein arrays. As a result, IGF1R was identified as a highly phosphorylated molecule by vimentin stimulation. In primary cultured mouse cortical neurons (ddY, E14), vimentin (1.75 μM) as well as IGF1 (1.3 μM) facilitated axonal growth at 6 days after the treatment. Vimentin-elicited axonal growth was completely inhibited by pretreatment with an IGF1R inhibitor (IGF1-analog) and with a neutralizing antibody of IGF1R. Phosphorylation level of IGF1R by vimentin was peaked at 30 min after the stimulation. The level was higher than that by IGF1 treatment at a similar dose. ELISA experiment confirmed that IGF1 was not released from neurons into culture medium within 30 min after the vimentin stimulation. These results suggest that vimentin stimulates IGF1R, leading to axonal growth. Our results show the new signaling of axonal growth via IGF1R by extracellular vimentin. The finding may provide a novel strategy to induce axonal growth in neurodegenerative diseases.

Disclosures: **M. Shigyo:** None. **T. Kuboyama:** None. **C. Tohda:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.15/B43

Topic: A.05. Axon and Dendrite Development

Title: Goα protein modulates the neuritogenesis in primary neurons

Authors: *H.-H. OH[1,2], J.-M. CHOI[1], S.-P. YOON[1], Y.-D. LEE[1,2], S.-S. KIM[1], H. SUH-KIM*[1,2];
[1]Dept, of Anat., [2]Neurosci. Grad. Program, Ajou University, Sch. of Med., Suwon, Korea, Republic of

Abstracts: Heterotrimeric G-proteins mediate signal transduction generated by numerous neurotransmitters and hormones. Among all G-proteins, Goα, a member of the Go/i family, is the

most abundant G protein in brain. Although Goα has been implicated in neuronal differentiation, the mechanism of how Go modulates neuronal differentiation has not been defined. We previously showed that Goα may modulates neurite outgrowth in F11 cells. Expression of Goα decreased the average length of neurites but increased the number of neurites per cell by interfering cAMP-PKA-CREB signaling (Ghil et al., 2000 and 2006). In this study, we investigated the roles of Goα during the neuritogenesis in primary cultured neurons through the aspects of cytoskeletal filament such as microtubule and F-actin. Short protrusions or neurites were found to be less extended in Goα knock-out neurons. Our data showed that the formation of protrusions/neurites is delayed in the absence of Goα. The data also suggest that Goα may induce the formation of protrusions/neuritis at earlier time. Further, we will discuss the mechanisms by which Goα regulate the neuritogenesis during differentiation and maturation of neuronal cells.

Disclosures: H. Oh: None. **J. Choi:** None. **S. Yoon:** None. **Y. Lee:** None. **S. Kim:** None. **H. Suh-Kim*:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.16/B44

Topic: A.05. Axon and Dendrite Development

Title: Dual roles of Unc-51-like kinase 1/2 in embryonic and postnatal mouse brain development

Authors: *B. WANG[1,2], M. KUNDU[2];
[1]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Dept. of Pathology, St Jude Children's Res. Hosp., Memphis, TN

Abstracts: Unc-51-like kinases 1 and 2 (ULK1/2), the mammalian homologues of *C. elegans* unc-51, are highly conserved serine/threonine kinases. Previous *in vitro* studies revealed important function of ULK1/2 and their homologues in regulating both invertebrate and mammalian neurodevelopment and in regulating autophagy, a bulk lysosome-mediated degradation pathway. ULK1 or ULK2 single-knockout (KO) mice show no overt phenotype, and ULK1/2 double-KO (DKO) mice die perinatally; therefore, the physiological role of ULK1/2 *in vivo* remains largely unknown. To evaluate embryonic neurodevelopmental defects and the contribution of ULK1/2-mediated autophagy to the maintenance of homeostasis in mature neurons, we generated central nervous system specific ULK1/2 conditional DKO (cDKO) mice. Approximately half of the ULK1/2 cDKO mice survived to adult age but showed severe growth

retardation and compromised motor performance. Distinct from Atg7 cKO mice, in which axonal guidance is normal, the ULK1/2 cDKO mice consistently show general defects in the axonal guidance in the forebrain, causing dysgenesis of corpus callosum and anterior commissure. However, no overt defects in neurogenesis and migration were observed. Adult cDKO mice also developed degenerative axonal swelling in the deep cerebellum nuclei, hippocampal pyramidal neuron loss in the CA1 region, and subsequently degeneration in the CA3 region. The phenotypic difference between ULK1/2 KO mice and the previously characterized Atg7 KO mice highlights essential dissimilarities between the function of ULK1/2 and canonical autophagy. Understanding the autophagy-related and -unrelated roles of ULK1/2 in physiologic and pathologic conditions is important since drugs targeting these kinases are being developed to inhibit autophagy for therapeutic purposes.

Disclosures: B. Wang: None. **M. Kundu:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

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Topic: A.05. Axon and Dendrite Development

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NEI grant P30-EY022589

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Research to Prevent Blindness

Title: Characterization and differential expression analysis of polyadenylated and non-polyadenylated riboRNA-depleted transcripts during maturation of defined population of CNS neurons

Authors: *E. F. TRAKHTENBERG[1], D. W. PITA-THOMAS[2], D. VELMESHEV[3], S. M. DOMBROWSKI[4], J. L. GOLDBERG[5];

[1]Neurosurg., Boston Children's Hospital, Harvard Med. Sch., Boston, MA; [2]Anat. and Neurobio., Washington Univ., St. Louis, MO; [3]Mol. and Cell. Pharmacol., Univ. of Miami Sch. of Med., Miami, FL; [4]Genomatix Software; and Wayne State Univ. Sch. of Med., Ann Arbor; and Detroit, MI; [5]Shiley Eye Ctr., Univ. of California San Diego, La Jolla, CA

Abstracts: Molecular changes during maturation of the central nervous system (CNS) neurons are associated with multiple developmentally regulated biological processes, such as decline in intrinsic axon growth capacity, regulation of axon pathfinding, maturation of dendritic arborization, and establishment of neural circuits. Thus, understanding the intrinsic molecular changes involved in maturation of CNS neurons is important for studying their development and function. Retinal ganglion cell (RGC) is a prototypical CNS projection neuron, previously used for studying transcriptional changes associated with neuronal maturation. However, the developmental changes in RGCs were studied using older version of microarray chips, which were limited by gene probes' availability and reliability, by most could not discern between alternatively spliced transcripts from the same locus, and the probes for tens of thousands of small and long-non coding RNAs were not available. Next Generation Sequencing (NGS) using RNA sequencing (RNA-seq) overcame these limitations by affording the ability to analyze expression of coding and non-coding RNAs, discern between alternatively spliced transcripts, and identify novel transcripts. Here, we purified by immunopanning embryonic and postnatal mouse RGCs, and used the NGS analysis to evaluate the prevalence of non-coding transcripts in the RGC transcriptome, and to investigate the developmental changes in coding and non-coding transcripts expression during RGC maturation. For identification of developmentally regulated mRNA transcripts we used a polyA-specific RNA isolation kit, for non-coding RNAs (ncRNA) we used total RNA after ribosomal RNA depletion. We found 19872 differentially expressed transcripts (2-fold or greater) across 5332 loci in the polyA-selected RNA samples, and 29391 differentially expressed transcripts (2-fold or greater) across 14347 loci in the ribosomal RNAs depleted samples. We also provided evidence supporting the hypothesis that polyA-selected- and ribosomal RNA-depleted transcriptomes are more dissimilar than similar, and that the difference is accounted for primarily by non-coding RNAs. These NGS analyses characterize the transcriptome diversity and developmental changes in a population of CNS primary neurons, as well as point to novel potential regulators of neuronal maturation and axon growth.

Disclosures: E.F. Trakhtenberg: None. **D.W. Pita-Thomas:** None. **D. Velmeshev:** None. **S.M. Dombrowski:** None. **J.L. Goldberg:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.18/B46

Topic: A.05. Axon and Dendrite Development

Title: Involvement of thyroid hormone receptor alpha in GnRH neuronal migration

Authors: *A. LAMARCA, E. FLANNERY, S. WRAY;
Celular& Developmental Neurobio. Section, Natl. Inst. of Hlth., Bethesda, MD

Abstracts: Migration of gonadotropin-releasing hormone-1 (GnRH) neurons from the nasal placode to their correct location within the brain during mammalian development is a critical step for sexual maturation and reproductive function. It is well documented that these neurons migrate from the nasal placode to the hypothalamus where GnRH is released during puberty. It is also known that a failure of this processes leads to hypogonadotropic hypogonadism (HH), resulting in delayed puberty and infertility. However, our understanding of the mechanisms controlling GnRH migration is still limited. Microarray analysis revealed novel molecules that were highly expressed in prenatal migrating GnRH neurons, including thyroid hormone receptor alpha (THRα). Co-expression of THRα has been reported in a subset of GnRH neurons in adult hamster and sheep (Heiko et al, 1997), but the physiological role of signaling via this receptor in GnRH cells remains unclear. Notably, thyroid hormone influences neuronal migration and differentiation, such as migration of postmitotic granule cells and terminal differentiation of purkinje cells (Morte et al., 2002). The aim of this work is to determine whether signaling via THRα plays a role in the migration of GnRH neurons. Double label immunofluorescence confirmed expression of THRα in mouse primary prenatal GnRH neurons maintained in explant tissue. Using this model system and *in situ* migration assays, functional analysis with pharmacological and genetic approaches is being performed to determine the action of thyroid hormone via THRα in the neuronal migration of GnRH cells. The importance of thyroid hormones during brain development has been appreciated for many decades. Yet, a limited number of genes activated by thyroid hormone in developing neurons have been identified as compared to genes activated in glial cells. As such, examining the role of thyroid hormone in GnRH neuronal migration may reveal novel molecules modulated by thyroid hormone during development. References: Morte, B., Manzano, J., Scanlan, T., Vennström, B., Bernal, J. (2002) Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. 19, 99-106 Heiko, T., Lubbers, L., Macchia, E., DeGroot, L.J., Lehman, M, N. (1997). Thyroid hormone receptor distribution in hamster and sheep brain:colocalization in gonadotropin-releasing hormone and other identified neurons. Endocrinology 138, 11.

Disclosures: **A. Lamarca:** None. **E. Flannery:** None. **S. Wray:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.19/B47

Topic: A.05. Axon and Dendrite Development

Support: New Jersey Commission on Brain Injury Research 07-3204-BIR-E-0

NSF CAREER CBET-0747615

Title: Mild and persistent axon stretch upregulates developmental and regenerative associated genes

Authors: *J. R. LOVERDE[1], P. SOTEROPOULOS[2], B. J. PFISTER[1];
[1]Dept. of Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [2]Ctr. for Applied Genomics, Rutgers Univ. - New Jersey Med. Sch., Newark, NJ

Abstracts: The integration of long nerve fibers throughout the body is distinctive among somatic tissues. Single, individual motor neurons grow to a meter in length or more as they maintain connectivity with sacral areas of the spinal cord from the brain during development. Similarly, homologous growth also occurs within the peripheral nervous system. The most widely studied aspect of axon development has been the extension and migration of growth cones. However, following growth cone extension, axonal fibers continue to grow in synchrony with the expansion of somatic tissues. A known regulatory mechanism which accommodates for such symbiotic interaction is the biomechanical stretching of axons, a formidable stimulus of neuronal growth. Here, utilizing custom designed bioreactors, unidirectional axon stretch growth of embryonic rat dorsal root ganglia (DRG) neurons was optimized for gene expression profiling *in vitro*. A daily strain of 25% was applied until a maximum stretch of 3mm/d was reached and sustained. Genome-wide microarray analysis was performed following 8d of persistent stretch growth. Bioinformatics analysis using Ingenuity® pathway analysis software revealed the most significant molecular changes in lipid metabolism, molecular transport, and small molecule biochemistry. Further, stress response and regenerative associated genes including SPRR1a and ATF3 were upregulated 5 and 3-fold, respectively, implicating a novel role in developmental growth in addition to their transient expression following injury. These results suggest that axon stretch growth is a process whereby neuronal expansion is regulated by developmental stress, which leads to a unique transcriptional profile that accommodates for axonal growth.

Disclosures: **J.R. Loverde:** None. **P. Soteropoulos:** None. **B.J. Pfister:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.20/B48

Topic: A.05. Axon and Dendrite Development

Support: NIA-NIH grant 5R01AG031524

Title: LKB1 and NUAK1 kinases regulate terminal axon branching in cortical neurons through control of presynaptic mitochondria capture and function

Authors: ***J. COURCHET**, T. L. LEWIS, F. POLLEUX;
Neurosciences, Columbia Univ., New York, NY

Abstracts: The molecular mechanisms underlying axon branching and circuit formation in the developing brain are still poorly understood. Impairment of these processes can lead to socially-devastating neurodevelopmental defects such as autism spectrum disorders or mental retardation. The polarity kinase LKB1/STK11 is required for the polarization of cortical neurons and in particular axon formation, both *in vitro* and *in vivo* (Barnes et al., Cell 2007). Using a CRE-based inactivation of the Lkb1 gene after axon specification, we uncovered a previously unknown function for LKB1 in controlling the outgrowth and terminal branching of the axon of cortical neurons (Courchet, Lewis et al., Cell 2013). We also identified that this novel function of LKB1 involves the phosphorylation and activation of NUAK1/ARK5, a poorly characterized downstream target of the AMPK-related kinase family. In parallel, we discovered that the LKB1-NUAK1 kinases promote the capture of mitochondria specifically at nascent presynaptic sites and demonstrated through a series of rescue experiments that direct control of mitochondrial immobilization in axons mediates the function of the LKB1-NUAK1 kinase pathway during axon branching. To determine what function of presynaptic mitochondria underlie their function during axon branching, we have developed novel approaches to probe dynamics of ATP homeostasis in cortical neurons and their axons. Our results suggest that LKB1 or NUAK1 deficiency impairs the production of ATP by axonal mitochondria, which play a critical role during axon morphogenesis.

Disclosures: **J. Courchet:** None. **T.L. Lewis:** None. **F. Polleux:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.21/B49

Topic: A.05. Axon and Dendrite Development

Support: R01-N5071056

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: HDAC6 inhibition promotes axon growth by selectively modifying the acetylome in the distal axon

Authors: *A. L. KALINSKI[1,2], P. BRITO-VARGAS[1], M. RIVECCIO[3], B. LANGLEY[3], J. L. TWISS[1];
[1]Univ. of South Carolina, Columbia, SC; [2]Drexel Univ., Philadelphia, PA; [3]Burke Med. Res. Inst., White Plains, NY

Abstracts: Histone deacetylase 6 (HDAC6) is a class IIb deacetylase that has preference for non-histone proteins. Known substrates in other cellular systems include α-tubulin, HSP90 and cortactin. Cytoplasmic functions for HDACs have been implicated in many biological processes including cell migration, degradation of misfolded proteins, cell spreading and stress granule formation. Both HDAC6 and HDAC5 have been implicated in axon regeneration. We previously showed that chemical inhibition of HDAC6 allows axons to grow on non-permissive substrates, and this occurred independent of ongoing transcription (Riveccio et al., 2009). This implicates an axon-intrinsic mechanism for growth inhibiting affects of HDAC6. However, the intra-axonal targets for HDAC6 have not been determined. Cultured DRG neurons treated with tubastatin A, a specific HDAC6 inhibitor, show a rapid redistribution of post-translationally modified α-tubulin. In contrast to control cultures, the tubastatin A-treated neurons show growth cones filled with acetylated and tyrosinated α-tubulin by quantitative immunofluorescence. The overall levels of these modified α-tubulins does not appear to change with HDAC6 inhibition. Acetylated HSP90 and cortactin show similar changes suggesting that HDAC6 activity selectively deacetylates distal axon and growth cone substrates. Interestingly, mitochondrial mobility is decreased with even short term HDAC6 inhibition, with organelles accumulating in the distal axon and growth cone where the acetylated α-tubulin, HSP90 and cortactin proteins accumulate. Our data suggests that the post-translational modifications associated with HDAC6 inhibition

alter transport and structure of the distal axon, which may help to explain how this manipulation supports axonal growth on non-permissive substrates.

Disclosures: A.L. Kalinski: None. **P. Brito-Vargas:** None. **M. Riveccio:** None. **B. Langley:** None. **J.L. Twiss:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.22/B50

Topic: A.05. Axon and Dendrite Development

Support: Jerome Lejeune Foundation

Innovation Research Seed Award, Kent State University

Title: Local translation of Down syndrome cell adhesion molecule in axon growth and guidance

Authors: *S. JAIN, K. WELSHHANS;
Dept. of Biol. Sci., Kent State Univ., Kent, OH

Abstracts: Down syndrome cell adhesion molecule (*Dscam*) is known to play an important role in many neurodevelopmental processes such as axon guidance, dendrite arborization and synapse formation. *DSCAM* is located in the Down syndrome trisomic region of human chromosome 21 and implicated as one of the genes directly contributing to the Down syndrome brain phenotype, which includes a reduction in the formation of long-distance connectivity. Here, we find that overexpression of DSCAM in mouse cortical pyramidal neurons results in a decrease in axon outgrowth and branching. This finding directly implicates DSCAM as a contributor to the formation of improper neuronal connectivity in Down syndrome. Thus, it is of significant interest to understand the underlying molecular mechanisms by which *Dscam* regulates axon pathfinding. The local translation of a select group of mRNA transcripts within growth cones is necessary for the formation of appropriate neuronal connectivity. Interestingly, we have found that *Dscam* mRNA is localized to growth cones of C57BL/6J mouse hippocampal pyramidal neurons. Localization of *Dscam* mRNA to growth cones decreases in response to the axon guidance molecule, netrin-1. However, netrin-1 stimulation results in an increase in locally translated DSCAM protein in growth cones. Locally translated mRNAs are transported in a translationally dormant state as a part of a ribonucleoprotein complex. We find that two RNA

binding proteins, fragile X mental retardation protein (FMRP) and cytoplasmic polyadenylation element binding protein (CPEB), colocalize with *Dscam* mRNA in growth cones, suggesting their regulation of *Dscam* mRNA localization and translation. We are currently examining the specific connectivity defects that occur during neural development in Down syndrome model mice, and how dysregulated local translation of *Dscam* mRNA contributes to this process. Taken together, these results have implications for Down syndrome, because dysregulated local translation of *Dscam* during embryonic development may contribute to inappropriate neural connectivity and the etiology of this neurodevelopmental disorder.

Disclosures: S. Jain: None. **K. Welshhans:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.23/B51

Topic: A.05. Axon and Dendrite Development

Support: NINDS grant 9R01NS089456-06 (FP)

JSPS (YH)

Title: Exploring the role of the mitochondria/endoplasmic reticulum (ER) interface in axonal development

Authors: *Y. HIRABAYASHI, F. POLLEUX;
Neurosci., Columbia Univ., New York, NY

Abstracts: Axon arborization and assembly of presynaptic terminals are critical for the formation of functional neural circuits. Our group studies the role of the serine/threonine kinase LKB1 (also called STK11 or Par4) in axon morphogenesis (Barnes et al. Cell 2007) and we recently discovered that LKB1 and one of its 14 downstream kinase, NUAK1, controls axon branching by promoting mitochondria immobilization at nascent presynaptic sites (Courchet, Lewis et al. Cell 2013). Importantly, the LKB1-NUAK1 pathway immobilized mitochondria specifically at nascent presynaptic sites. These results suggest that presynaptic mitochondria capture plays an important role in axonal/presynaptic development. However, what anchors mitochondria at specific presynaptic sites is currently unknown. The physical coupling of mitochondria with ER represents one of the best characterized organelle interface at least in

simple eukaryotic cells such as yeast. We are currently testing the potential role of mitochondria/ER coupling in the control of axon and presynaptic development. In the current study, we developed novel ways to visualize this organelle interface in developing neurons and we examined the dynamics of ER/mitochondria interaction in developing axons. Our results suggest that the mitochondria-ER interface might play a role in mitochondria localization presynaptically and thereby might play a role during axon morphogenesis.

Disclosures: Y. Hirabayashi: None. **F. Polleux:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

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Topic: A.05. Axon and Dendrite Development

Support: NEI Grant R01-EY022129

NEI Grant P30-EY022589

an unrestricted grant from research to prevent blindness

Title: The scaffold protein mAKAPα regulates retinal ganglion cell survival and axon growth

Authors: *J. L. GOLDBERG[1], Y. WANG[1], J. LI[2], T. L. STILES[1], J. HERTZ[3], M. D. KRITZER[2], T. NGUYEN[1], M. S. KAPILOFF[2];
[1]Shiley Eye Ctr., UC San Diego, La Jolla, CA; [2]Departments of Pediatrics and Medicine, Univ. of Miami, Miami, FL; [3]Bascom Palmer Eye Inst., Miami, FL

Abstracts: Retinal ganglion cells (RGCs) and other central nervous system neurons die after axon injury. Many molecular signaling pathways, including cAMP, Ca2+, and neurotrophin-dependent pathways, have been implicated in the regulation of neuronal survival and apoptosis. Muscle A-kinase anchoring protein (mAKAP) is the scaffold for a large 'signalosome' located at the nuclear envelope in neurons and striated myocytes that in myocytes orchestrates localized cAMP, Ca2+, hypoxia, phospholipid and MAPK-regulated stress signaling. We now show that the neuronal α form of mAKAP is expressed in the retina in RGCs. By conditional ablation of a mouse floxed allele, mAKAPα deletion early in prenatal RGC development did not affect RGC density in the adult retina, indicating that mAKAPα was not necessary for RGC differentiation or survival through development. In contrast, when RGCs were stressed by optic nerve axon injury,

loss of mAKAPα exacerbated RGC death, revealing a new neuroprotective function for this scaffold. Furthermore, intravitreal injection of BDNF and/or cAMP, which promote RGC survival after axotomy, had no such effect in the absence of mAKAPα. These results show that the mAKAPα scaffold is required in RGCs after optic nerve injury for the transduction of pro-survival signaling, including that induced by BDNF and cAMP, and suggest a broader role for mAKAPα signalosomes in neuroprotection after nerve trauma and stroke.

Disclosures: J.L. Goldberg: None. **Y. Wang:** None. **J. Li:** None. **T.L. Stiles:** None. **J. Hertz:** None. **M.D. Kritzer:** None. **T. Nguyen:** None. **M.S. Kapiloff:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.25/B53

Topic: A.05. Axon and Dendrite Development

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Kavli Institute for Neuroscience at Yale

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CTSI-CN

Title: Plexin-a4 mediates the maintenance of postnatal callosal axons

Authors: *A. I. SON[1], F. SUTO[2], Y. MOROZOV[3], S. ISHII[1], P. RAKIC[4], P. LEVITT[5], K. HASHIMOTO-TORII[1], M. TORII[1];
[1]Ctr. for Neurosci. Res., Children's Natl. Hlth. Syst., Washington, DC; [2]Dept. of Ultrastructural Res., Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [3]Dept. of Neurobio., [4]Yale Kavli Inst. for Neurosci., Yale Univ. Sch. of Med., New Haven, CT; [5]Keck Sch. of Med., USC, Los Angeles, CA

Abstracts: The corpus callosum is the largest axonal tract within the mammalian brain and plays a pivotal role in interhemispheric communication, with deficits being associated with an assortment of cognitive disabilities. Development of this structure is a sophisticated multistage process, first involving the genesis and migration of cells with distinct upper layer neuronal phenotypes and the initial elaboration of their axons during prenatal stages, followed by the overproduction and subsequent pruning of axons and their synapses. However, the molecular mechanisms underlying the refinement of this tract during the postnatal pruning period remain largely unknown and grossly understudied. We have recently found that a member of the plexin-A subfamily of signaling molecules, plexin-A4, plays an integral role in the maintenance of the corpus callosum at early postnatal stages. Plexin-A4-/- mice develop normal callosal projections, but is followed by hypoplasia during the second week. Plexin-A4 expression is prominent within both the neocortex and corpus callosum during early postnatal stages. Silencing of plexin-A4 in cortical neurons by *in utero* electroporation causes the pruning of crossed callosal axons, whereas overexpression of plexin-A4 appears to suppress pruning. Together, these data indicate that plexin-A4 plays an important role in the selective stabilization of callosal axons postnatally during the period when fine-tuning of cortical projections occurs.

Disclosures: A.I. Son: None. **F. Suto:** None. **Y. Morozov:** None. **S. Ishii:** None. **P. Rakic:** None. **P. Levitt:** None. **K. Hashimoto-Torii:** None. **M. Torii:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.26/B54

Topic: A.05. Axon and Dendrite Development

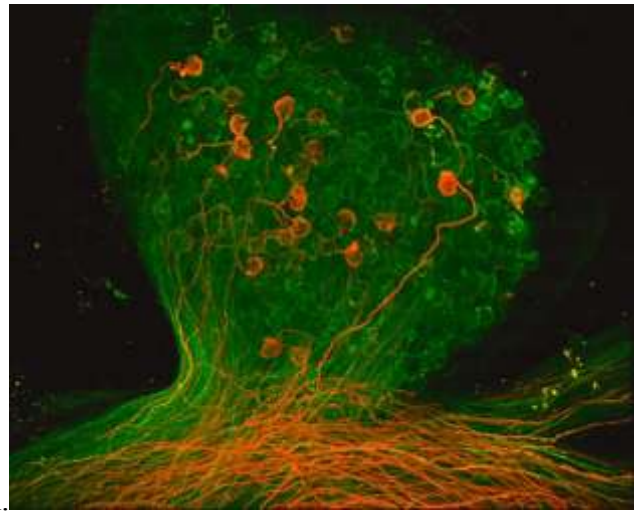
Support: KAKENHI (20722226)

Title: Developmental connectomics of axonal reorganization in avian ciliary ganglion

Authors: *R. EGAWA, S. HOSOSHIMA, T. ISHIZUKA, H. YAWO;
Tohoku Univ. Grad Sch. Life Sci., Sendai, Japan

Abstracts: During the later developmental stages of both central and peripheral nervous systems, axonal branches and synapses are massively reorganized to form mature connections. Although it is a classically-known process, the morphology at single axon level and the molecular mechanisms are not fully understood due to technical difficulties which come from the

complexity of neural circuitry. Here, with a combination of sparse expression system and tissue clearing method, we revealed the morphological characteristics of axonal reorganization in developing chick ciliary ganglion (CG), a conventional model system of synaptic development. Plasmid vectors, which express tdTomato under the modulation of sparse expression promoter Thy1s (Ako et al, 2011), were introduced into the midbrain presynaptic neuron using in ovo electroporation method (Odani et al, 2008). The CG was isolated at E8-14, cleared with ScaleA2 protocol (Hama et al, 2011) and imaged as a whole under two-photon microscopy. Each intertangled axon was three-dimensionally traced and quantitatively analyzed. We found that the total length and branching number of the axons are gradually decreased with development and that many axons lose their branches before E14. Our results were complementary to the previous electromicroscopic/electrophysiological studies carried out by Landmesser and Pilar in 1970s which showed the decrease in presynaptic inputs from multiple to single during E8-14. It is suggested that the excess presynaptic branches are pruned to form one-to-one connections without any "winners" of the synaptic competition which form oligopoly connections over postsynaptic targets. With the combination of genetic manipulations, our system of developmental connectomics would facilitate further understanding of the fundamental principles and molecular mechanisms underlying developmental axonal reorganization.



Disclosures: **R. Egawa:** None. **S. Hososhima:** None. **T. Ishizuka:** None. **H. Yawo:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

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Program#/Poster: 593.27/B55

Topic: A.05. Axon and Dendrite Development

Support: NIMH Intramural Research Grant

Title: Regulation of axonal trafficking of nuclear-encoded mitochondrial COXIV mRNA

Authors: *A. KAR[1], N. KHOSHAB[2], N. M. GERVASI[2], S. S. SCOTT[2], C. Y. CHEN[2], J. KOWALAK[3], A. E. GIOIO[2], B. B. KAPLAN[2];
[1]SNB, LMB, Natl. Inst. of Mental Hlth., [2]SNB, LMB, NIMH, [3]NINDS-NIMH Proteomics Core, NIMH, NIH, Bethesda, MD

Abstracts: It is now well-established that a distinct subset of nuclear-encoded mitochondrial mRNAs such as Cytochrome C oxidase IV (COXIV), are selectively transported and translated in the distal structural/functional domains of neurons (i.e. axons and presynaptic terminals). Local translation of the COXIV mRNA, which encodes a key subunit of oxidative phosphorylation complex IV, plays an important role in axonal energy metabolism, function and growth. Disruption of local COXIV expression leads to compromised mitochondrial membrane potential, decreased ATP levels and generation of reactive oxygen species (ROS) in the axon. However, relatively little is known about the mechanisms involved in regulating the axonal trafficking of these nuclear-encoded mitochondrial transcripts. In previous studies, we identified a putative 38-nucleotide stem-loop structure (zipcode) in the 3'-untranslated region of the COXIV transcript that was necessary and sufficient for the axonal localization of COXIV mRNA in superior cervical ganglion (SCG) neurons. To identify proteins involved in the axonal transport of the COXIV transcript, we used this 38-nucleotide COXIV RNA zip-code as bait for RNA-protein binding studies. Gel-shift assays of the biotinylated COXIV-zipcode incubated with retinoic-acid differentiated SHSY5Y cell lysates showed that the zipcode binds endogenous proteins and forms nucleoprotein complexes. Mass spectrometric analysis of proteins isolated by affinity purification using biotinlylated COXIV-zipcode oligomer incubated with SHSY5Y cell-lysates lead to the identification of a number of RNA-binding and mitochondria-associated proteins such as fused in sarcoma/translated in liposarcoma (FUS/TLS) and Parkinson disease protein 7 (PARK7/DJ-1) respectively. Validation using western blotting analyses confirmed the presence of the candidate proteins in the COXIV-zipcode affinity purified complexes from SHSY5Y lysates. In addition, using immunohistochemical and western blotting analyses, we also established the presence of these candidate COXIV-zipcode binding proteins in SCG neurons. Future experiments are aimed at confirming the interaction of these candidate binding proteins with the endogenous COXIV mRNA in SCG neurons and assessing the *in vivo* significance of the candidate proteins in axonal transport of COXIV mRNA.

Disclosures: **A. Kar:** None. **N. Khoshab:** None. **N.M. Gervasi:** None. **S.S. Scott:** None. **C.Y. Chen:** None. **J. Kowalak:** None. **A.E. Gioio:** None. **B.B. Kaplan:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.28/B56

Topic: A.05. Axon and Dendrite Development

Title: RACK1 regulates local translation at point contacts in growth cones

Authors: *L. J. KERSHNER, J. SERRE, K. WELSHHANS;
Dept. of Biol. Sci., Kent State Univ., Kent, OH

Abstracts: The formation of appropriate connectivity in the developing nervous system is dependent on the process of local translation. In developing neurons, a population of mRNAs is transported to and locally translated within growth cones, which are the pathfinding structures located at the tip of extending axons. However, the specific regions in which local translation takes place and the molecular mechanisms underlying this process are not well understood. Growth cone adhesion to the extracellular matrix occurs at point contacts, which play a critical role in regulating axon growth and guidance. Here, we investigate whether local translation occurs at point contacts, and examine the role of receptor for activated C kinase (RACK1), a member of the point contact complex, in axon growth and guidance. We have previously demonstrated that the local translation of β-actin mRNA is dependent on the phosphorylation of RACK1, a ribosome scaffolding protein, and zipcode binding protein 1 (ZBP1), an mRNA binding protein. ZBP1 binds to the 3'UTR of β-actin mRNA, represses its translation, and localizes it to growth cones. In response to stimulation with brain-derived neurotrophic factor (BDNF), RACK1 binding of the ZBP1/β-actin mRNA complex on ribosomes is increased. Phosphorylation of RACK1 then facilitates the release of β-actin mRNA from ZBP1 and its subsequent translation. Interestingly, we have now found that RACK1 is localized to point contacts, which led us to hypothesize that point contacts might be a site of β-actin mRNA local translation in growth cones. Thus, we examined the location of the ZBP1/β-actin mRNA complex relative to point contacts under both basal and growth factor stimulated conditions in cortical neurons of embryonic day 17 C57BL/6J mice. β-actin mRNA, RACK1 and ribosomes colocalize with paxillin, a marker of point contacts in growth cones. BDNF stimulation increases the colocalization of β-actin mRNA and RACK1 with paxillin. Additionally, BDNF stimulation increases the number of point contacts within growth cones, and this increase is dependent on RACK1 phosphorylation. RACK1 also plays a critical role in the regulation of axon growth and growth cone area. Taken together, these data suggest that point contacts are a site of local translation within the growth cone, and that RACK1 is critical to the formation of point contacts,

the local translation process, and appropriate neuronal development. These data provide further insight into how and where local translation is regulated within growth cones, and thereby leads to appropriate connectivity formation in the developing nervous system.

Disclosures: L.J. Kershner: None. **J. Serre:** None. **K. Welshhans:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 593.29/B57

Topic: A.05. Axon and Dendrite Development

Title: The UNC-23-HSP-1 chaperone system determines localization of Parkinson disease-related kinase LRK-1 in *C. elegans*

Authors: *T. ALAM, T. FUKUZONO, S. PASTUHOV, O. FUKUSHIMA, C. LI, A. HATTORI, H. HANAFUSA, K. MATSUMOTO, N. HISAMOTO;
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Abstracts: Neuron is a polarized cell that contains distinct sets of proteins in its axon and dendrite. Synaptic vesicles (SV) proteins are exclusively localized in the presynaptic regions but not in dendrites. Previously, we have shown that LRK-1, a *Caenorhabditis elegans* homolog of the familial Parkinsonism gene *PARK8/LRRK2*, determines the polarized sorting of SV proteins to axons at Golgi apparatus. However, the molecular mechanism that regulates localization of LRK-1 at the Golgi remained unclear. Here, we identified two human proteins, BAG2 and HSC70, as LRRK1/2-binding proteins. LRRK1/2 associate directly with HSC70, and indirectly with BAG2 via HSC70. BAG2 induces the release of LRRK1/2 from HSC70. We further characterized the role of their *C. elegans* homologs, UNC-23 and HSP-1, in the regulation of LRK-1. We found that in *unc-23* mutants , SV proteins are mislocalized, a phenotype also observed in *lrk-1* mutants. In addition, we identified mutations in the *hsp-1* gene as suppressors for the SV mislocalization phenotype of *unc-23* mutants. In *C. elegans* sensory neurons, LRK-1 exclusively localizes at Golgi apparatus, which acts as a platform that controls the proper delivery of SV proteins to axons. In *unc-23* mutants, however, the LRK-1 localization at Golgi was significantly reduced. This reduction was suppressed by the *hsp-1* mutations. Thus, the UNC-23–HSP-1 chaperone system has an important role for intracellular localization of LRK-1 in the control of polarized sorting of SV proteins.

Disclosures: **T. Alam:** None. **T. Fukuzono:** None. **S. Pastuhov:** None. **O. Fukushima:** None. **H. Hanafusa:** None. **K. Matsumoto:** None. **N. Hisamoto:** None. **C. Li:** None. **A. Hattori:** None.

Poster

593. Axon Growth and Guidance: Intrinsic Mechanisms

Location: Halls A-C

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Program#/Poster: 593.30/B58

Topic: A.05. Axon and Dendrite Development

Support: MRC C.D.Award to K. Franze

Title: Investigating the role of mechanical cues in axon guidance

Authors: ***G. SHERIDAN**[1], H. SVOBODA[2], D. E. KOSER[2], A. DWIVEDY[2], A. J. THOMPSON[2], M. P. VIANA[4], L. F. COSTA[4], J. GUCK[3], C. E. HOLT[2], K. FRANZE[2]; [1]PDN, [2]Physiol. Develop. & Neurosci., [3]Dept. of Physics, Univ. of Cambridge, Cambridge, United Kingdom; [4]Inst. of Physics at Sao Carlos, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstracts: During Xenopus laevis embryogenesis, development of the visual system involves the projection of retinal ganglion cell (RGC) axons from eye primordia to correct synaptic contacts in the optic tectum of the brain. There are well-characterised chemical attractant and repellent cues that guide axons to their target locations. During chemotactic migration, however, growing axons also physically interact with their surrounding environment. Therefore, it is possible that mechanical interactions could also impact their guidance to the tectum. To probe RGC mechanosensitivity, we cultured stage 35/36 Xenopus eye primordia *ex vivo* on compliant polyacrylamide hydrogels with increasing rigidities. We found clear morphological differences in axons grown on soft and rigid substrates. RGC axons on stiff hydrogels grow relatively straight and appear longer. RGC axons cultured on softer gels, however, grow faster and display a more contoured trajectory as they emanate from the eyeball. Moreover, axons cultured on softer hydrogels fasciculate to a greater extent and display thicker axon bundles than on rigid substrates. Finally, RGC axons grown on stiffness gradients turn away from the hard surface and grow toward the softer side. This suggests that RGC axons are mechanosensitive and can sense the mechanical properties of the extracellular environment during axon path-finding. Using *in vivo* atomic force microscopy-based stiffness mapping of Xenopus exposed brains, we found stiffness gradients along the path RGC axons grow. We reveal, for the first time, how RGC axons migrate through relatively soft brain structures until they reach the more rigid optic tectum, where they terminate. Presently, we are investigating if the tectum acts as a durotactic

landmark for RGC axon termination in addition to chemotactic cues present. Moreover, Xenopus brains exposed to chondroitin sulphate undergo softening and experience changes in the observed stiffness gradients, leading to axon guidance defects. Furthermore, exposing developing Xenopus embryos (stages 32-40) to inhibitors of mechanosensitive ion channels causes RGC miswiring. Treated RGC axons de-fasciculate before reaching the optic tectum; strongly suggesting that the mechanical properties of the extracellular environment may significantly contribute to axon guidance in the developing brain.

Disclosures: G. Sheridan: None. **H. Svoboda:** None. **D.E. Koser:** None. **A. Dwivedy:** None. **A.J. Thompson:** None. **M.P. Viana:** None. **L.F. Costa:** None. **J. Guck:** None. **C.E. Holt:** None. **K. Franze:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

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Program#/Poster: 594.01/B59

Topic: A.05. Axon and Dendrite Development

Support: Hartwell Foundation

March of Dimes

Alfred P. Sloan Foundation

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Canada Foundation for Innovation

Brain Canada and the W. Garfield Weston Foundation

National Science Council NSC102-2320-B-038-056-MY2

Title: α2-chimaerin function in spinal motor axon guidance

Authors: *T.-J. KAO[1,2], G. C. B. NICHOLL[3,4], J. A. JOHANSEN[5], A. KANIA[2,6,7], A. BEG[3,4]; [1]Taipei Med. Univ., Taipei, Taiwan; [2]Inst. de Recherches Cliniques de Montréal, Montréal, QC, Canada; [3]Univ. of Michigan Med. Sch., Ann Arbor, MI; [4]Univ. of Michigan, Ann Arbor, MI; [5]Central Michigan Univ., Mt. Pleasant, MI; [6]McGill Univ., Montréal, QC, Canada; [7]Univ. de Montréal, Montréal, QC, Canada

Abstracts: The relay of axon guidance signals to the actin cytoskeleton at the growth cone remains poorly understood. We are studying this question in the context of spinal lateral motor column (LMC) motor axons innervating ventral and dorsal limb muscles. This trajectory choice by LMC axons is, in part, controlled by a repulsive ephrin:Eph signaling: EphA4-expressing lateral LMC axons are repulsed into the dorsal limb from ephrin-As expressed in the ventral limb, whereas EphB1-expressing medial LMC axons are repulsed into the ventral limb from ephrin-Bs in the dorsal limb. The Rac-GAP protein, α2-chimaerin, has been implicated to participate in Eph signal relay; however, its function in the context of motor axon pathfinding has not been established prompting us to examine the role of α2-chimaerin in LMC axon guidance. Using *in situ* hybridization technique, we determined that α2-chimaerin mRNA is expressed in LMC motor neurons of chick and mouse embryos at the time of limb trajectory selection. The loss of α2-chimaerin function leads to the selection of inappropriate lateral LMC axon trajectories in both chick and mouse embryos. In addition, α2-chimaerin knockdown attenuated the retargeting of LMC axons caused by EphA, but not EphB over-expression, suggesting that α2-chimaerin participates in EphA-mediated lateral LMC motor axon guidance. To determine the extent to which α2-chimaerin participates in other axon guidance events, such as those mediated by c-Ret-GDNF, which also contribute to LMC axon guidance, we will examine the effect of α2-chimaerin knockdown in c-Ret-overexpressing LMC motor neurons.

Disclosures: T. Kao: None. **G.C.B. Nicholl:** None. **J.A. Johansen:** None. **A. Kania:** None. **A. Beg:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

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Program#/Poster: 594.02/B60

Topic: A.05. Axon and Dendrite Development

Support: 3DNeuroN project in the European Union's Seventh Framework Programme, Future and Emerging Technologies, grant agreement no. 296590

Title: A simple technique for directed axonal guidance on multi-electrode arrays with arbitrary topology

Authors: *C. FORRO, L. DEMKO, H. DERMUTZ, J. VOROS;
Lab. of Biosensors and Bioelectronics, ETH Zurich, Zurich, Switzerland

Abstracts: The use of cultured neuronal networks as a model for their *in vivo* counterparts allows researchers to study the central nervous system, especially the brain, in a controlled environment. Specific neuronal connectivity patterns in the brain are implicated to play a role in the perception, processing and storage of information, so the building of small neuronal networks with controlled topology is a promising approach to investigate the basic circuits of the nervous system. Realizing such bottom-up systems on multi-electrode arrays (MEA) makes a way to study the dynamics of functional neural networks systematically as a function of the network structure. The present work focuses on a simple and robust technique developed in our group to realize small neuronal networks with well-defined topology, directly on MEA chips with arbitrary electrode arrangements. The technique utilizes special PDMS sheets which provide control over the type, number, position of the neurons, as well as the connections between them, and the main emphasis is on the pre-defined directions of these connections achieved by simple geometric constraints. We demonstrate the applicability of the developed method on MEA chips with different electrode patterns. The results show the proof of concept by realizing directed connections between rat hippocampal neurons sitting on neighboring electrodes, and analyzing the resulted neuronal activity from the measured extracellular signals. By creating reproducible neuronal networks with the well-defined topology, our vision is to bridge the gap between micro and macro, the molecular or few cell level and whole brain experiments. With the results, we aim to provide insights into the role of neuronal and synaptic properties such as synapse development, plasticity and connectivity, and extract the algorithms that support information processing and neurocomputation in brain tissue.

Disclosures: **C. Forro:** None. **L. Demko:** None. **H. Dermutz:** None. **J. Voros:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

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Program#/Poster: 594.03/C1

Topic: A.05. Axon and Dendrite Development

Support: Pro Retina Foundation, Pro-Re/KP/Engelhardt.1-2014

Title: Activity-dependent structural maturation of the cisternal organelle in the axon initial segment of visual system neurons *in vivo*

Authors: ***M. ENGELHARDT**[1], A. SCHLÜTER[1], S. ROSSBERGER[2], S. VORWALD[1], C. SCHULTZ[1];

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Abstracts: Normal CNS maturation fundamentally depends on correct network formation during postnatal 'critical periods', which shape and manifest the mature wiring of neurons by permitting certain levels of neuronal plasticity in dependence of experienced sensory activity. The structural components that allow a neuron to function properly within such dynamic networks are organized in electrogenic microdomains such as the axon initial segment (AIS), which is required for action potential generation, and has been shown to be dynamically regulated during development and maturation. In fact, we recently demonstrated that maturation of visual system AIS is subject to a complex tri-phasic development regulated by visual experience. It remains unclear, which axonal features drive this dynamic remodeling of the AIS. A possible candidate is the cisternal organelle (CO), a Ca2+-storage/release compartment specifically localized in the AIS. The CO is composed of stacks of smooth endoplasmic reticulum. An involvement of the CO in neuronal AIS plasticity is conceivable since it has a structural and functional homologue in the highly dynamic dendritic spine apparatus (SA), which is involved in remodeling mechanisms aimed at adjusting the strength of excitatory synapses to persisting changes in network activity. Whether synpo and the CO in AIS undergo similar remodeling events during development and neuronal plasticity is unknown. Furthermore, the precise subcellular localization of the CO, especially in relation to AIS-specific protein complexes, is currently unidentified. Therefore we investigated the developmental maturation of the CO by comprehensive immunofluorescence and biochemical analysis of the expression of synaptopodin (synpo), an actin-associated protein specifically localized in the SA and CO, respectively. Utilizing murine visual cortical and retinal neurons, we present data indicating (1) a dynamic regulation of the CO during visual system development, and (2) an activity-dependent structural remodeling of the CO. Finally, to further elucidate the subcellular localization and potential interaction of synpo with other essential AIS molecules, we employed high resolution microscopy (structured illumination in combination with single molecule localization microscopy) of CO clusters in the AIS of retinal neurons. Taken together, our findings indicate that CO maturation in the visual system is subject to activity-dependent structural remodeling. Future studies aim at providing more conclusive information on the potential interaction of synpo with other structurally essential AIS protein clusters.

Disclosures: M. Engelhardt: None. **A. Schlüter:** None. **S. Vorwald:** None. **C. Schultz:** None. **S. Rossberger:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

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Program#/Poster: 594.04/C2

Topic: A.05. Axon and Dendrite Development

Support: NINDS NIH F31NS077620

Title: RNA-binding protein SFPQ regulates axonal bclw mRNA to promote axon viability

Authors: *S. J. FENSTERMACHER[1,2], K. E. COSKER[1,2], M. F. PAZYRA-MURPHY[1,2], R. A. SEGAL[1,2];
[1]Dana Farber Cancer Inst., Boston, MA; [2]Neurobio., Harvard Med. Sch., Boston, MA

Abstracts: Localized translation of axonal mRNAs has important roles in axonal growth and long term viability. While numerous cis-acting elements and trans-acting factors have been identified that regulate axonal localization of mRNAs, little is known about the RNA-binding proteins that coordinate transcription, transport, and translation of axonal mRNAs to promote axonal viability. We now demonstrate that the RNA-binding protein, splicing factor proline/glutamine rich (SFPQ), is present in nuclei and axons of sensory neurons and is necessary for neurotrophin-mediated axonal survival. Neurotrophin stimulation increases phosphorylation of SFPQ and regulates binding of SFPQ to select mRNAs, including c-fos and bclw mRNA. While SFPQ functions in neurotrophin-dependent transcription of cfos, interestingly SFPQ instead functions in the intracellular trafficking of bclw mRNA. We find that SFPQ binds to multiple regions of the long 2.7kb bclw 3'UTR, and this region is critical for neurotrophin-dependent axonal localization. Previous studies have demonstrated that bclw is a major anti-apoptotic regulator of axonal viability, suggesting that the effects of SFPQ on axonal maintenance may reflect changes in Bclw synthesis. Together these studies suggest a model whereby SFPQ bound bclw mRNA exits the nucleus and is assembled into an RNA transport granule to localize bclw into axons for localized translation to promote the health and viability of long sensory axons.

Disclosures: S.J. Fenstermacher: None. **K.E. Cosker:** None. **M.F. Pazyra-Murphy:** None. **R.A. Segal:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.05/C3

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS085285

Title: Netrin 5 expressed by boundary cap cells prevents motor neuron migration out of the ventral horn of the spinal cord

Authors: *A. M. GARRETT, T. J. JUCIUS, S. L. ACKERMAN, R. W. BURGESS;
The Jackson Lab., Bar Harbor, ME

Abstracts: The Netrins are a family of evolutionarily conserved secreted proteins that act as guidance cues for migrating cells and axons during development. Netrins are bidirectional cues; they can be both chemoattractant and chemorepellant depending on the receptors present on the responding cells. Three classical Netrins have been described in mice - Netrin 1, 3, and 4 - as well as two GPI-linked G-Netrins. Here we describe Netrin 5, a previously unstudied classical Netrin. The mouse *Ntn5* gene is on chromosome 7 where its exons interdigitate with *Sec1,* a gene encoding a fucosyl transferase, on the opposite strand. We examined the pattern of *Ntn5* expression by *in situ* hybridization during development and found restricted labeling at the motor exit point (MEP) and dorsal root entry zone (DREZ) of the developing spinal cord. These cells co-expressed *Krox20*, a marker for boundary cap cells. Boundary cap cells are a transient neural-crest-derived population that forms a functional boundary between the central and peripheral nervous system during development. To investigate the function of *Ntn5,* we generated knockout mice through standard homologous recombination targeting exons 2-6 without disrupting *Sec1*. Whole embryo staining for neurofilament at E11.5 demonstrated normal gross dorsal root organization and cranial nerve guidance. Boundary cap cells have been previously studied by ablation, and we reasoned that *Ntn5* knockouts would have a subset of the phenotypes reported in the ablation studies. Dorsal boundary cap cells differentiate into a subset of sensory neurons in the DRG, but we did not find any reduction in sensory neurons *Ntn5* mutants. We also have not observed invasion of oligodendrocytes into spinal roots. We did, however, find ectopic motor neuron cell bodies migrating out of the ventral horn into the ventral roots, consistent with ablation studies. In addition, these ectopias have also been observed in chicks and mice mutant for members of the semaphorin family of guidance molecules and their plexin/neuropilin receptors. In those mutants, more ectopic motor neurons were observed caudally, whereas we found more ectopic motor neurons rostrally in *Ntn5* knockouts. Furthermore, the motor neurons found in the ventral roots in *Ntn5* mutants are not strongly positive for neuropilin 2, suggesting that separate populations of motor neurons depend on Netrin 5 signaling and semaphorin signaling to maintain cell body position. Currently, we are analyzing mutants for known Netrin receptors to find which transduces the Netrin5 signal to maintain motor neuron position in the ventral horn.

Disclosures: **A.M. Garrett:** None. **T.J. Jucius:** None. **S.L. Ackerman:** None. **R.W. Burgess:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.06/C4

Topic: A.05. Axon and Dendrite Development

Support: NIH RO1 grant NS28182 to K.G.Z.

Gordon Ross Postdoctoral Fellowship (Caltech) to N.B.

Title: Identification and genetic characterization of new ligands for receptor tyrosine phosphatases

Authors: ***N. BALI**, H.-K. LEE, K. G. ZINN;
Div. of Biol., Caltech, Pasadena, CA

Abstracts: Axon guidance during *Drosophila* development is controlled by attractive and repulsive signals, some of which are transduced through receptor tyrosine kinases and receptor tyrosine phosphatases (RPTPs). *Drosophila* RPTPs are expressed in CNS axons and are required for normal motor and CNS axon guidance. To understand the regulation of axon guidance, it is necessary to determine how RPTP activity is controlled by interactions with extracellular ligands. Only a few RPTP ligands have been identified thus far, making this an important subject for research. Our group showed that Syndecan is a ligand for the Lar RPTP in 2005. More recently, we found that a transmembrane receptor called Stranded at second (Sas) is a ligand for Ptp10D (Lee et al., Neuron 78, 813-826 (2013)). In the same screen that identified Sas, we also found ~25 candidate ligands for four different RPTPs (Ptp10D, Lar, Ptp69D, and Ptp99A). To identify the ligands, lines bearing insertions of 'UAS'- element-containing transposable elements upstream of 311 genes coding for cell surface and secreted (CSS) proteins were crossed with GAL4 'driver' lines expressed in a variety of tissues, including embryonic muscle. The resulting embryos with ectopic expression of individual CSS proteins were incubated with dimeric RPTP-placental alkaline phosphatase (AP) fusion proteins, followed by antibody staining to detect AP. Candidate ligands were those which conferred RPTP-AP fusion protein staining of muscles and other tissues. In wild-type embryos, each RPTP-AP fusion binds only to CNS axons. We further characterized these candidate RPTP-ligand interactions by 'reverse-binding' experiments, in which the RPTPs were ectopically expressed using tubulin-GAL4 or engrailed-GAL4 driver lines. We then analyzed whether ligand-AP fusion proteins were able to bind to the ectopically

expressed RPTPs. In the published paper, we performed these tests for Sas. Since then, we have further confirmed 9 novel RPTP-ligand interactions by reverse-binding. We are now examining whether these ligands bind to the RPTPs *in vitro* using a modified ELISA assay. We have been able to detect binding for a number of ligands in this manner. Many of the candidate ligands, like the RPTPs themselves, are highly conserved between flies and mammals, indicating that insights derived from this work should be applicable to human systems. We are employing similar approaches to find novel ligands for extracellular leucine-rich repeat proteins (LRRs) which play important roles in axon guidance and synaptogenesis.

Disclosures: **N. Bali:** None. **H. Lee:** None. **K.G. Zinn:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.07/C5

Topic: A.05. Axon and Dendrite Development

Title: Endocannabinoids modulate cortical development by configuring Slit2/Robo1 signaling

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Abstracts: Local environmental cues are indispensable for axonal growth and guidance during brain circuit formation. Here, we show that excess 2-arachidonoylglycerol, an endocannabinoid affecting directional axonal growth, triggers corpus callosum enlargement due to errant CB1 cannabinoid receptor (CB1R)-containing corticofugal axon spreading. This phenotype mechanistically relies on the premature differentiation and end-feet proliferation of CB2R-

expressing oligodendrocytes. The binding of secreted Slit ligands to Roundabout (Robo)1/2 receptors evokes repulsive axon guidance decisions. We combine genetic and pharmacological tools, as well as systems neuroanatomy in human fetuses and mouse models to hierarchically link endocannabinoid and Slit/Robo signaling systems to show the dependence of both axonal Robo1 positioning and oligodendroglial Slit2 production on cell-type specific cannabinoid receptor activation. Accordingly, Robo1 and/or Slit2 manipulation limits endocannabinoid modulation of axon guidance. We conclude that endocannabinoids can configure focal Slit2/Robo1 signaling to modulate directional axonal growth, which provides a novel basis for brain wiring deficits associated with prenatal illicit drug abuse and metabolic deficits.

Disclosures: **D. Calvigioni:** None. **A. Alpár:** None. **M.J. Niphakis:** None. **I. Milenkovic:** None. **J. Bakker:** None. **G.A. Cameron:** None. **J. Hanics:** None. **C.V. Morris:** None. **J. Fuzik:** None. **G.G. Kovacs:** None. **B.F. Cravatt:** None. **J.G. Parnavelas:** None. **W.D. Andrews:** None. **Y.L. Hurd:** None. **E. Keimpema:** None. **T. Harkany:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.08/C6

Topic: A.05. Axon and Dendrite Development

Support: NIDCD Intramural Research Program

Title: Targeting of cochlear inner hair cells by type I spiral ganglion neurons is controlled by Sema3f/Nrp2 signaling

Authors: *T. M. COATE[1,3], K. T. ISGRIG[2], M. W. KELLEY[2];
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Abstracts: The mouse cochlea provides an excellent model for investigating molecular mechanisms associated with sensory organ innervation. To establish appropriate hearing function in the cochlea, type I spiral ganglion neurons (SGNs) must synapse with the mechanosensory inner hair cells (IHCs), whereas type II SGNs must synapse with outer hair cells (OHCs). In addition, low-spontaneous rate (low-SR) SGNs target the medial side of IHCs, whereas high-SR SGNs target the lateral side of IHCs. To better understand cochlear innervation, we developed a live imaging model in which sparse numbers of SGNs express tdTomato, while all hair cells

express GFP. Using this we have determined that supernumerary type I SGNs project to the OHCs around E15, but then retract to the IHCs through P0. These data support the hypothesis that cues within the hair cell environment direct different SGN subtypes to different hair cell regions. Cells within the OHC region express the axon guidance factor Semaphorin-3F (Sema3F) while the SGNs express its receptor, Neuropilin-2 (Nrp2). To characterize the role of these factors with high optical resolution, we combined Nrp2 mutants with our imaging model and documented the projection of each labeled SGN. Nrp2 deletion leads to the ectopic projection of type I SGNs into the OHC region, but does not alter the arrangement of low- and high-SR type I SGNs. The loss of Nrp2 also leads to increased numbers of OHC ribbon synapses and changes in auditory brainstem response thresholds, suggesting this receptor is necessary for the establishment of normal hearing function. In addition, treating cultured cochleae with Sema3F causes SGN branches to collapse, suggesting Sema3F normally acts as a chemorepellant. These data support a model whereby Sema3F within the cochlear epithelium activates Nrp2 on type I SGNs to sequester them at the IHC. We are currently examining how processing of the co-receptor Plexin-A3 dictates differential targeting by SGNs. Funding by NIDCD.

Disclosures: **T.M. Coate:** None. **K.T. Isgrig:** None. **M.W. Kelley:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.09/C7

Topic: A.05. Axon and Dendrite Development

Title: The pattern of activity in injured and non-injured axons after selected axonal microdissection in microchannels

Authors: *R. HABIBEY, A. GOLABCHI, M. NANNI, F. DIFATO, A. BLAU;
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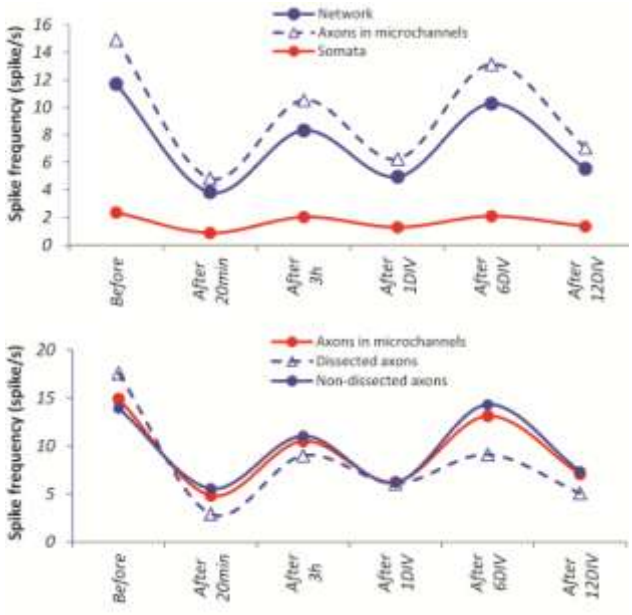
Abstracts: An *in vitro* method for monitoring axonal and network activity after controlled axonal injury would help in investigating network response to injury as well as understanding axonal function during repair (1). Laser microdissection is used for highly controlled ablation of microchannel-confined axons while other parts of the network remain intact (2). In addition, axonal activity and propagation of action potentials could be recorded by custom-made or commercial electrode arrays (3). In the present study, we combined these techniques. We cultured cortical neurons on top of commercial microelectrode arrays (MEAs) and forced axons

to grow into polydimethylsiloxane (PDMS) microchannels. Axons were accessible for laser microdissection while recording from both the whole network and axons inside the microtunnels. The PDMS devices included two 100 µm-high rectangular-shaped reservoirs (400 µm X 1600 µm). Reservoirs were connected by eight rows of 30 µm wide and < 5 µm high microtunnels. Neurons were seeded from above into one of the freely accessible reservoirs. A PDMS device was aligned with the MEA electrodes recording electrophysiological activity from neurons inside the reservoir and from growing axons inside the microtunnels. Electrical activity was recorded before and for 12 DIVs after axonal dissection. The activity pattern and morphology of injured and non-injured axons and within the network was evaluated at different time points. Figure 1 Spike frequency before and until 12 DIVs after laser microdissection of some axons in microchannels. Data are represented as mean frequency. References: 1.Difato F et al. J Biomed Opt. 2011;16(5):051306. 2.Hellman AN et. al. Lab Chip. 2010;10(16):2083-92. 3.Dworak BJ, Wheeler BC. Lab on a Chip. 2009;9(3):404-10.



Disclosures: **R. Habibey:** None. **A. Golabchi:** None. **M. Nanni:** None. **F. Difato:** None. **A. Blau:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.10/C8

Topic: A.05. Axon and Dendrite Development

Title: Mathematical model of the epidermal axon growth

Authors: ***Y. KOBAYASHI**[1], M. TSUTSUMI[2], J. KUMAMOTO[2], M. DENDA[2], M. NAGAYAMA[1];
[1]Hokkaido Univ., Sapporo, Japan; [2]Shiseido Co., Ltd., Yokohama, Japan

Abstracts: Itchiness is one of our fundamental sensations caused by various internal and/or external stimuli. While histamine-caused itch that can be suppressed by dosing the histamine antagonist is well understood, little is known about the itchiness accompanied by atopic dermatitis (AD), where the histamine antagonist has little effect. Recent experiments have indicated that scratching behavior in mice is associated with the increase in the density of axons penetrating into the epidermis. It has also been reported that axons tend to intrude into the epidermis in the case of AD. These findings might indicate the possibility that abnormal growth of the nerve axons in AD skins alters the spatio-temporal propagation pattern of the nerve pulses, leading to sustained itchiness. Developing a mathematical model of the axons in the epidermis and simulating propagation patterns of the nerve pulses for various nerve growth patterns would therefore be a great help to understand the mechanism of the generation of itchiness. For this purpose, it is necessary to have an appropriate mathematical model of growing axons inside the epidermis. In this study, we construct such a model where the axon is approximated by a bead-spring system, which consists of particles connected by springs with the bending rigidity. The tip of the axon is guided by the chemical attractant, the gradient of which induces the torque on the tip so that it tends to direct to the high concentration of the attractant. The growth of the axon is represented by the continuous addition of new particles at the tip. We check the validity of our model by simulating an AXIS experiment, in which dorsal root ganglions are cultivated in one chamber and various chemicals are put in the other chamber separated by the slit to see how the growth pattern changes depending on the added chemicals. Performing simulations with various conditions, we also present a quantitative measure for the degree of the axon guidance that can be applicable in the AXIS experiment.

Disclosures: Y. Kobayashi: None. **M. Nagayama:** None. **M. Tsutsumi:** None. **J. Kumamoto:** None. **M. Denda:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.11/C9

Topic: A.05. Axon and Dendrite Development

Support: NIH DP2 MH10008

March of Dimes

Title: Serotonin regulates midline axon crossing via ephrinb2 and is involved in remodeling after hypoxia

Authors: *J. BONKOWSKY[1], L. XING[1], J.-H. SON[2], T. STEVENSON[2];
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Abstracts: Serotonin neurons and their axon projections are widespread in the CNS in early development. Disruption of serotonergic signaling leads to diffuse CNS abnormalities with a wide range of behavioral phenotypes. This suggests a non-classical role for serotonin in development. In mice, serotonin can modulate axon responsiveness to netrin-1, causing thalamocortical axons to adopt a more ventral pathway and altered fasciculation of medial pre-frontal cortex neurites. While serotonin appears necessary for axon guidance, no one has explored its role in commissure formation. Moreover, the mechanism of serotonin action in pathfinding is poorly understood. To determine the role of serotonin in commissure formation and characterize the molecular mechanism, we used embryonic zebrafish to visualize axon guidance decisions, in transgenic lines with precise expression in subsets of well-defined axon pathways. Using pharmacological blockade of serotonin signaling, genetic ablation of the serotonergic neurons, or knock-down of serotonin receptors, we found that axons of telencephalic neurons fail to cross the midline, indicating a role for serotonin in commissural axon guidance. We further demonstrate that serotonin acts through its main excitatory G protein-coupled receptor htr2a. Blockade of htr2a elevates receptor-tyrosine kinase ephrinB2 levels, causing failure of commissural axon midline crossing. Blockade of serotonin signaling rescues commissural axon errors induced by hypoxia. Thus, our study demonstrates an unexpected instructive role for serotonin in axon pathfinding choice, and identifies ephrinB2 as a key mediator in serotonin-regulated pathfinding.

Disclosures: J. Bonkowsky: None. **L. Xing:** None. **J. Son:** None. **T. Stevenson:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

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Program#/Poster: 594.12/C10

Topic: A.05. Axon and Dendrite Development

Support: the Sloan Foundation

UVa Fund for excellence in science and technology

NIH-NINDS 1R01NS072388

Title: Coronin-1 mediates hierarchal neurotrophin signaling required for sympathetic neuron target innervation

Authors: *D. SUO, J. PARK, C. DEPPMANN;
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Abstracts: Developing axons traverse multiple growth niches; first moving in fascicles along blood vessels then ramifying in the final target. In postganglionic sympathetic neurons this transition is driven by two neurotrophins acting through the same receptor tyrosine kinase, TrkA: vascular derived neurotrophic factor 3 (NT3) and final target derived nerve growth factor (NGF). We find that the NGF-induced gene, Coronin-1, represents a molecular switch critical for axon transition from intermediate to final targets. Both neurotrophins are capable of inducing MAPK and PI3K through TrkA, however; only NGF induces coronin-1 dependent calcium signaling. Prior to final target innervation NT3-TrkA promotes axon growth and inhibits branching through MAPK, whereas upon coronin-1 upregulation, Coronin-1 dependent calcium signaling decreases axon growth rate and branching in the final target. Neither intermediate nor final destination niche displays branching commensurate with patterning of a final target, suggesting the existence of an additional niche. We provide evidence for a “transition window” where axons are exposed to NGF but coronin-1 expression has not yet been induced. In this niche NGF-TrkA-PI3K induces a remarkable increase in PI3K-dependent growth rate and branching, which we suggest is a key determinant for axon patterning in target organs.

Disclosures: D. Suo: None. **J. Park:** None. **C. Deppmann:** None.

Poster

594. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 594.13/C11

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

Support: NIH R01NS079569

Title: Elimination of cortico-motoneuronal connections during development by Sema6D-PlexA1-mediated axon pruning in mice

Authors: *J. KALAMBOGIAS[1], Z. GU[2], M. UENO[2], A. KUMANOGOH[3], J. MARTIN[4], Y. YOSHIDA[2];
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Abstracts: The corticospinal tract (CST), originating primarily in motor cortex (M1), is a key spinal pathway for voluntary movements. In many monkey species and humans, the CST makes mostly contralateral and some ipsilateral monosynaptic connections directly with motoneurons (MN) as well as indirectly via interneurons. In mice and most other species CST connections are made primarily with contralateral interneurons. Establishment of these circuits is preceded by transient projections that are subsequently eliminated, leading to the mature pattern. Whereas the role of activity- and use-dependent processes in refining CST connections and motor development is well-studied, the molecular mechanisms are poorly understood. By labeling the CST with GFP using mouse genetics, we find that CST projections in wild-type mice descend in the dorsal, ventral and lateral columns at cervical levels at P2 and terminate throughout much of the gray matter, including the motor pools. By P14 most of the ipsilateral descending and motor pools projections are eliminated, leading to a predominantly contralateral projection to spinal interneurons. By contrast, PlexA1, as well as Sema6D (a PlexinA1 receptor), mutant mice fail to eliminate transient ipsilateral descending and motor pool projections. CST contacts remain on motoneurons, producing a bilateral projection to interneurons and MNs. To assess CST physiological actions in mature mice, we used M1 microstimulation (14 stim; 333 Hz) to evoke limb movements and facilitation of sustained forelimb EMG activity (single stim; post-stimulus facilitation, PStF). In the PlexA1 mice, threshold stimulation at most M1 sites evoked bilateral forelimb movements, consistent with bilateral CST projections, while threshold stimulation in controls evoked contralateral responses. Remarkably, PlexA1 mice displayed shorter PStF latencies compared with controls, consistent with maintenance of direct MN connections into maturity. PlexA1 mice reached with both forelimbs, instead of one or the other, and displayed a grasping impairment, suggesting disrupted left-right and inter-joint coordination. Our results show the importance of PlexA1-Sema6D in refinement of exuberant CST projections in the white matter and ventral horn, as well as development of voluntary motor skills. These findings complement the role of motor experience and activity in CST axon refinement. Importantly, the anatomical and physiological data suggest cortico-motoneuronal connections in the PlexA1

mice. This finding provides new insights into why the mouse CST does not have monosynaptic cortico-motoneuronal connections, whereas the human CST does.

Disclosures: J. Kalambogias: None. **Z. Gu:** None. **M. Ueno:** None. **A. Kumanogoh:** None. **Y. Yoshida:** None. **J. Martin:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 595.01/C12

Topic: A.08. Transplantation and Regeneration

Support: SNRP GRANT # NS041073

RCMI-NIH G12

NIH 5 R01NS37060-14

F32 NS054511-03

Title: Extracellular histones, a putative inhibitor of cns axonal regeneration, are blocked by activated protein c (apc)

Authors: M. M. SIDDIQ[1], S. S. HANNILA[2], Y. ZORINA[3], V. RABINOVICH[1], E. NIKULINA[4], *W. MELLADO[5], R. IYENGAR[1], M. T. FILBIN[6];
[1]Mount Sinai Sch. of Med., New York, NY; [2]Univ. of Manitoba, Winnipeg, MB, Canada; [3]Acorda Therapeut., Ardsley, NY; [4]The Feinstein Inst. for Med. Res., Manhasset, NY; [5]Burke Med. Res. Inst., White Plains, NY; [6]Hunter Col., New York, NY

Abstracts: Axons in the injured adult CNS do not regenerate, in part due to the inflammatory response. Outside the nervous system, released histones are detected in response to inflammation. In the CNS, up-regulation of a cytoplasmic isoform of histone H1 was reported in neurons and astrocytes in a mouse model of prion disease and in humans with Alzheimer's disease. Adult mouse ischaemic brain slices released histones H1 and H2B into the media. Examination of conditioned media from naïve astrocytic cultures revealed secretion of Histones H2A and H4. This suggests that histones are released extracellularly in the CNS under pathological conditions. Here, we show that primary rat cortical and hippocampal neurons extended long neurites when grown on permissive monolayers of CHO cells; however, when we

simultaneously added exogenous histones (a mix of all isoforms) to the co-cultures, we observe significantly shorter neurites (up to 70% shorter). Using microfluidic chambers, a technique which isolates the cell bodies from the neurites, we plated cortical neurons on PLL and after one week we observed long neurites growing across the micro-grooves of the chamber. In contrast, when histones were applied to either the cell body- or neurite-containing parts of the chambers, we observed that neurites were unable to grow a significant distance past the micro-grooves. Furthermore, exogenous application of histones to primary cortical neurons activates Rho GTPase. Using western blot analysis, we determined that levels of extracellular histones are increased *in vivo* after dorsal column lesion and are released into the CSF within 24hrs. Together with our *in vitro* data, these findings suggest that extracellular histones may inhibit axonal regeneration in spinal cord injury as well. Extracellular histones can be cleaved by APC, an enzyme that is part of coagulation system. In microfluidic chambers with cortical neurons, the inhibitory effect of histones on neurite outgrowth can be blocked with the addition of APC. In addition, delivery of APC after dorsal column injury or optic nerve crush promotes axonal regeneration *in vivo*. Our data suggests that extracellular histones released after injury to the CNS are inhibitory to axonal regeneration and administration of APC can block this inhibitory effect and promote regeneration.

Disclosures: **M.M. Siddiq:** None. **S.S. Hannila:** None. **Y. Zorina:** None. **V. Rabinovich:** None. **E. Nikulina:** None. **W. Mellado:** None. **R. Iyengar:** None. **M.T. Filbin:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 595.02/C13

Topic: A.08. Transplantation and Regeneration

Support: NIH Grant 5R01GM054508

Title: Systems therapeutic approach for axonal regeneration in the injured CNS

Authors: ***M. M. SIDDIQ**[1], Y. ZORINA[3], V. RABINOVICH[1], E. KAPLAN[2], M. T. FILBIN[4], R. IYENGAR[1];
[1]Pharmacol. and Systems Therapeut., [2]Ophthalmology, Mount Sinai Sch. of Med., NEW YORK, NY; [3]Acorda Therapeutics, Inc, Ardsley, NY; [4]Biol., Hunter Col., New York, NY

Abstracts: Axons in the injured CNS do not regenerate, in part due to the inhibitory environment. Neurons must integrate the signals that they receive from multiple pathways in order to respond to this inhibitory environment in order to promote axons to regenerate. Taking a systems therapeutic approach, we investigated the cannabinoid-1 receptor (CB1R) signaling cascade to stimulating neurite outgrowth and determined that it may be regulated by a much larger signaling network, allowing for many potential points of signal integration. The CB1R signaling cascade involves activation of signal transducer and activator of transcription 3 (STAT3) and cAMP response element-binding protein (CREB), both are transcription factors involved with neurite outgrowth and axonal regeneration. We studied how integration of CB1R and other parallel signaling pathways, in this case the Interleukin-6 receptor (IL-6R) could induce axonal regeneration. We found in primary cortical neurons that combinatorial activation of the CB1R and IL-6R pathways converge on STAT3 activation for promoting neurite outgrowth even in the presence of myelin inhibitors. We also observed that lower dosages of the agonists for CB1R and IL-6R that are normally ineffective individually, in combination promoted neurite outgrowth of cortical neurons. To determine if the combinatorial activation of CB1R and IL-6R can promote *in vivo* axonal regeneration we utilized the rat optic nerve model. Using HU-210 as the agonist for CB1R, and IL-6 which we delivered intra-vitreally immediately after optic nerve crush we determined the extent of axons regenerating with cholera toxin-B (CTB) labeling and GAP-43 staining. We found using sub-optimal concentrations of HU-210 or IL-6 individually did not promote axonal regeneration in the crushed optic nerve, however in combination we observed robust CTB-labeled axons and GAP-43 staining that were significantly past the crush site. Currently we are using electroretinograms (ERGs) to determine if there are physiological improvements detectable in the retinal ganglion cell bodies that could be indicative of the potential axonal regeneration in the injured optic nerve. Our preliminary data suggests that after HU-210 and IL-6 treatment there are improvements in the pattern and flash ERGs. Our combinatorial approach offers us an opportunity to elucidate signaling pathways to integrate and induce axonal regeneration in the injured CNS using lower dosages of agonists and potentially decreasing unwanted side effects.

Disclosures: **M.M. Siddiq:** None. **Y. Zorina:** None. **V. Rabinovich:** None. **E. Kaplan:** None. **M.T. Filbin:** None. **R. Iyengar:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 595.03/C14

Topic: A.08. Transplantation and Regeneration

Support: NS061293

Title: Microglial depletion reduces neurogenesis in response to QA-induced lesioning in adult zebrafish telencephalon

Authors: *K. SKAGGS[1], D. GOLDMAN[2], J. M. PARENT[3];
[1]Neurol., [2]Biol. Chemistry, Mol. and Behavioral Neurosci. Institutie, Neurosci. Program,
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Abstracts: Zebrafish represent an attractive system for the study of neurogenesis following injury because, unlike mammals, they regenerate damaged neurons. We developed a novel brain injury model using quinolinic acid (QA) excitotoxic telencephalic lesioning in adult zebrafish to study neural regeneration. After lesioning, initial cell death is followed by robust inflammation 1-2 days post-injury and a marked increase in proliferation or radial glia-like progenitors that peaks 3-4 days post-injury. The neural progenitors give rise to increased numbers of newborn neurons that migrate to injury and integrate, likely contributing to repair of the lesioned brain. In order to study the role of the early inflammatory response on neurogenesis, we ablated microglia that responded to injury though injection of liposomes containing Clodronate at the time of lesioning. Proliferation and neurogenesis were markedly reduced following Clodronate treatment. These effects persisted over time, resulting in incomplete repair in Clodronate-treated, lesioned brains compared to control liposome-treated, lesioned brains. These results indicate that the early inflammatory response following QA-induced excitotoxic lesioning is an important signaling event that stimulates neurogenesis and repair of adult zebrafish telencephalic brain injury.

Disclosures: K. Skaggs: None. **D. Goldman:** None. **J.M. Parent:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 595.04/C15

Topic: A.08. Transplantation and Regeneration

Support: Wings for Life Foundation

Canadian Institutes of Health Research (CIHRA)

Title: Aging diminishes the regenerative capacity of PTEN deleted rubrospinal neurons following spinal cord injury

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Abstracts: The devastating paralysis that occurs following spinal cord injury (SCI) is largely a result of transected central nervous system (CNS) axons failing to regenerate. Previous research has demonstrated that phosphatase and tensin homolog (PTEN) is a significant contributor to regenerative failure, with its deletion within retinal ganglion cells and corticospinal neurons resulting in robust regenerative responses in the optic nerve and corticospinal tract respectively. Here, we assessed whether PTEN deletion would promote axon regeneration in the rubrospinal tract (RST) and whether the age at which PTEN is deleted has an impact on rubrospinal regenerative outcome. Four week (young) and 7-8 month (old) aged floxed PTEN mice were injected with adeno-associated virus serotype 2 expressing Cre and GFP (AAV2-Cre) or GFP alone for control (AAV2-GFP) into the right red nucleus. Four weeks later, mice underwent a left dorsolateral crush at cervical level C4/C5 to fully axotomize the rubrospinal tract. Eight weeks later, mice were sacrificed. While young AAV-Cre injected mice showed rubrospinal axon regeneration up to 1 mm past the injury site, old AAV-Cre injected mice showed only decreased dieback back to the lesion site relative to controls and significantly less axon growth caudal to the lesion site relative to young mice. In analysis of rubrospinal neuron cell bodies within the midbrain, we observed no difference in GFP or phospho-S6 labelling between young and aged AAV-Cre injected mice, suggesting that the decreased regenerative response following ageing is independent of mammalian target of rapamycin (mTOR) signaling. Thus, although PTEN deletion promotes rubrospinal axon regeneration, ageing significantly diminishes the regenerative capacity of PTEN deleted rubrospinal neurons following SCI.

Disclosures: B.J. Hilton: None. **W. Tetzlaff:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.05/C16

Topic: A.08. Transplantation and Regeneration

Support: NIH AG20047

Title: Direct conversion of adult spinal cord-derived oligodendrocyte progenitor cells (OPCs) to spinal motor neurons

Authors: *S. BAZAREK[1], R. A. MARR[1], C. A. BRIGGS[1], G. E. STUTZMANN[1], R. M. HOWARD[2], S. R. WHITTEMORE[2], D. A. PETERSON[1];
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Abstracts: The lack of cell replacement following neurological injury limits the regenerative response of the CNS. Progress in understanding the biology of neural stem cells has raised interest in using stem cells for replacing neurons lost to injury or to disease. As existing committed and uncommitted cells in the CNS do not naturally progress to a neuronal fate, it will be necessary to engineer a conversion to a neuronal fate. Advances in cellular reprogramming provide new tools for re-specification of cell fate and provide a potential alternative to cell transplantation, namely the direct *in vivo* conversion of resident CNS cell populations for neuronal replacement. Success in this approach will require the generation of relevant neuronal subtypes. The aim of this study was to evaluate the effect of various neurogenic transcription factors (sox2, mash1, olig2VP16, pax6, and neurogenin2) that are related to cell specification during development on fate induction and subtype specificity on resident glia in the spinal cord. An O4+ Oligodendrocyte Progenitor Cell (OPC) population was enriched from the adult rat spinal cord using Magnetic Activated Cell Sorting (MACS). OPCs are the most abundant cycling population in the adult CNS and their isolation provides an ideal *in vitro* assay for screening neuronal fate determinants. Retroviral-GFP delivery of the single factor, neurogenin2, or the combination of sox2 and mash1 to cultured OPCs resulted in co-expression of GFP with the early neuronal marker, B-III-tubulin at 7 days. Neurogenin2-transduced cells expressed mature neuronal markers, NeuN and MAP2, and cholinergic marker, ChAT, at 7 days. These cells also exhibited typical spinal motor neuron morphology and repetitive action potentials were evoked at 5 weeks post-transduction. In showing that adult-derived OPCs can be directly converted to functional neurons *in vitro*, we provide support for investigating the direct *in vivo* conversion of resident OPCs to a neuronal fate. This approach may provide an alternative therapeutic strategy for neuronal replacement in the adult spinal cord.

Disclosures: S. Bazarek: None. **D.A. Peterson:** None. **G.E. Stutzmann:** None. **R.A. Marr:** None. **C.A. Briggs:** None. **S.R. Whittemore:** None. **R.M. Howard:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.06/C17

Topic: A.08. Transplantation and Regeneration

Support: NIH EY013879

Title: N-cadherin function in the adult zebrafish optic nerve regeneration

Authors: S. BHATTARAI[1], R. L. LONDRAVILLE[2], *Q. LIU[3];
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Abstracts: Injured optic nerves in adult mammals have very limited ability to regenerate due to unfavorable extracellular conditions and reduced intrinsic regenerative ability. Unlike mammals, injured optic nerves in fish and amphibians can successfully regenerate. Molecules implicated to promote regeneration include growth promoting factors, transcription factors and cell adhesion molecules such as cadherins. Cadherins are calcium dependent cell adhesion molecules that play important roles in development and maintenance of adult tissues. We previously showed that N-cadherin expression in the adult zebrafish visual system was limited mainly to the germinal zone and outer plexiform layer of the retina, but following an optic nerve crush, its expression became significantly increased in both the retinal ganglion cell layer and the optic nerve for more than two weeks. To determine N-cadheirn function in zebrafish optic nerve regeneration, we blocked N-cadherin expression by electroporating a lissamine-tagged N-cadherin morpholino antisense oligonucleotide (MO) into the fish eye one day after the optic nerve crush, and examined retinal axons regeneration at various time points (e.g. 7 and 14 days). We found that optic nerve regeneration was severely perturbed in these fish compared to fish with a control MO electroporated into the eye. The vast majority of the regenerating optic axons in the N-cad MO treated fish were found between the optic nerve head region and the crush site (immediately behind the eye) 7 days after the optic nerve crush, while the regenerating retinal axons in the control MO treated fish arrived in the optic tectum. Our results suggest that N-cadherin plays an important role in the fish optic nerve regeneration.

Disclosures: S. Bhattarai: None. **R.L. Londraville:** None. **Q. Liu:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: U.S Army W81XWH-12-1-0319

NEI

Pew Charitable Trust

Ziegler Foundation

Title: Characterization of AAV vectors' potentials to promote long distance optic nerve regeneration, path-finding, and restoration of visual functions after injury

Authors: *B. YUNGHER[1], T. SCHMIDT[3], K. CHEW[3], X. LUO[1], K. LYAPICHEV[1], T.-H. CHOU[2], V. PORCIATTI[2], M. BLACKMORE[4], S. HATTAR[3], K. PARK[1];
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Abstracts: Recent studies have demonstrated the effectiveness of several Cre-mediated gene deletions at enhancing axon regeneration in adult retinal ganglion cells (RGCs), though such transgenic manipulations have limited therapeutic relevance and practicability. Given the successes of gene therapy in treating ocular disorders, in the present study we sought to evaluate the potentials of AAV vectors in promoting i) long distance RGC axon regeneration, ii) target-specific guidance of RGC axons to their visual targets, and iii) recovery of visual functions after optic nerve injury. To this end, we use a novel combinatorial approach in which we combine AAV vectors expressing short hairpin RNA against phosphatase and tensin homolog (PTEN) with ciliary neurotrophic factor (CNTF) along with pharmacological elevation of a cyclic adenosine monophosphate (cAMP) analog. We observe the efficacy of this RNA interference both at the suppression of endogenous PTEN expression and at enhancing RGC axon regeneration within the optic nerve of adult mice. Furthermore, combining these three treatments synergistically promotes extensive RGC axon regeneration beyond the optic chiasm and into the brain, an effect dependent on the mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3) pathways. Regenerating axons display misguidance at multiple levels, frequently turning and looping in the optic nerve and projecting to non-target areas in the brain. Nevertheless, we find this treatment sufficient to induce axon regeneration into the suprachiasmatic nucleus (SCN), the retino-recipient circadian pacemaker normally innervated by highly-melanopsin expressing intrinsically photosensitive RGCs. The number of axon fibers reaching the SCN is even higher in apoptosis-deficient bcl2-associated X (BAX) knockout mice. Visualization of the retina by optical coherence tomography reveals outer nuclear layer swelling. Photoentrainment of circadian rhythms, as measured by wheel-running activity, does not occur following AAV treatment in wild-type or BAX null mice, demonstrating

the importance of type-specific regeneration and maintenance of retinal physiology, rather than simply total axon numbers regenerating.

Disclosures: **B. Yungher:** None. **T. Schmidt:** None. **K. Chew:** None. **X. Luo:** None. **K. Lyapichev:** None. **T. Chou:** None. **V. Porciatti:** None. **M. Blackmore:** None. **S. Hattar:** None. **K. Park:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.08/C19

Topic: A.08. Transplantation and Regeneration

Support: Janssen R&D

Title: Unraveling the therapeutic mechanisms of human umbilical cord tissue-derived cells (hUTC) in retinal degenerative diseases

Authors: ***S. KOH**[1], N. KIM[2], N. DEJENEKA[3], I. HARRIS[3], H. YIN[2], C. EROGLU[1]; [1]Cell Biol., [2]Psychology and Neurosci., Duke Univ., Durham, NC; [3]Janssen Res. & Develop., Spring House, PA

Abstracts: Age-related macular degeneration (AMD) is the leading cause of vision loss in people over the age of 50. Subretinal administration of hUTC to a model of retinal degeneration preserved photoreceptors and visual function; however, the therapeutic mechanism of hUTC has not been defined. Here we characterized the effects of hUTC on functional synapse formation, neuronal survival and outgrowth. Retinal ganglion cells (RGCs), purified from P7 rats,were co-cultured with hUTC or cortical astrocytes (ASCs) as a positive control. A normal human dermal fibroblast (NHDF) cell line was used as negative control. Synapse formation was assessed by immunocytological co-localization of pre- (Bassoon) and post-synaptic (Homer-1) markers and we found that RGC exhibited significant increases on both number and size of synaptic puncta, comparable to astrocytes (positive control). Subsequent electrophysiological recording of the RGCs treated under same conditions revealed that hUTC also increase the amplitude of miniature excitatory postsynaptic currents (mEPSCs). To confirm the effects of co-culture, RGCs were fed with various concentrations of hUTC-conditioned medium (hUCM). Synapse analysis showed that hUCM is sufficient to induce synapse formation in concentration dependent manner, similar to astrocyte-conditioned media (ACM). hUCM also strengthened functional

synapses as shown by increased amplitude and frequency of mEPSCs. Besides its effects on synapse formation, hUCM was sufficient to promote RGC survival in the absence of any other growth factors. hUCM also enhanced RGC neurite outgrowth as demonstrated by significant increase in total process length, number of processes and number of branches. In conclusion, we found that hUTC secrete factors that promote development of functional synapses between purified RGCs *in vitro*. Moreoever, hUTC also support neuronal survival and neurite outgrowth. Our findings suggest that hUTC may affect multiple aspects of retinal cell health and connectivity. The work described was performed under a sponsored research agreement between Duke University and Janssen R&D.

Disclosures: S. Koh: None. **N. Kim:** None. **N. Dejeneka:** A. Employment/Salary (full or part-time):; Janssen R&D. **I. Harris:** A. Employment/Salary (full or part-time):; Janssen R&D. **H. Yin:** None. **C. Eroglu:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.09/C20

Topic: A.08. Transplantation and Regeneration

Support: SHC-85220

SHC-84293

Title: Expression of RhoA in lamprey brain after spinal cord injury

Authors: *K. G. ZHANG[1], J. HU[1], W. RODEMER[1], M. E. SELZER[2,1];
[1]Shriners Hosp. Pediatric Res. Ctr., [2]Neurol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstracts: Disability following spinal cord injury (SCI) is due to failure of axonal regeneration. It is believed that axon growth is inhibited by the presence of several types of inhibitory molecules in CNS, including the chondroitin sulfate proteoglycans (CSPG), myelin-associated glycoprotein (MAG), Nogo and oligodendrocyte-myelin glycoprotein (OMgp). In the lamprey CNS, there are 18 pairs of individually identified reticulospinal neurons with heterogeneous axon regenerative abilities, among which “bad-regenerating” neurons often experience a very delayed form of apoptosis. Previously we reported that the putative CSPG receptors, PTPsigma and LAR, are expressed selectively in the bad-regenerating neurons both in normal lampreys and after SCI,

when those same neurons were also undergoing apoptosis, as indicated by labelling with fluorescently labelled inhibitors of caspase activation (FLICA). Many studies indicate that the intracellular signaling pathways downstream of those inhibitory molecules converge on RhoA. CSPG can activate RhoA via their receptor LAR, and after SCI, activated RhoA has been implicated in both apoptosis and axon growth inhibition. To study the role of RhoA in SCI-induced retrograde neuronal death, we cloned lamprey RhoA, which was highly homologous to mammalian RhoA, and investigated its distribution in lamprey CNS by wholemount *in situ* hybridization (ISH) before and after SCI. RhoA expression was observed in both neurons and glial cells, and increased developmentally in normal lamprey CNS. RhoA was continuously expressed in bad-regenerating neurons and in microglia/macrophages on cord surface after SCI. However, RhoA expression decreased greatly in most reticulospinal neurons after SCI. Moreover, RhoA mRNA expression was correlated with caspase activation in brain at 2 weeks after SCI. The inverse correlation of RhoA expression with the intrinsic regenerative ability and survival post-axotomy is consistent with a role for RhoA signaling in triggering apoptosis and restricting regeneration in lamprey reticulospinal neurons after SCI.

Disclosures: **K.G. Zhang:** None. **J. Hu:** None. **W. Rodemer:** None. **M.E. Selzer:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.10/C21

Topic: A.08. Transplantation and Regeneration

Support: Wings for Life

IRC 268282

Title: Anisotropic capillary hydrogels with cellular growth factor delivery promote linear axonal growth in the injured spinal cord

Authors: ***M. GÜNTHER**[1], M. MOTSCH[1], N. WEIDNER[1], R. MÜLLER[2], A. BLESCH[1]; [1]Spinal Cord Injury Ctr., Univ. Hosp. Heidelberg, Heidelberg, Germany; [2]Inst. of Physical and Theoretical Chem., Univ. of Regensburg, Regensburg, Germany

Abstracts: Despite recent progress in enhancing axonal growth in the injured spinal cord, the guidance of regenerating axons across an extended lesion site remains a major challenge. To

determine whether axons can be guided in a rostrocaudal direction within a spinal cord lesion site, we implanted alginate-based anisotropic capillary hydrogels seeded with bone marrow stromal cells (BMSCs) into the injured rat spinal cord. Adult female Fischer344 rats underwent a C5 lateral hemisection. Immediately following the lesion, hydrogels (2x2x1.3 mm) seeded with BMSCs (2 µl; 100,000 cells/µl) were implanted into the lesion site. BMSCs were genetically modified to express brain-derived neurotrophic factor (BDNF; 170 ng/ 10^6 cells/ 24 h) or GFP as a control. Four weeks post-lesion, numerous GFP-labeled BMSCs survived inside the scaffold capillaries, providing a cellular matrix for axonal growth. Axons were primarily found in the center of each channel, often in bundles, whereas BMSCs appeared to cover the channel wall. In addition, blood vessels extended into the gels, identified by von Willebrandt factor-labeling. Quantification of the number of axons growing into channels with BDNF- and GFP-expressing cells (n = 5-6 animals/group) demonstrated 3.3 times ($p < 0.01$) higher numbers of β-III-tubulin-labeled axons in BMSC-BDNF seeded hydrogels at 100 µm and 4.2 times ($p < 0.05$) higher numbers at 500 µm inside the hydrogel. Axons were also found in the center of the scaffold (~ 1 mm away from host tissue). Increasing the capillary diameters of hydrogels seeded with BDNF expressing BMSCs from 41 µm to 65 µm did not lead to significant differences in the number of regenerating axons. The orientation of axonal growth in hydrogel capillaries was measured using a new semiautomatic image analysis system and compared to BMSC suspension grafts without hydrogels. In lesions without hydrogels, regenerating axons exhibited random orientations, whereas axons were oriented parallel to the channel walls inside the hydrogels. To examine how far axons can grow into the hydrogel channels and to determine whether axons reach the caudal end of the lesion site, anterograde tracer BDA was injected above the lesion site at level C2/3. The results of this experiment are currently being determined.

Disclosures: **M. Günther:** None. **M. Motsch:** None. **N. Weidner:** None. **R. Müller:** None. **A. Blesch:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.11/C22

Topic: A.08. Transplantation and Regeneration

Support: NSF Grant 1003907

Title: Implantable fibrin hydrogels as novel engineered microenvironments that recruit and guide endogenous neural stem cells for cell replacement therapy in the brain

Authors: A. R. CLARK, A. B. CARTER, *E. M. PRICE;
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Abstracts: Cell replacement therapy is a promising approach to treat numerous neurodegenerative disorders such as Parkinson's disease, stroke and traumatic brain injury. Unfortunately, direct stem cell transplantation into areas of the brain where new neural cells would be beneficial has proven to be problematic due to issues such as tumor formation and the inability for the implanted cells to integrate into existing circuitry. Our long-term goal is to exploit endogenous adult neural stem cells and redirect migration from their usual destination to new regions of the brain where they will reconstitute lost neural function. The subventricular zone (SVZ) is one source of adult neural stem cells, and their usual function is to continually provide the olfactory bulb with new neurons, which arrive from the SVZ via the rostral migratory stream (RMS). We have engineered cylindrical fibrin hydrogels which contain immobilized neurotrophins, extracellular matrix molecules and aprotinin, and we have implanted these cylinders into one hemisphere of the rat brain with the goal of creating a new migratory path for SVZ-derived neural stem cells. Hydrogel cylinders (0.7 mm dia x 6 mm long) were made with fibrin containing VEGF, NGF, aprotinin and laminin immobilized with the homobifunctional chemical crosslinker disuccinmidyl suberate (DSS). Stereotaxic coordinates were used to implant the cylinder at a location 17 degrees off midline on a path from the RMS to the striatum. Animals were sacrificed 6 weeks following surgery and sections of brain were analyzed via immunohistochemistry with DAPI, doublecortin (Dcx), a marker for immature neurons, and 160 kD Neurofilament (NF 160), a marker for mature neurons. DAPI-labeled cells were found concentrated in the implant region in a pattern that was suggestive of a controlled migratory response to the hydrogel. Dcx labeling demonstrated that migrating neuroblasts (Dcx+) were abundant in the brain region surrounding the cylinder implant region. NF 160 labeled neurons were also observed coincident with Dcx+ cells, indicating that mature neurons, possibly derived from the Dcx+ precursors, were also present in the region of the fibrin hydrogel implant. There was no evidence of undegraded fibrin in any of the samples analyzed. Except for the surface of the brain corresponding to the area of the implant, no overt signs of damage from the procedure were apparent. We are currently evaluating the efficacy of these implants in correcting the 6-OHDA hemi-Parkinson rat model.

Disclosures: **A.R. Clark:** None. **A.B. Carter:** None. **E.M. Price:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Support: German Federal Ministry of Education and Research (BMBF, 01GQ1206, BG)

National Science Foundation (NSF, LH)

National Institute of Health (NIH, 2T15LM009451, LH)

Title: The hanalyzer: A tool to model neurodegenerative and -regenerative events after spinal cord injury

Authors: W. BAUMGARTNER JR[1], D. M. WALDERA-LUPA[2], D. PAPE[3], I. GEORGIEV[1], I. GRICHTCHENKO[1], L. HUNTER[1], K. STUEHLER[2], K. COHEN[1], *B. GRIMPE[3];
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Abstracts: The suffering of millions of spinal cord injury (SCI) patients, worldwide, has been the motivation for numerous studies for over a century. Despite extensive success in identifying genes and proteins involved in the complex processes after SCI, no satisfying treatment is available. Our work focuses on understanding the entire complex biochemical processes and their interplay with pathways to unravel the mechanisms leading to regeneration failure, with the goal to find a treatment for SCI. As our central hypothesis is that major functional changes after trauma to the spine are reflected on the proteome level, we perform quantitative proteomics experiments of contused spinal cords at different time points. Therefore, we will present analysis of results, which were generated 1 hour after injury. The results are integrated into a systems biology tool, called Hanalyzer, which was modified to work in the SCI field. For this purpose we extend the tool to draw on information sources for the rat in addition to those for the mouse and human that have been used up to now. The system further implements various ontologies; links molecular functions, biological processes and cellular component to genes, metabolic pathways, and small molecule participants; and retrieves information from various proteinprotein, protein-DNA interaction or metabolic data bases. It also interfaces with the published biomedical literature, extracting facts that are relevant to the biological problem at hand. The comprehension of the complex system behavior and unravel key proteins responsible for regeneration failure holds promise to find a treatment for SCI in the near future.

Disclosures: **W. Baumgartner Jr:** None. **D.M. Waldera-Lupa:** None. **D. Pape:** None. **I. Georgiev:** None. **I. Grichtchenko:** None. **L. Hunter:** None. **K. Stuehler:** None. **K. Cohen:** None. **B. Grimpe:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Program#/Poster: 595.13/C24

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH P01 NS055976

Craig H. Neilsen Foundation 280850

Title: Creating a migratory stream of transplanted glial restricted progenitors as a therapeutic strategy for spinal cord injury

Authors: *X.-B. YUAN, C. HAAS, I. FISCHER;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstracts: Transplantation of glial restricted progenitors (GRP) and derived astrocytes is a promising therapeutic strategy for treatment of spinal cord injury, capable of creating a supportive environment for regeneration of injured host axons. Our previous studies have demonstrated that GRP transplantation allows for the regeneration of lesioned, ascending sensory axons into but not out of the graft. Given the permissive nature of GRP, we hypothesized that regenerating axons become trapped within GRP grafts by: 1) encountering an excess of permissive factors within GRP grafts, stalling further regenerative attempts, or 2) forming connections/adhesions with graft-derived cells (e.g., astrocytes or NG2 cells). To address these issues, we targeted GRP migration as a means to enhance axonal regeneration, hypothesizing that by extending the permissive migratory GRP stream we would extend axonal regeneration by: 1) shifting the concentration of the permissive environment or 2) “towing” sensory axons along migrating GRP. We reasoned that during development, gradients of chemotactic factors direct the migration of neural progenitors, and building upon these principles, we examined the effects of related factors on migration of grafted GRP. To screen chemotactic molecules that augment GRP migration, we developed an *in vitro* agarose gradient migration assay and confirmed our results utilizing a standard transwell assay, identifying stromal derived factor-1 (SDF1) and basic fibroblast growth factor (bFGF) as potent pro-migratory agents. In addition, we also discovered that chondroitin sulfated proteoglycans (CSPG) presented a strong inhibitory cue to GRP/astrocyte migration that could be overcome by the CSPG-digesting enzyme, Chondroitinase. Following transplantation into the injured spinal cord, we found that the direction-specific migration of GRP could be significantly enhanced following administration of

lentiviral gradients of chemotactic factors SDF1 and bFGF, as well as CSPG-digesting Chondroitinase. However, the increased migration was not associated with increased regeneration, indicating that other interventions may be needed to prevent connections with graft-derived cells (e.g., targeting LAR receptors) or to increase the regenerative capacity of the sensory axons (e.g., mTOR, SOCS3). These results highlight the ability of GRP grafts to serve as a therapeutic platform and future studies will be directed at identifying additional chemoattractive molecules and evaluating combinatorial strategies for promoting axonal regeneration to reconnect the injured spinal cord. Support by Craig H. Neilsen Foundation 280850 and NIH P01 NS055976.

Disclosures: X. Yuan: None. **C. Haas:** None. **I. Fischer:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: International Foundation for Research in Paraplegia, International Spinal Research Trust and the EU (IRG268282 to A. B)

Wings for Life postdoctoral fellowship to B.S.

Title: Limited functional effects of subacute syngenic bone marrow stromal cell transplantation after rat spinal cord injury

Authors: *B. SANDNER, M. CIATIPIS, M. MOTSCH, N. WEIDNER, A. BLESCH;
Spinal Cord Injury Ctr., Univ. Hosp. Heidelberg, Heidelberg, Germany

Abstracts: Cell transplantation might be one means to improve motor, sensory or autonomic recovery after traumatic spinal cord injury (SCI). Among different cell types, bone marrow stromal cells (BMSCs) have received considerable interest due to their potential neuroprotective properties. However, large discrepancies exist in studies published to date due inadequate animal models, a lack of adequate cellular controls, and limited outcome measures. Therefore, we analyzed motor function, bladder recovery and tissue sparing after syngeneic BMSC transplantation in a rat spinal cord contusion injury model. Adult Fischer 344 rats underwent a T9 contusion injury (200kDy) followed by grafting of syngeneic GFP-expressing BMSCs into

the lesion epicenter 3 days post-injury (n=17). Animals receiving a contusion injury and no cellular graft (n=16), or an injury followed by grafts of syngeneic fibroblasts (n=16) served as control. Eight weeks post transplantation, BMSC-grafted animals showed only a minor functional improvement in hindlimb motor function of 1-1.5 points in the BBB open field locomotor rating scale, which was only significant compared to injured control animals without cellular grafts at this time point ($p<0.05$). However, no differences in sensorimotor recovery (number of footfalls) were observed when animals crossed a horizontal ladder. Both cell types survived in the lesion site, but the volume of BMSCs was significantly smaller compared to fibroblast grafts already one week post-grafting ($p<0.05$). Cell survival was further reduced in both groups at the latest assessment, eight weeks post-grafting ($p<0.05$). Eriochrome cyanine staining revealed only minor differences in spared white matter between all three groups, which did not reach significance. Urodynamic measurements of baseline, maximum and intermicturition pressure as well as micturition frequency and micturition volume indicated similar bladder dysfunction in all groups 8 weeks post-injury. Thus subacute grafting of BMSCs has only a minor effect on functional recovery, questioning the rationale for autologous intraspinal BMSC transplantation after SCI.

Disclosures: **B. Sandner:** None. **M. Ciatipis:** None. **M. Motsch:** None. **N. Weidner:** None. **A. Blesch:** None.

Poster

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Topic: A.08. Transplantation and Regeneration

Support: Craig H Neilsen Foundation

Title: The immune receptor dectin-1 participates in conditioning injury-induced axonal regeneration of sensory neurons

Authors: ***C. YOON**, K. CARBAJAL, K. BALDWIN, B. SEGAL, R. GIGER;
Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstracts: Under certain circumstances, activation of innate immunity promotes robust regeneration of severed axons following injury to the optic nerve or spinal cord for which, however, the underlying cellular and molecular mechanisms remain to be elucidated. Our lab

recently identified that the interaction of curdlan (a particular from of β-(1,3)glucan) with the pattern recognition receptor dectin-1 on myeloid cells triggers an inflammatory cascade that enables injured retinal ganglion cells (RGC) to extend lengthy axons that grow beyond the lesion site in the adult mouse optic nerve. Curdlan, an FDA approved food supplement, mimics the pro-regenerative effects of zymosan when injected into the vitreous of wildtype mice, but not dectin-1 null mice. In the current study, we hypothesized that dectin-1 participates in the conditioning injury-induced growth response of dorsal root ganglion (DRG) neurons. Our preliminary results show a decrease in macrophage infiltration into DRGs following conditioning injury in dectin-1 null mice compared to wild type controls. Flow cytometric analysis confirmed that following sciatic nerve injury, neutrophil, dendritic cell, and macrophage accumulation in DRGs is reduced. Importantly, conditioning injury-induced neuronal growth of DRG neurons in dectin-1 mutants is reduced compared to wildtype controls: neurite outgrowth, as assessed by NF200 immunolabeling, is significantly decreased in dectin-1 null cultures. Together these findings suggest that dectin-1 participates in immune responses that contribute to conditioning injury-induced axonal growth of sensory neurons. Our current focus is on determining whether dectin-1 is required for conditioning injury-induced growth of severed dorsal column axons in the mouse spinal cord. The long-term goal of this research is to identify the signaling pathways that are activated by the innate immune system to enable CNS axon regeneration following injury or disease.

Disclosures: C. Yoon: None. **K. Carbajal:** None. **K. Baldwin:** None. **B. Segal:** None. **R. Giger:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 595.16/C27

Topic: A.08. Transplantation and Regeneration

Title: Unconventional granulocytes are recruited by zymosan and promote central nervous system regeneration

Authors: *K. CARBAJAL[1], K. T. BALDWIN[2], R. J. GIGER[2], B. M. SEGAL[1];
[1]Neurol., [2]Cell & Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstracts: Regeneration in the central nervous system (CNS) can be enabled by inflammation, however the underlying molecular mechanism is poorly understood. Intra-ocular injection of

zymosan, a yeast cell-wall extract, following optic nerve crush injury promotes local inflammation in association with robust retinal ganglion cell (RGC) axon growth. We have previously shown that immune cell expression of Dectin-1 and TLR2, pattern recognition receptors (PRRs) that bind components of zymosan, is critical for regeneration in this experimental model. In the present study, we assess the contribution of different leukocyte subsets to zymosan-induced axon regrowth. Flow cytometric analysis of intraocular infiltrates demonstrate that neutrophils are the most prominent cell type early post zymosan injection. To directly determine their pro-regenerative potential, we adoptively transferred zymosan-stimulated, purified neutrophils into the eyes of mice with O.N. crush only and observed robust axon regeneration comparable to positive controls injected intraocularly with zymosan. We next blocked recruitment of neutrophils to the eye following O.N. crush and intraocular zymosan injection with the expectation of thwarting axonal regrowth. Surprisingly, this resulted in a significant increase in regeneration of axons compared to vehicle treated controls. Detailed flow cytometric analysis confirmed blockade of conventional neutrophil recruitment following treatment but also revealed an unexpected enrichment of a subset of unconventional, CCR2+ granulocytes in the intraocular infiltrate. These unconventional granulocytes, which became the dominant population of infiltrating cells following treatment, have features of immature myeloid cells. Our study raises the possibility of using regenerative, CCR2+ granulocytes as a neuroprotective intervention in patients with CNS injury.

Disclosures: **K. Carbajal:** None. **R.J. Giger:** None. **B.M. Segal:** None. **K.T. Baldwin:** None.

Poster

595. Extrinsic Mechanisms Controlling CNS Regeneration

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 595.17/C28

Topic: A.08. Transplantation and Regeneration

Support: Charles A. Dana Foundation

Title: Beta-glucan/dectin-1 signaling in immune cells activates the canonical NF-kB pathway to induce long-distance cns axon regeneration

Authors: *K. T. BALDWIN[1], K. S. CARBAJAL[2], B. M. SEGAL[2], R. J. GIGER[1];
[1]Cell and Developmental Biol., [2]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstracts: Following injury to the adult mammalian central nervous system (CNS), severed axons fail to undergo spontaneous regeneration, resulting in permanent neurological deficits. Retro-orbital crush injury to the optic nerve in adult mice is a well-established animal model to study neuronal responses to CNS injury. Intra-ocular injection of zymosan, a yeast cell-wall extract, triggers an inflammatory response in the eye, and enables retinal ganglion cells (RGCs) to extend lengthy axons following optic nerve injury. Despite its relatively common use, the molecular mechanism of zymosan-elicited axonal regeneration is unknown. Here we identify particulate *β*-(1,3)glucan as the active ingredient in zymosan, sufficient to promote RGC axon regeneration. In mice, loss of the pattern recognition receptor *dectin-1*, but not *toll-like receptor-2 (TLR2)* or *complement receptor-3,* greatly attenuates zymosan-mediated RGC regeneration. The combined loss of *dectin-1* and *TLR2* completely blocks the pro-regenerative effects of zymosan, suggesting that these receptors act in a collaborative manner. Dectin-1 is found on retina-resident microglia and dendritic cells, as well as blood-derived myeloid cells which accumulate in the vitreous following intraocular inflammation. In *dectin-1-/-* mice, intraocular *β*-(1,3)glucan fails to induce optic nerve regeneration. Studies with chimeric *dectin-1* mice revealed a requirement for *dectin-1* in both retina-resident cells as well as bone marrow-derived immune cells for *β*-(1,3)glucan-elicited optic nerve regeneration. Ligation of dectin-1 with particulate *β*-(1,3)glucan leads to activation of multiple downstream signaling pathways, including both canonical and noncanonical NF-kB pathways, and the mitogen-activated protein kinase (MAPK) pathway. Studies with mice deficient for *caspase recruitment domain 9 (CARD9)* revealed that dectin-1 signals primarily through the canonical NF-kB pathway to elicit *β*-(1,3)glucan-induced optic nerve axon regeneration. Ongoing studies are focusing on a detailed characterization of myeloid cell types necessary for immune-mediated neuroregeneration, as well as chemokines, cytokines, and growth factors that enable long-distance axonal growth in the injured adult mammalian CNS.

Disclosures: K.T. Baldwin: None. **K.S. Carbajal:** None. **B.M. Segal:** None. **R.J. Giger:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.01/C29

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: European Research Council Grant 243153

The Wellcome Trust International Senior Research Fellowship 090946/Z/09/Z

Momentum Program LP2013-54/2013

NIH NS74432

Title: Differential nanoscale organization of cannabinoid signaling at perisomatic and dendritic GABAergic synapses uncovered by cell type-specific STORM superresolution imaging

Authors: *B. DUDOK[1], L. BARNA[1], S. I. SZABÓ[1], E. SZABADITS[1], B. PINTÉR[1], S. G. WOODHAMS[1], C. M. HENSTRIDGE[1], G. Y. BALLA[1], R. NYILAS[1], C. VARGA[2], S.-H. LEE[2], M. MATOLCSI[3], J. CERVENAK[4], I. KACSKOVICS[4,5], M. WATANABE[6], M. MELIS[7], M. PISTIS[7,8], I. SOLTESZ[2], I. KATONA[1];
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Abstracts: Retrograde endocannabinoid signaling via presynaptic CB_1 cannabinoid receptors controls neurotransmitter release at most synapse types. Despite the specific functions of CB_1 receptors in various forms of synaptic plasticity, the principles characterizing the precise subcellular distribution and density of this GPCR on given axon terminal types are poorly understood. Therefore, in the present study, we employed cell type-specific STORM superresolution microscopy to address the general question of how the nanoscale localization and amount of CB_1 receptors on boutons contribute to synapse-specific properties of cannabinoid signaling. For example, perisomatic and dendritic GABAergic synapses on CA1 pyramidal neurons exhibit striking quantitative differences in the efficacy of exogenous cannabinoids and in endocannabinoid signaling. Thus, we aimed to ascertain in the mouse hippocampus whether axon terminals of two major forms of CB_1-containing GABAergic interneurons, which are specialized to target either the perisomatic or dendritic regions of pyramidal cells, are equipped with a differential nanoscale molecular architecture for cannabinoid signaling. First, we developed a novel methodology based on the combination of whole-cell patch-clamp recording, single-cell biocytin labeling, and STORM superresolution microscopy, which allowed the cell type-specific correlation of physiological and morphological parameters with nanoscale molecular imaging data. By using this approach, we found that presynaptic CB_1 abundance correlated tightly with bouton size as well as axon terminals of perisomatically-projecting interneurons were larger and possessed more CB_1 receptors compared to dendritically-targeting cells. In contrast, the level of bassoon, an integral component of the presynaptic release machinery known to organize voltage-gated calcium channels at the active zone, did not differ between the two axon terminal types. These findings imply that a higher CB_1 receptor/effector ratio may contribute to stronger cannabinoid signaling at perisomatic synapses. Furthermore, chronic Δ^9-tetrahydrocannabinol treatment, which is known to reduce cannabinoid efficacy on

GABA release, resulted in a robust overall decrease in CB_1 levels and increased receptor internalization in axon terminals derived from perisomatically-projecting interneurons. Together, these findings suggest that the number of available CB_1 receptors on the presynaptic plasma membrane critically determines the efficacy of cannabinoid inhibition of neurotransmitter release.

Disclosures: **B. Dudok:** None. **L. Barna:** None. **S.I. Szabó:** None. **E. Szabadits:** None. **B. Pintér:** None. **S.G. Woodhams:** None. **C.M. Henstridge:** None. **G.Y. Balla:** None. **R. Nyilas:** None. **C. Varga:** None. **S. Lee:** None. **M. Matolcsi:** None. **J. Cervenak:** A. Employment/Salary (full or part-time):; ImmunoGenes Ltd. **I. Kacskovics:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ImmunoGenes Ltd.. **M. Watanabe:** None. **M. Melis:** None. **M. Pistis:** None. **I. Soltesz:** None. **I. Katona:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.02/C30

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: European Research Council Grant 243153

The Wellcome Trust International Senior Research Fellowship 090946/Z/09/Z

Momentum Program LP2013-54/2013

Hungarian Scientific Research Fund grant K101364

Title: Polyclonal and monoclonal antibody production for CB_1 cannabinoid receptors in FcRn transgenic animals

Authors: *G. Y. BALLA[1], J. CERVENAK[2], C. M. HENSTRIDGE[1], A. ILIÁS[3], E. SZABADITS[1], M. LEDRI[1], S. I. SZABÓ[1], B. DUDOK[1], B. LÁSZLÓ[1], I. KACSKOVICS[2,3], I. KATONA[1];
[1]Inst. of Exp. Med., HAS, Budapest, Hungary; [2]ImmunoGenes Kft, Budakeszi, Hungary; [3]Eötvös Loránd Univ., Budapest, Hungary

Abstracts: Antibodies are the best tools to determine the position and density of endogenous proteins in the brain. However, it is often difficult to study low copy number proteins due to limitations in antibody sensitivity and specificity. For example, presynaptic CB_1 cannabinoid receptors are visualized by most conventional antibodies on forebrain GABAergic boutons, but CB_1 expression levels are usually too low to be investigated in a quantitative manner on most other axon terminal types. To overcome this limitation, we used transgenic rabbits and mice overexpressing the neonatal Fc receptor (FcRn) to produce highly sensitive polyclonal and monoclonal antibodies against the mouse CB_1 receptor. Transgenic animals carrying and expressing extra copies of the FcRn exhibit an enhanced immune response. Accordingly, CB_1 antisera obtained from FcRn transgenic rabbits had higher titers and exhibited better dissociation kinetics compared to antisera collected from wild-type rabbits. Moreover, each antisera obtained from FcRn transgenic rabbits (n=3) revealed dense axonal CB_1-immunostaining pattern in wild-type mouse hippocampus. The specificity of this staining pattern was validated in CB_1 knockout animals. In contrast, only one antiserum collected from wild-type rabbits (n=3) could visualize CB_1 receptors. Similarly, we could successfully obtain 10 positive, high affinity anti-CB_1 IgG-expressing clones from FcRn-overexpressing mice, but none from wild-type mice. Immunostaining with monoclonal antibodies generated from these clones also revealed extensive immunolabeling of CB_1-positive GABAergic axons. To further improve the sensitivity of CB_1-immunostaining, the FcRn transgenic rabbit, producing antisera with the best dissociation constant, was further immunized and the last three bleeds were mixed and purified. Immunohistochemistry with this new antibody resulted in unprecedentedly dense CB_1-immunostaining outlining the laminar topography of glutamatergic pathways. Electron microscopic analysis demonstrated that the vast majority of a randomly selected population of excitatory terminals carried CB_1 receptors in the inner molecular layer of dentate gyrus and in the stratum oriens of CA3 subfield. STORM superresolution imaging of CA3 pyramidal cells confirmed this result at the single cell level by visualizing CB_1 in most recurrent collaterals. These findings demonstrate that FcRn-overexpressing transgenic animals are ideal tools to generate highly sensitive and specific antibodies, and were instrumental to obtain new monoclonal and polyclonal antibodies which allow quantitative investigation of CB_1 receptors in the brain.

Disclosures: **G.Y. Balla:** None. **J. Cervenak:** A. Employment/Salary (full or part-time):; ImmunoGenes Ltd.. **C.M. Henstridge:** None. **A. Iliás:** None. **E. Szabadits:** None. **M. Ledri:** None. **S.I. Szabó:** None. **B. Dudok:** None. **B. László:** None. **I. Kacskovics:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ImmunoGenes Ltd.. **I. Katona:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.03/C31

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: European Research Council Grant 243153

The Wellcome Trust International Senior Research Fellowship 090946/Z/09/Z

Momentum Program LP2013-54/2013

Title: A new approach for correlated confocal and superresolution microscopy visualizes the distribution of presynaptic CB_1 cannabinoid receptors at the nanoscale

Authors: *L. BARNA[1], B. DUDOK[1], V. MICZÁN[1], S. I. SZABÓ[1], A. HORVÁTH[2], M. MATOLCSI[3], I. KATONA[1];
[1]IEM HAS, Budapest, Hungary; [2]Pázmány Péter Catholic Univ., Budapest, Hungary; [3]Alfred Renyi Inst. of Mathematics, Budapest, Hungary

Abstracts: How synapse-specific differences in signaling protein density determine the qualitative and quantitative properties of synaptic transmission is a fundamental question in neuroscience. However, our understanding of the molecular parameters underlying synaptic processes remains limited due to methodical limitations. Therefore, in the present study, we exploited whole-cell patch-clamp recordings, confocal microscopy and STORM superresolution microscopy to allow the integrated analysis of physiological and morphological properties of a single neuron with quantitative molecular imaging within its axon terminals. Importantly, the whole experimental and analysis process can be carried out in a few days and reveals the localization of synaptic proteins with excellent sensitivity and specificity, ultra-high spatial resolution, and in large sample sizes. We first employed methodical improvements for STORM superresolution imaging in mouse hippocampal sections, which revealed the subcellular distribution of CB_1 cannabinoid receptors with a fluorophore localization precision of 6 nm and 41 nm in the lateral and axial dimensions, respectively. The specificity of CB_1 immunolabeling in STORM images was validated by using CB_1 knockout animals. To make physiological characterization and anatomical classification possible in combination with superresolution imaging, CB_1-positive GABAergic interneurons were filled with biocytin during patch-clamp recording and their morphology was visualized by confocal microscopy. To provide a cellular context for the 3D coordinates of CB_1 localization points obtained by STORM, high magnification confocal microscopy was used to detect biocytin-labeled axon terminals and then CB_1 distribution within the selected axon terminal was imaged by STORM. To facilitate the correlative visualization and analysis of confocal and STORM microscopy data, we also developed a new software with an easy-to-use graphical user interface. A correlation analysis of

confocal and STORM images demonstrated that 3-D STORM provides a reliable quantitative readout of protein abundance in HEK cells and in GABAergic axon terminals. Finally, we applied new algorithms allowing nanoscale measurements of relative CB_1 density and intermolecular distances along the surface of GABAergic axon terminals. Taken together, our new methodology including improvements for brain tissue processing and imaging, as well as new software tools for data analysis represents a powerful approach for cell type-specific nanoscale molecular investigations in association with physiological and anatomical characterization in intact brain circuits.

Disclosures: **L. Barna:** None. **B. Dudok:** None. **V. Miczán:** None. **S.I. Szabó:** None. **A. Horváth:** None. **M. Matolcsi:** None. **I. Katona:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.04/C32

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR grant MOP123256

Title: Corticosterone-endocannabinoid signaling for memory recovery from general anesthesia

Authors: F. WANG[1], L. TONG[1], X. LI[1], R. KINDEN[1], L. XIONG[2], H. DONG[2], *X. ZHANG[1]; [1]Inst. Mental Hlth. Res., Univ. Ottawa, Ottawa, ON, Canada; [2]Dept. of Anesthesiol., Fourth Military Med. Univ., Xi'an, China

Abstracts: The inaugural clinical practice of general anesthesia in 1840's revolutionized medicine by allowing surgery to be performed in a humane manner, and now approximately 234 million of patients each year undergo anesthesia for surgery worldwide. However, how memory recovers from anesthesia remains completely unknown. In the present study, we investigated how contextual fear memory recovers from general anesthesia with the volatile anesthetic isoflurane, a routine anesthetic in both humans and animals. We found that rats received isoflurane anesthesia for 2 h showed an approximate 25% of contextual fear memory recovery at 1 hour, 67% of memory recovery at 4 hour, and a full recovery at 12 hour after cessation of anesthesia. Contextual fear memory recovery at 1 hour after cessation of anesthesia was abolished by pretreatment of rats with the mineralocorticoid receptor (MR) antagonist RU28318 (but not by glucocorticoid receptor antagonist), calcium channel blocker, endocannabinoid

(eCB) 2-arachidonoylglycerol (2-AG) synthesis enzyme inhibitor, and the cannabinoid type-1 receptor (CB_1R) antagonist AM281. The abolishment effects produced by RU28318 and AM281 were mimicked and blocked by gamma-aminobutyric acid (GABA) receptor agonist and antagonist, respectively. All the findings together lead us to propose a corticosteroid-eCB signaling ---- composed of MR activation, calcium influx, 2-AG mobilization, presynaptic CB_1R activation, and GABAergic synaptic disinhibition ---- that drives memory recovery from general anesthesia.

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Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.05/C33

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: FAPESP 2012/00390-0

CAPES

FAEPA

Title: Medial prefrontal cortex endocannabinoid system modulates autonomics response in rats submitted to restraint stress

Authors: ***T. B. MORAES NETO**, A. FASSINI, F. M. A. CORREA, L. B. M. RESSTEL; Dept. of Pharmacol., Univ. of Sao Paulo - Sch. of Med. of Ribeirao Preto, Ribeirao Preto, Brazil

Abstracts: Goals: Acute restraint is an unavoidable stress situation that evokes autonomics changes, characterized by elevated mean arterial pressure (MAP), intense heart rate (HR) increases and decrease in the tail skin temperature. The ventral portion of medial prefrontal cortex (vMPFC), witch is compound by prelimbic (PL) and infralimbic (IL) cortices, is involved with modulation of autonomics responses associated with stress. The vMPFC glutamatergic system is involved with modulation of autonomics responses evoked by acute restraint stress. Moreover, the endocannabinoid CB1 receptors activation reduces the local vMPFC glutamate releases. The mainly endocannabinoid is anandamide (AEA), witch is hydrolyzed by enzyme fatty acid amide hydrolase (FAAH). Therefore, the objective of the present work was to

investigate the involvement of endocannabinoid in the modulation of autonomic responses evoked by restraint stress in rats. Methods: Male Wistar rats (250-270g) had guide cannulae bilaterally implanted in the PL or IL for drug injection. A polyethylene catheter was implanted in the femoral artery for MAP and HR recording. Tail skin temperature was measured using a thermal camera. The animals were submitted to acute restraint, which was initiated by introducing animals into a small plastic cylindrical restraining tube (diameter =6.5cm and length =15cm) and lasted for 60 minutes. The FAAH inhibitor URB597 (100 pmol/ 200 nL) was administrates 10 minutes before the restraint stress. Results: The acute restraint stress was able to increase MAP ($F_{35, 350} = 15.14$, $P<0.001$) and HR ($F_{35, 350} = 16.65$, $P<0.001$), and decrease the tail skin temperature ($F_{17, 180} = 60.99$, $P<0.001$). The microinjection of URB597 (n=6) reduces the pressor (PL: $F_{1, 350} = 16.13$, $P<0.01$; IL: $F_{1, 350} = 16.80$, $P<0.01$) and tachycardiac response (PL: $F_{1, 350} = 6.517$, $P<0.01$; IL: $F_{1, 350} = 6.446$, $P<0.01$), and increases the tail skin temperature drop (PL: $F_{1, 180} = 44.86$, $P<0.01$; IL: $F_{1, 180} = 69.36$, $P<0.01$) when compared with vehicle treated animals (n= 6). Conclusion: The present a result shown that vMPFC endocannabinoid system through AEA has an inhibitory influence in the cardiovascular responses and an excitatory influence on the tail skin temperature responses evoked by restraint stress.

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Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

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Program#/Poster: 596.06/C34

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CONACYT, Mexico Grant 152326

Title: Activation of CB1 cannabinoid receptors stimulate transmitter release in striatopallidal terminals of the rat mouse brain when Gi Protein-Receptor coupling is restricted

Authors: R. CABALLERO-FLORAN[1], L. LOPEZ-SANTIAGO[4], L. ISOM[4], J. ACEVES[2], D. ERLIJ[5], *G. B. FLORAN[3];
[1]Pharmacol., [2]Physiol., [3]CINVESTAV IPN, Mexico DF, Mexico; [4]Pharmacol., Univ. of Michigan Med. Ctr., Ann Arbor, MI; [5]Dept. of Physiol. and Pharmacol., SUNY Dowstate Med. Ctr., Brooklyn, NY

Abstracts: The most widespread neuronal effect of CB1 cannabinoid receptor agonists is inhibition of transmitter release from presynaptic terminals. In a previous study, using neurochemical techniques, we found that the decrease in GABA release produced by activating CB1 cannabinoid receptors in striatopallidal terminals was transformed into stimulation of release by restricting Gi/o protein interactions. In the present experiments we investigated the effect of stimulating the CB1 receptor with the selective cannabinoid type 1 receptor (CB1) agonist arachidonyl-2-chloroethylamide (ACEA) on whole-cell recordings of inhibitory postsynaptic currents (IPSCs) in neurons of the globus pallidus. ACEA reduced IPSC amplitude. The effect was associated with paired-pulse facilitation, indicating a presynaptic action. When Gi/o receptor-protein interactions were limited by treatment with pertussis toxin, the inhibitory effects of ACEA were transformed. ACEA now increased IPSC amplitude instead of reducing it. This effect was associated with paired-pulse depression indicating stimulation of GABA release. Because it has been shown that stimulation of D2 receptors may reduce Gi/o availability we investigated the effects of activation of dopamine D2 receptors (D2Rs) on the response to stimulation of CB1 receptors. Treatment with the selective D2R agonist quinpirole depressed IPSC amplitude and caused paired pulse facilitation. In quinpirole treated slices ACEA increased IPSCs amplitude and depressed the paired-pulse ratio. These results show that when Gi protein-receptor coupling is restricted, activation of CB1 receptors leads to transmitter release stimulation. They also show that increasing D2R activation can transform the inhibitory effects of CB1 receptor stimulation into a stimulatory response. We suggest that this effect is also associated with decreased Gi protein-receptor interactions.

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Poster

596. Synaptic Signaling: Retrograde Messengers

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Topic: B.01. Neurotransmitters and Signaling Molecules

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The Strategic Research Program for Brain Sciences, project D, from MEXT, Japan

Title: The endocannabinoid 2-arachidonoyl glycerol suppresses seizures by decreasing excitatory synaptic input around the inner molecular layer of the dentate gyrus

Authors: *Y. SUGAYA[1], M. YAMAZAKI[2], K. SAKIMURA[2], M. KANO[1];
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Abstracts: Previous studies revealed that the endocannabinoids in the dentate gyrus and the surrounding structures have protective effect on kainate-induced status epilepticus. However, it is still unclear which of the two major endocannabinoids, anandamide or 2-arachidonoyl glycerol (2-AG), suppresses seizures and how the endocannabinoid signaling changes excitability in the hippocampus during seizures. We have recently demonstrated that 2-AG produced by diacylglycerol lipase α (DGLα) is the endocannabinoid that mediates retrograde synaptic suppression. The present study aimed at elucidating roles of 2-AG in modulating neural circuit activity in the hippocampal dentate gyrus and in the generation of seizure. Under urethane anesthesia (1.75g/kg BW, i.p.), a stimulus electrode was implanted into the angular bundle of adult DGLα knockout mice and their wild-type littermates. A 16-channel silicon probe was implanted into the dentate gyrus of the mice. Afterdischarges were evoked by burst stimuli (20 Hz for 10 seconds) to the perforant path and the current source density during afterdischarges was calculated from the local field potentials. We found that the afterdischarges were significantly longer in DGLα knockout mice than in their wild-type littermates. The afterdischarges in the dentate gyrus consisted of repetitive burst discharges of granule cells with inter-burst interval of about 350 msec. At the start of each burst discharge, sink current around the inner molecular layer (IML) of the dentate gyrus was significantly larger in DGLα knockout mice than in their wild-type littermates. Optogenetic stimulation of excitatory projection fibers to the IML significantly increased the duration of afterdischarges in wild-type mice. These results suggest that 2-AG suppresses the excitatory synaptic input around the IML of the dentate gyrus and shortens afterdischarges. We also investigated the suppressive effect of 2-AG on spontaneouse seizures during kainate-induced epileptogenesis (0.4 μg dissoleved in 100 nl, injected to the dentate gyrus). We found that the blockade of 2-AG hydrolysis by JZL184 (4 mg/kg, i.p.), an inhibitor of monoacylglycerol lipase, significantly reduced the number of spontaneous seizures. By contrast, blockade of 2-AG-mediated signaling by AM251 (4mg/kg, i.p.), a blocker of cannabinoid CB_1 receptor, significantly increased the number of spontaneous seizures and mortality rate. These results indicate that 2-AG mediated signaling strongly suppresses seizures and may be a possible target for the treatment of epilepsy.

Disclosures: **Y. Sugaya:** None. **M. Yamazaki:** None. **K. Sakimura:** None. **M. Kano:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

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Program#/Poster: 596.08/C36

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Macamides as inhibitors of fatty acid amide hydrolase (FAAH)

Authors: M. ALAMOUDI, M. BOHLKE, T. J. MAHER, *A. J. PINO-FIGUEROA; MCPHS, Boston, MA

Abstracts: Lepidium meyenii (Maca), a Peruvian plant, has been used as a folk medicine for centuries. It has been found that the pentate extract of Maca possesses neuroprotective effect *in vitro* and *in vivo*. Maca contains unique compounds called macamides which are claimed to be responsible for its pharmacological effects. Since there is a structure similarity between macamides and anandamide, the endogenous ligand of the endocannabinoid system, macamides might exert their effects through the endocannabinoid system. Our preliminary results suggested that macamides act as inhibitors of fatty acid amide hydrolase (FAAH), an enzyme that degrades anandamide, and results in the termination of endocannabinoids signaling. The aim of this study was to demonstrate and characterize the FAAH inhibitory effects of the most abundant macamides, N-benzyl-palmitamide, and their derivatives, N-(3-methoxybenzyl)-palmitamide, N-(4-fluorobenzyl)-palmitamide, and N-(4-chlorobenzyl)-palmitamide. An FAAH inhibitory activity assay, which is a fluorescence-based method, was performed to test each compound using concentrations from 1 to 100 μM. Fluorescence was measured at different time points, using a microplate reader at an excitation wavelength of 340-360 nm and an emission wavelength of 450-465 nm, at 37 °C, during 60 minutes after initiation of the enzymatic reaction. The percentage of inhibition of FAAH produced by 100 μM of N-benzyl-palmitamide, N-(3-methoxybenzyl)-palmitamide, N-(4-fluorobenzyl)-palmitamide, and N-(4-chlorobenzyl)-palmitamide was 53.3±1.8%, 47.7±1.3%, 28.0±1.2%, and 43.1±1.6%, respectively (n=3). IC50 values of N-benzyl-palmitamide, N-(3-methoxybenzyl)-palmitamide, N-(4-fluorobenzyl)-palmitamide, and N-(4-chlorobenzyl)-palmitamide were 11.6±1.6 μM, 4.7±0.3 μM, 5.7±0.7 μM, and 7.7±0.6 μM, respectively. The results indicated that all studied macamides inhibit FAAH in a concentration-dependent manner. Macamides by inhibiting FAAH could offer a promising alternative for treatment of many CNS disorders such as pain, inflammation and neurodegenerative diseases.

Disclosures: M. Alamoudi: None. **M. Bohlke:** None. **T.J. Maher:** None. **A.J. Pino-Figueroa:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.09/C37

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: FONDECYT 1140108

Title: Neuronal localization of endothelial nitric oxide synthase

Authors: A. CAVIEDES[1], J. BRAVO[1], A. MASSMANN[2], J. FIGUEROA[2], F. NUALART[3], *U. WYNEKEN[1];
[1]Univ. De Los Andes, Santiago, RM, Chile; [2]Wake Forest Univ. Hlth. Sci., Winston-Salem, NC; [3]Ctr. for Advanced Microscopy Bio-Bio, Concepcion, Chile

Abstracts: Nitric oxide (NO) is a pivotal messenger in various brain processes. Its synthesis in the brain has been most commonly been ascribed to neuronal nitric oxide synthase (nNOS), however, the endothelial isoform (eNOS) has also been implicated as a retrograde messenger in cellular plasticity. A better characterization of eNOS localization in neurons and its possible functional consequences has not yet been performed. We thus studied the localization of eNOS by immunocytochemistry of primary hippocampal and cortical neurons and by Western Blots of subcellular fractions, while cell viability was assessed with the Tripan blue test. We show by confocal and super-resolution microscopy that eNOS co-distributes with post-synaptic markers (Shank2) and is localized in spines. Moreover, eNOS is enriched in synaptic membranes and in postsynaptic densities isolated from neuronal cultures and from the rat forebrain. eNOS inhibition in cortical cells has a negative impact on cell survival after excitotoxic stimulation with NMDA. In turn, hippocampal neuronal death depends on nNOS-dependent NO synthesis and eNOS inhibition does not affect neuronal viability. Our results show that eNOS is located at excitatory synapses where it could represent a major source for NO production able to modulate synaptic function and neuronal survival. Grant: FONDECYT 1140108

Disclosures: A. Caviedes: None. **U. Wyneken:** None. **J. Bravo:** None. **A. Massmann:** None. **J. Figueroa:** None. **F. Nualart:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.10/C38

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NINDS

Title: Slit2 as a β-Catenin-dependent retrograde signal for presynaptic differentiation

Authors: ***A. BARIK**[1], H. WU[1], Y. LU[2], W.-C. XIONG[3], L. MEI[3];
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Abstracts: Neuromuscular junction (NMJ) formation requires proper interaction between motoneurons and muscle cells. Our earlier work indicated that β-catenin in muscle is critical for motoneuron differentiation, probably via a retrograde mechanism. β-Catenin could regulate cell adhesion by bridging the interaction between cadherin and a-catenin. Alternatively, β-Catenin regulates transcription by associating with TCF/LEF1 transcription factors, a major biological response in the Wnt canonical pathway. To investigate how muscle β-Catenin controls presynaptic differentiation, we generated transgenic mice expressing wild type β-catenin or mutants impaired in either of the two functions. In a set of *in vivo* rescue experiments, we found that the effect of β-Catenin requires the C-terminal transactivation domain (TAD), but not the α-catenin binding motif, suggesting a necessary role of β-Catenin target genes. Analysis of β-Catenin -dependent transcriptome in the muscle led to the identification of Slit2, a factor that has been implicated in axon navigation in the brain. A previous report demonstrated that Slit2 null mice showed NMJ deficits including characteristic mislocation of primary nerve branches in the diaphragms that were observed in muscle β-Catenin mutant mice. We found, first by chromatin immunoprecipitation (ChIP), that β-catenin bound to the 5'-UTR of the Slit2 gene. Second, Slit2 immobilized on beads were able to induce terminal differentiation of motoneurons as well as hippocampal neurons. Finally, we generated transgenic mice that express Slit2 specifically in the muscle. Our data suggest that Slit2 muscle expression was able to diminish presynaptic deficits in β-Catenin mutant mice, providing genetic evidence for a role of Slit2 in NMJ formation. Together, these observations identify Slit2 as a novel retrograde signal of muscle for presynaptic differentiation.

Disclosures: **A. Barik:** None. **H. Wu:** None. **Y. Lu:** None. **W. Xiong:** None. **L. Mei:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.11/C39

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Fondazione Istituto Italiano di Tecnologia

Italian Ministry of Health

Fondazione Compagnia di San Paolo

Fondazione Cariplo

Title: Pathway-specific neuromodulation of striatonigral and striatopallidal neurons in the pathophysiology of motor control

Authors: M. TRUSEL[1], *A. CAVACCINI[1], B. GRECO[1], A. GUIJARRO[2], P. P. SAINTOT[1], M. CEROVIC[3], I. M. MORELLA[4], R. BRAMBILLA[4], R. TONINI[1];
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Abstracts: In the dorsolateral striatum (DLS), complex motor information originating mainly from cortical and midbrain inputs converges on striatal medium spiny neurons (MSNs) of the striatonigral- (direct-dMSNs) and striatopallidal (indirect-iMSNs) pathways. Increasing evidence suggests that these two MSN subpopulations exert opposing control on aspects of motor behavior, including skilled motor learning and drug-induced locomotion. The apt and timely regulation of the relative balance between the activity of dMSNs and iMSNs neurons is, therefore, crucial to execute proper motor patterns. To this purpose, distinct classes of synaptic neuromodulators functionally integrate at corticostriatal synapses of the DLS during complex tasks of motion, hence tuning the coordinated outputs of striatonigral and striatopallidal pathways. However, a direct relationship between pathway-specific modulation of corticostriatal synaptic plasticity and aspects of motor behavior is still missing. We found that long-term synaptic depression (LTD) induced by high-frequency stimulation of layer V of the somatosensory cortex (HFS-LTD) was blocked by inhibition of endocannabinoid (eCB)- and dopamine D2 receptor-mediated signaling at cortical connections to iMSNs, but not to dMSNs in the DLS. HFS-LTD at dMSNs is expressed pre-synaptically and it involves the activation of local neuromodulators. To address whether striatal motor behavior displays similar pathway specificity, we examined how cell-type specific modulation of synaptic plasticity rescues motor deficits in the unilateral 6-hydroxydopamine (6-OHDA) lesioned mouse model of Parkinson's disease (PD). Under our experimental conditions, HFS-LTD was selectively lost at cortical connections to iMSNs of parkinsonian mice, while it was unaffected in striatonigral neurons. The in-vivo pharmacological manipulations of intracellular Ca^{2+} signal in the MSNs exerted a

dichotomous effect as it decreased the excitatory drive to the striatopallidal pathway while it increased the synaptic responsiveness in the striatonigral pathway. This associated with improved skilled-motor behavior in PD mice through a mechanism involving the release of eCBs from striatopallidal neurons, and it enhanced rotational behavior induced by administration of dopaminergic drugs. Our results reveal a direct relationship between neuromodulation of synaptic plasticity in segregated striatal circuits and the pathophysiology of motor control.

Disclosures: **M. Trusel:** None. **A. Cavaccini:** None. **B. Greco:** None. **A. Guijarro:** None. **P.P. Saintot:** None. **M. Cerovic:** None. **I.M. Morella:** None. **R. Brambilla:** None. **R. Tonini:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.12/C40

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant RO1 MH41256.

CIHR grant MOP 119502

NIH Grant RO1 MH090412

Title: Corticotropin releasing hormone increases 2-arachidonoylglycerol in the medial prefrontal cortex

Authors: A. B. KIM[1], M. GRAY[1], D. J. HERMANSON[3], R. J. MCLAUGHLIN[4], C. D. WILSON[5], H. A. VECCHIARELLI[1], B. S. MCEWEN[6], J. SCHULKIN[7], I. N. KARATSOREOS[8], S. PATEL[3], *M. N. HILL[2,1];
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Abstracts: Corticotropin releasing hormone (CRH) signaling within the prefrontal cortex (PFC) has been shown to modulate emotional behavior. Herein, we examined the interactions between CRH signaling and endocannabinoid signaling within the PFC to determine if an interaction between these systems in the PFC could be relevant for the regulation of emotional behavior. To this extent, we found that intra-cerebroventricular (icv) administration of CRH resulted in an

increase in the tissue levels of the endocannabinoid ligand 2-arachidonoylglycerol (2-AG) within the PFC. This effect appeared to be mediated by the CRH type 1 receptor (CRHR1) as this effect was recapitulated by icv administration of the CRHR1 agonist cortagine, but not the CRHR2 agonist urocortin II. We then sought to investigate if this relationship maintained under conditions of chronic exposure to corticosterone, which has been shown to upregulate extrahypothalamic levels of CRH. Similar to what has been established within the amygdala, we found that chronic exposure to corticosterone resulted in an upregulation of CRH mRNA within the PFC. This increase in CRH expression in the PFC was accompanied by a significant increase in the tissue content of 2-AG following chronic corticosterone exposure, which was completely reversed by concurrent exposure to the CRHR1 antagonist antalarmin. The role of this interaction with respect to the regulation of emotional behavior is currently under investigation.

Disclosures: **A.B. Kim:** None. **M.N. Hill:** F. Consulting Fees (e.g., advisory boards); Hill - consultant for Pfizer. **M. Gray:** None. **H.A. Vecchiarelli:** None. **I.N. Karatsoreos:** None. **C.D. Wilson:** None. **J. Schulkin:** None. **R.J. McLaughlin:** None. **S. Patel:** None. **D.J. Hermanson:** None. **B.S. McEwen:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.13/C41

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSFC

CIHR

Title: Increased NFκB signalling upregulates human PINK1 gene expression

Authors: ***X. DUAN**[1,2], J. TONG[1], Q. XU[1], Y. WU[2], F. CAI[1], W. SONG[1];
[1]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [2]Children's hospital, Chongqing Med. Univ., Chongqing, China

Abstracts: Parkinson’s disease (PD) is one of the major neurodegenerative disorders. However, its etiology and the mechanism underlying its pathogenesis remain elusive. Mitochondrial movement, distribution, and clearance are critical for cells to keep their energy balance and avoid oxidative stress. Mitochondrial malfunction is implicated in PD pathogenesis. Ser/Thr kinase

PINK1, a PD-associated protein, plays an important role in the regulation of mitochondrial movement. The regulation of PINK1 gene expression remains unknown. In the present study, we aimed to understand the transcriptional regulation of PINK1. Firstly, we identified the transcription start site (TSS) of PINK1 using RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE) strategy. The TSS is located at 94bp upstream of the translation start site (ATG). The 1825bp 5'-flanking region of the human PINK1 gene coding sequence and a series of nested deletions were cloned into the pGL3-Basic vector. The promoter activities of the nested deletions were analyzed by a dual luciferase assay. The region of 104bp (-78 to +26) was identified to be the minimal promoter region with functional transcriptional activity for the PINK1 gene. Secondly, transcriptional activation and gel-shift assay demonstrated that the PINK1 gene promoter contains functional cis-acting NFκB-binding sites. NFκB p65 overexpression led to up-regulation of PINK1 transcription and expression in HEK293 cells and SH-SY5Y cells. Meanwhile, lipopolysaccharide (LPS) treatment significantly increased the PINK1 protein level in SH-SY5Y cells. Taken together, our results clearly suggested that PINK1 expression is tightly regulated at its transcription level and NFκB is a positive regulator for PINK1 expression.

Disclosures: X. Duan: None. **J. Tong:** None. **Q. Xu:** None. **Y. Wu:** None. **F. Cai:** None. **W. Song:** None.

Poster

596. Synaptic Signaling: Retrograde Messengers

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 596.14/C42

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Office of Science (BER), U.S. Department of Energy.

Title: Brain distribution pattern of radiolabel from carbon-14 labeled anandamide

Authors: *K. QIAN, Y. MIAO, S. SONTI, R. DUCLOS, S. J. GATLEY;
Northeastern Univ., Boston, MA

Abstracts: Inhibitors of fatty acid amide hydrolase (FAAH), which inactivates the endocannabinoid anandamide (N-arachidonoylethanolamine), are candidate drugs against pain and inflammation. Mapping FAAH activity in the brain would facilitate evaluation of such drugs. Autoradiography of mouse brain after administration of [H-3]anandamide show a

heterogeneous distribution pattern which was considered to reflect regional FAAH activity (Glaser, Gatley et al. 2006). However, using carbon-14 instead of tritium we found that similar patterns were produced with labeled arachidonic acid and with [C-14-arachidonoyl]anandamide, suggesting that FAAH does not control disposition of radioactivity. To examine regional FAAH activity without the interference of other enzymatic activities related to the metabolism of the fatty acids, we undertook the radiosynthesis of anandamide labeled in the ethanolamine moiety instead of the acyl moiety, and used [C-14]ethanolamine in control experiments. Autoradiography experiments with [C-14-ethanolamine]anandamide showed a heterogeneous pattern of incorporation of radiolabel in the brain, which was distinct from the more homogenous distribution of radioactivity produced with [C-14]ethanolamine. (Abstract#: 639.25, 2012) Radio TLC and HPLC analyses showed that [C-14]ethanolamine was released from anandamide and then converted mainly to phosphatidylethanolamine (PE). In subsequent work we have tested the effects of the FAAH inhibitor URB597 on the regional uptake of radiolabel from anandamide labeled at either the acyl or the ethanolamine moiety. Even though URB597 (3mg/kg) blocked the production of labeled phospholipids (TLC analysis showed 0% radioactivity as phospholipids at 15 minutes after tracer injection); it did not prevent the formation of a heterogeneous pattern at both 15 minute and 100 minute time-points. The heterogeneous pattern changed over time in brains from URB597 treated but not vehicle treated mice. At 100 minutes after tracer injection into URB597 treated mice, there was increased accumulation of radioactivity in hippocampus, and a 40% decrease in total amount of radioactivity, relative to 15 minutes. Our results indicate that un-metabolized anandamide accumulates heterogeneously in different brain parts before it is hydrolyzed, but that FAAH activity does not control the brain distribution pattern of radiolabel from anandamide. We conclude that the change in brain distribution of label over time in URB597 treated mice is determined by a combination ofthe physical and/or biochemical properties of anandamide itself and of its labeled metabolites.

Disclosures: **K. Qian:** None. **Y. Miao:** None. **R. Duclos:** None. **S.J. Gatley:** None. **S. Sonti:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 597.01/C43

Topic: B.04. Ion Channels

Title: Expression of transient receptor potential ankyrin type 1 in the mouse brain

Authors: *S.-I. HONG, S.-X. MA, J.-Y. HWANG, J.-S. KIM, J.-Y. SEO, S.-Y. LEE, C.-G. JANG;
Dept. of Pharmacol. Sch. of Pharm., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstracts: Transient receptor potential ankyrin type 1 (TRPA1), responding to noxious cold and pungent compounds, was found in thermosensitive neurons, and hair cells of ear, but little is known about the expressions of the TRPA1 within the brain, specifically cortex, dorsal and ventral hippocampus, caudate putamen, nucleus accumbens, and ventral tegmental area. To address this issue, we used real-time PCR, Western blot analysis, and immunohistochemistry. Real-time PCR and Western blot analysis data showed that TRPA1 mRNA was expressed in the brain. TRPA1 mRNA was highly expressed in the cortex compared to brain other regions. We investigated that whether the location of TRPA1 protein is in the neuronal cell body or neurofilament of axons and dendrite. Double staining of TRPA1 with neuronal nuclei protein (NeuN), a neuronal marker, showed that TRPA1 was co-expressed with NeuN in the brain, especially hippocampus pyramidal region, caudate putamen and nucleus accumbens in which the cell bodies of neurons are located. Further, when combining TRPA1 and microtubule associated protein type 2 (MAP2) staining, a marker of neurofilament, both TRPA1 and MAP2 can be observed in the brain. Taken together, our results suggest that TRPA1 mRNA and protein be expressed in the mouse brain and further, TRPA1 be expressed in the neuronal cell body and neurofilament.

Disclosures: S. Hong: None. **S. Ma:** None. **J. Hwang:** None. **J. Kim:** None. **J. Seo:** None. **S. Lee:** None. **C. Jang:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 597.02/C44

Topic: B.04. Ion Channels

Support: Hacettepe University 013 D07105001

TUBİTAK 113S211

Title: Intense/prolonged sensory stimulation activates neuronal pannexin-1 channels in the mouse brain *in vivo*

Authors: *T. DALKARA[1], B. DÖNMEZ-DEMIR[2], K. KILIC[2], E. EREN-KOCAK[2], Y. GÜRSOY-ÖZDEMIR[2], P. MAGISTRETTI[3], H. KARATAS[2];
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Abstracts: We have recently demonstrated that spreading depolarization (SD) can activate neuronal pannexin-1 channels in the intact mouse brain *in vivo* (Science 2013, 339:1092-1095). The extracellular concentrations of glutamate and potassium substantially increase during SD and may contribute to opening of pannexin-1 channels. One may posit therefore that increases in extracellular glutamate and potassium to supra physiological levels during intense/prolonged synaptic activity may also activate pannexin-1 channels. Here, we tested this hypothesis in the intact mouse barrel cortex *in vivo*. We first identified the barrel cortex area that showed maximum CBF increase during whisker stimulation by laser speckle imaging. We stimulated whiskers with a brush driven by a motor and detected the stimulation parameters that induced a robust CBF increase in this area. We then placed extracranial Ag/AgCl electrodes over the thinned skull to record DC potential changes during whisker stimulation to make sure they do not induce SD, which itself activates pannexin-1 channels. Just before the stimulation, we injected propidium iodide, a membrane impermeable fluorescent dye, intracerebroventricularly to label neurons, whose pannexin-1 channels were activated. Pannexin-1 megachannel opening allows passage of large molecules up to 1 kD, therefore, the influx of small fluorescent dyes are used to monitor their activity. We found that prolonged and intense sensory stimulation caused opening of the neuronal pannexin-1 channels in the barrel cortex and thalamus. This effect was especially striking when glycogen mobilization or lactate transport to neurons were inhibited pharmacologically or by genetic means. During synaptic activity, glycogen in peri-synaptic astrocyte end-feet is used as an immediate energy supply for glutamate and potassium uptake and to fuel post-synaptic membrane pumps by supplying lactate. In conclusion, intense/prolonged sensory stimulation can activate neuronal pannexin-1 channels when energy supply cannot quickly match the increased demand.

Disclosures: T. Dalkara: None. **B. Dönmez-Demir:** None. **K. Kilic:** None. **E. Eren-Kocak:** None. **Y. Gürsoy-Özdemir:** None. **P. Magistretti:** None. **H. Karatas:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 597.03/C45

Topic: B.04. Ion Channels

Support: NRF Grant 20110018358

BK21+ program of Ministry of Education of Korea

Title: Anoctamin 1 mediates histamine-independent itch signaling in somatosensory neurons

Authors: H. KIM[1], *B. LEE[2], H. CHO[2], H. CHUN[2], J. Y. CHA[2], J. JUNG[3], U. OH[2,4];
[1]Sensory Res. Center, CRI, Col. of Pharmacy, Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Pharm., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Dept. of Physiology, Col. of Medicine, Korea Univ., Seoul, Korea, Republic of; [4]Dept. of Mol. Med. and Biopharmaceutical Sciences, Grad. Sch. of Convergence Sci. and Technology, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstracts: Itch is an unpleasant sensation that evokes a desire to scratch. Antihistamines are widely used for treating pruritus. However, in many cases, patients who suffer from chronic itch are insensitive to antihistamine treatment. Thus, better therapeutics is needed to treat the histamine-independent itch. Several molecules, cells, and circuitry for itch transmission have been identified. TRPV1 is implicated in mediating histaminergic itch. Mas-related G protein-coupled receptors are itch receptors for non-histaminergic pruritogens, such as chloroquine or SLIGRL. TRPA1 is known to be a downstream target of Mas-related G protein-coupled receptors in mediating itch signaling. Anoctamin 1 (ANO1/TMEM16A) is a Ca2+ activated chloride channel expressed highly in nociceptors. Recently, we found that ANO1 is a heat sensor necessary for mediating acute, inflammation, or nerve-injury induced pain. Because of high expression in nociceptors, ANO1 may be involved in itch signals. Thus, this study aims to determine if ANO1 mediates itch. Using Adv/Ano1fl/fl mice that have a functional ablation of Ano1 mainly in DRG neurons, we determined a functional role of ANO1 in itch. To induce itch, pruritogenic substances were injected into the nape of the neck subcutaneously. Surprisingly, compared to control mice, Adv/Ano1fl/fl displayed a significant reduction in scratching behaviors to chloroquine or SLIGRL injection, but not histamine injection. These results demonstrate that ANO1 possibly mediate histamine-independent itch signaling in sensory neurons. Supported by a grant from the National Research Foundation of Korea (No. 20110018358) and a grant from BK21+ program of Ministry of Education of Korea.

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Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 597.04/C46

Topic: B.04. Ion Channels

Support: CSIR India

Title: Inhibition of recombinant $GABA_A$ receptor by sulphated neurosteroids - a study of molecular mechanism and elucidation of binding sites

Authors: *D. SACHIDANANDAN, T. AHMED, A. K. BERA;
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Abstracts: γ- amino butyric acid ($GABA_A$) receptor is negatively modulated by two structurally similar neurosteroids, Pregnenolone sulphate (PS) and Dehydroepiandrosterone sulphate (DHEAS). However, the exact binding site and mechanism still remains elusive. Using human $GABA_A$ receptors expressed in HEK293 cells, we performed electrophysiology experiments to study the molecular mechanisms governing neurosteroid mediated GABA-ergic inhibition. We showed that presence γ subunit in $GABA_AR$ enhances the efficacy of DHEAS without altering its binding affinity. Saturating concentrations of DHEAS blocked about 80% of currents mediated by $GABA_AR$, composed of α_1, β_1 and γ_{2S} subunits. The inhibition was only 30% in case of α_1 and β_1 containing $GABA_AR$. IC_{50} of DHEAS was identical with or without γ subunit. In contrast to DHEAS, neither the affinity nor the efficacy of PS was altered by the γ subunit. When Val256 of α_1 was mutated to Ser, the mutant channel became resistant to inhibition by both DHEAS and PS. PS exerted its inhibitory effect by enhancing the desensitization kinetics of $GABA_AR$, possibly by promoting the interaction between M2-M3 linker and extracellular loop 7/ loop 2. Mutant α_1, containing double Cys in loop 2/loop 7 and M2-M3 linker formed disulphide bond twice as much faster, when treated with saturating GABA + PS, compared to GABA alone or GABA + DHEAS. Taken together, we demonstrated that inhibition of $GABA_AR$ by DHEAS and PS follows similar but not identical inhibitory mechanism. For elucidating the neurosteroid binding sites, we built a homology model of $GABA_AR$ based on *C. elegans* Glutamate Gated Chloride Channel (GluCl, PDB i.d.3RHW). The two proteins were aligned using EMBOSS Needle and the structure was built using MODELLER 9.9. The model thus generated was used for docking after refinement and verification with experimental data. Docking was performed with PS and DHEAS on the built model using AUTODOCK. Results suggest that sulphated neurosteroids bind at α (-)/β (+) interface. Residues predicted by docking were mutated to alanine and the mutants subjected to PS and DHEAS concentrations to estimate the IC_{50}. We observed that PS and DHEAS have different binding sites as well, apart from molecular mechanism.

Disclosures: **D. Sachidanandan:** A. Employment/Salary (full or part-time):; IIT Madras, India, MHRD, India. 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.; CSIR, India. **T. Ahmed:** None. **A.K. Bera:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 597.05/C47

Topic: B.04. Ion Channels

Title: The ClC-2 channel: contribution to neuronal excitability in the thalamocortical network

Authors: *C. E. NITURAD, N. DAMMEIER, U. B. S. HEDRICH, S. MALJEVIC, H. LERCHE;
Neurol. and Epileptology, Hertie Inst. For Clin. Brain Res., Tübingen, Germany

Abstracts: Chloride homeostasis is essential for effective inhibition in the brain. Intracellular chloride accumulation in neurons can lead to an excitatory GABAergic response, emphasizing the importance of effective chloride extrusion mechanisms. The potassium chloride co-transporter 2 (KCC2) provides the major chloride extrusion pathway and a similar role has been proposed for the chloride channel ClC-2. Recently, loss of function mutations of ClC-2 channels were found in leukoencephalopathic patients and none of them was affected by epilepsy. Nevertheless we are interested in verifying and understanding whether the absence of the ClC-2 channels can be considered as susceptibility factor of some epileptic phenotypes. Since previous studies suggested a role of ClC-2 channels in mediating chloride extrusion or in a direct regulation of neuronal excitability by chloride influx, we used ClC-2 knock-out, heterozygous and WT littermates to study the contribution of the ClC-2 channel to chloride homeostasis in thalamocortical brain slices. Chloride currents were recorded using the whole cell patch clamp technique in the thalamic reticular nucleus (NRT), in relay neurons within the ventrobasal complex (VB) of the thalamus and in the cortex. Regarding the distribution pattern of ClC-2, our recordings did not show obvious differences in current amplitudes of NRT inhibitory neurons in WT compared to heterozygous animals. ClC-2 KO mice did not show any chloride currents. However, NRT inhibitory neurons showed decreased firing activities in KO animals in

comparison to the WT, indicating reduced inhibition in thalamocortical networks, the mechanisms of which have to be further explored.

Disclosures: C.E. Niturad: None. **N. Dammeier:** None. **U.B.S. Hedrich:** None. **S. Maljevic:** None. **H. Lerche:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Topic: B.04. Ion Channels

Support: National Natural Science Foundation of China (Grant 31270882 to HZ)

National Basic Research Program of China (Grant 2013CB531302 to HZ)

Title: Characterization of Cl^- channel modulators on the currents of TMEM16A and Bestrophin1

Authors: *H. ZHANG[1], Y. LIU[1], H. ZHANG[1], D. HUANG[1], X. DU[1], N. GAMPER[2,1]; [1]Pharmacol., Hebei Med. Univ., Hebei, China; [2]Sch. of Biomed. Sciences, Fac. of Biol. Sci., Univ. of Leeds, Leeds, United Kingdom

Abstracts: The Ca^{2+} activated Cl^- channels (CaCCs) play multitude of important physiological functions including regulation of neuronal excitability. A number of candidate proteins have been proposed to form CaCC, but only two families, the bestrophins and the TMEM16 (ANO) proteins, recapitulate properties of native CaCC in expression systems. In sensory neurons, both bestrophin1and TMEM16A have been suggested to be the molecular basis of CaCC. Studies of endogenous CaCCs are hindered by the lack of information for the profiles of pharmacology modulators of CaCC and a systematic comparison of the effects of these modulators on TMEM16A and bestrophin1 is missing. In the present study, we studied seven Cl^- channel inhibitors: niflumic acid (NFA), NPPB, flufenamic acid (FFA), DIDS, tannic acid, $CaCC_{inh}$-A01 and $T16A_{inh}$-A01 for their effects on TMEM16A and bestrophin-1(Best1) stably expressed in CHO cells using patch clamp technique. Among seven inhibitors studied, NFA showed highest selectivity for TMEM16A (IC_{50} of 7.40 ± 0.95 μM) over Best1 (IC_{50} of 102.19 ± 15.05 μM). In contrast, DIDS displayed a reverse selectivity inhibiting Best1 with IC_{50} of 3.93 ± 0.73 μM and TMEM16A with IC_{50} of 548.86 ± 25.57 μM). $CaCC_{inh}$-A01 was the most efficacious blocker for

both TMEM16A and Best1 channels. $T16A_{inh}$-A01 partially inhibited TMEM16A currents but had no effect on Best1 currents. Tannic acid, NPPB and FFA had variable intermediate effects. We are testing the effects of these modulators on endogenous CaCC of sensory neurons.

Disclosures: **H. Zhang:** None. **Y. Liu:** None. **H. Zhang:** None. **D. Huang:** None. **X. Du:** None. **N. Gamper:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Topic: B.04. Ion Channels

Support: NIH Intramural program PDN

Title: The influence of intracellular ATP on rat P2X2a receptor desensitization

Authors: ***M. ROKIC**[1], C. CODDOU[2], E. LEIVA-SALCEDO[1], S. STOJILKOVIC[1]; [1]NIH, Bethesda, MD; [2]Dept. of Biomed. Sciences,, Fac. of Medicine, Univ. Catolica del Norte, Coquimbo, Chile

Abstracts: The activated P2X2 receptor (P2X2R) desensitizes in a calcium-independent and -dependent manner. The former reflects the structure of the C-terminal sequence, whereas the nature and mechanism of the later process are not known. Using the whole-cell recording with potassium as an intracellular ion, and rat P2X2aR expressed in HEK293 cells and bathed in 2 mM calcium-containing medium, here we show that receptor desensitization is facilitated during repetitive agonist application (termed use-dependent desensitization); receptor desensitization was practically abolished in the whole-cell recording in the absence of bath calcium and in perforated patch clamp recording independently of bath calcium concentration. Addition of ATP but not GTP in the pipette solution also abolished bath calcium-dependent receptor desensitization in whole-cell recording, suggesting that a leak of ATP from cytosol to pipette facilitates transition of channels from open to desensitized state. Substitution of intrapipette potassium with cesium facilitated receptor desensitization as well as the addition of 10 mM staurosporine to ATP-containing intracellular solution, indicating the relevance of phosphorylation status of receptors on channel gating. In a search for amino acid residues accounting for effects of ATP, we used three spliced forms of receptors, the V439-Q459 C-terminal deletion mutant, and numerous intracellular single residue mutants. These experiments

excluded the relevance of the V370-Q459 amino acid sequence in calcium-dependent desensitization. Furthermore, no consistent effect was observed with single residue D15N, E17A, and several T354 mutants. On the other hand, six Y16 mutants showed the presence of significant positive correlation between residue volume and desensitization rate. Furthermore, T18V, T18D and T18P mutants desensitized rapidly, whereas the receptor function was rescued by substitution of T18 with arginine or tyrosine. Finally, receptor desensitization was facilitated by substitution of the S363 residue with five other amino acids, but used-dependent desensitization was lost in all mutants. These findings indicate the importance of intracellular ATP in P2X2R function by providing the phosphorylation/dephosphorylation equilibrium and suggest the importance of S363 amino acid residues in use-dependent desensitization, whereas the role of T18 residue in calcium-dependent and -independent desensitization requires further work.

Disclosures: **M. Rokic:** None. **E. Leiva-Salcedo:** None. **S. Stojilkovic:** None. **C. Coddou:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Program#/Poster: 597.08/C50

Topic: B.04. Ion Channels

Title: Effects of chronic caffeine administration on brain sodium-potassium atpase activities in streptozotocine-induced diabetic female wistar rats

Authors: *A. O. MAHMUD-IMAMFULANI, O. K. BAMIKOLE, B. V. OWOYELE; Physiol., Univ. of Ilorin, Ilorin, Nigeria

Abstracts: Diabetes mellitus (DM) may affect the morphology and plasticity of the brain, leading to cognitive, memory, and electrophysiological impairment. Streptozotocine-Induced (STZ-induced) diabetes leads to a sustained up-regulation of facilitatory adenosine A2A receptors in the hippocampus. Brain Sodium-Potassium ATPase (Na+/K+ATPase) enhance neuroprotection, memory consolidation and diminishes cognitive deficits. Thus, it has been observed that brain Na+/K+-ATPase activities is reduced in diabetic state in male animals. We therefore aim to determine the effects of caffeine (an adenosine receptor antagonist) on brain Na+/K+ATPase activities in STZ-induced diabetic female rats. Eleven groups of 7 female Wistar rats weighing between 150-200g were used for the study. Groups 1, 2 and 3 were administered

15, 20 and 25 mg/kg caffeine intraperitoneally (i.p) per day respectively for five weeks; groups 4, 5 and 6 were administered 15, 20 and 25 mg/kg caffeine (i.p) per day respectively for five weeks and were maintained on caffeine for three weeks thereafter; groups 7, 8 and 9 were DM rats placed on caffeine immediately after induction of DM at 15, 20 and 25 mg/kg (i.p) per day respectively for 3 weeks; groups 10 and 11 were control and DM respectively that were administered normal saline. DM was induced with 50 mg/kg STZ. At the end of the experiments, rats brains were removed and Na+/K+ATPase activities was determined. The ATpase activity was expressed as μmolPi/mg protein/hour x 10-3. The results showed a significant ($P < 0.05$) decrease in Na+/K+ATPase activities of untreated DM group (250.9 ± 0.26) compared with control group (415.6 ± 0.26). Also groups 1, 2 and 3 pretreated with caffeine showed increase in Na+/K+ATPase activities (1022.0 ± 0.51, 825.0 ± 0.22 and 498.6 ± 0.04 respectively) with significant ($P < 0.05$) observation in groups 1 and 2 when compared with control group. Groups 4 and 6 showed significant ($P < 0.05$) decrease in Na+/K+ATPase activities (280.0 ± 0.40 and 232.4 ± 0.34 respectively) when compared with control and a significant increase in group 5 (374.0 ± 0.34) compared with DM group. DM rats in groups 7, 8 and 9 showed increase in Na+/K+ATPase activities (468.8 ± 0.51, 428.5 ± 0.50 and 259.9 ± 0.20 respectively when compared with diabetic group, with significant increase ($P < 0.05$) in groups 7 and 8. The result shows that Na+/K+ATPase activity is reduced in STZ-induced diabetic female Wistar rats. In conclusion, the findings from this study shows that caffeine at low to moderate doses improves and restores the brain Na+/K+ATPase activities in diabetic rats. Thus moderate caffeine intake could be beneficial to brain functions in diabetic animals and man.

Disclosures: **A.O. Mahmud-Imamfulani:** None. **O.K. Bamikole:** None. **B.V. Owoyele:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Topic: B.04. Ion Channels

Support: Indian Council of Medical Research,Government of India

Title: Modulation of p2x7 receptor mediated calcium signaling by pannexin hemichannel

Authors: *V. SIRISHA, A. K. BERA, A. MANI;
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Abstracts: Pannexins are the members of the family of gap junction proteins in vertebrates, expressed in many organs including brain. In the central nervous system, Pannexin1 (Panx1) participates in both autocrine and paracrine signaling by working as an ATP release channel. It is also involved in calcium signaling by activating P2X7 receptor through the released ATP. A physical interaction between Panx1 and P2X7 receptor (P2X7R) has been reported, though the functional consequences of such interaction have not been studied. In the present study, we demonstrate that over-expression of Panx1 attenuates P2X7R mediated intracellular calcium rise without altering expression of the latter. By sequentially deleting amino acids from the carboxy terminus of Panx1, we identified that the region from amino acid 370 to 406 in the carboxy terminus is responsible for this inhibition. CHO-K1 cells which are known to express endogenous functional P2X7 receptor were transiently transfected with wild type Pannexin1 and Pannexin1 C terminus truncated mutants. Intracellular calcium was measured ratiometrically using Fura2-AM.Endogenous expression of P2X7 was checked by western blotting and Real time PCR after transfecting with Pannexin1 hemi channel.

Disclosures: **V. Sirisha:** None. **A.K. Bera:** None. **A. Mani:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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The Korean Health Technology R&D Project: A092042

The Asan Institute for Life Sciences: 2012-193

Title: Angiopoietin-1 inhibits zinc entry through PIP2 hydrolysis-induced ion channel blockage

Authors: ***J. LIM**[1], J.-Y. KOH[1,2];
[1]Neural Injury Res. Lab., [2]Dept. of Neurol., Asan Med. Ctr., Seoul, Korea, Republic of

Abstracts: Release of synaptic zinc and its entry into cortical cells during hypoxic ischemia or seizure result in extensive neuronal death. Hence, neuroprotective measures targeting zinc toxicity may prove useful in reducing acute brain injury. Angiopoietin-1 (Ang1), a potent vascular growth factor that binds to Tie2 and integrins, exhibits substantial protective effect

against various kinds of cytotoxic insults. Thus, we investigated whether Ang1 is able to protect cortical cells against zinc-induced cell death. In our neuron-glia co-culture system, transient exposure to zinc (150 μM) induced necrotic death of approximately 70% of the cells; co-treatment with Ang1 (200 ng/μl) markedly reduced zinc-induced toxicity to 20%. Interestingly, whereas zinc toxicity occurs largely through oxidative stress, Ang1 did not protect cortical cells against direct oxidative stress induced by H_2O_2_rather, fluorescent staining of intracellular zinc revealed that Ang1 dramatically reduced the entry of zinc via ion channels, thereby nullifying the effect of added extracellular zinc ion in the first place. We then tested the hypothesis that Ang1 blocks ion channels by activating phospholipase C (PLC), which induces hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) and ion channel blockage: notably, PLC inhibitor U73122 completely negated Ang1-induced inhibition of zinc entry and the subsequent cytotoxicity. Lastly, the protective action of Ang1 was effectively reversed by anti-integrin α5 antibody and FAK inhibitor PF573228, indicating that binding of Ang1 to α5 integrin and the subsequent activation of FAK signaling pathway is needed for blockage of zinc entry. Taken together, these results demonstrate a potent protective effect of Ang1 against zinc-induced necrotic cortical cell death, and at the same time reveal a novel role of Ang1/integrin pathway in regulating ion channel activity by PLC activation and subsequent PIP_2 hydrolysis. Keywords: zinc, angiopoietin-1, ion channel, phosphatidylinositol 4,5-biphosphate

Disclosures: J. Lim: None. **J. Koh:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Topic: B.04. Ion Channels

Support: NINDS R01NS11613

Title: Software for model-based design of experiments

Authors: *S. G. CARVER[1,2], M. HINES[3];
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Abstracts: We have written software to aid in the design of experiments that test hypotheses in the form of models. The models to be tested must include a representation of both the biological

system (e.g. currents and/or cells) and the experimental protocols. The models can have adjustable parameters. A successful experiment will reveal that one of the hypothesized models is substantially more likely than the others to reproduce the experimental data. Our software addresses the question: do the different hypotheses make different enough predictions---different enough to be testable with the planned experimental protocols? With the answer to this question in hand, the experimenter can decide to proceed with the experiment as planned, or alternatively, to reformulate the hypotheses, and/or adjust the protocols. To arrive at the answer, our software computes, with Monte Carlo integration and optimization, (1) the minimum Kullback-Leibler divergence between an assumed model and an alternative model structure, (2) the probability that the Akaike information criterion (AIC) selects each candidate model (based on data simulated from the assumed model), and (3) the number of repetitions of the experiment needed to make the selection correctly, a desired fraction of the time (e.g. 95%). As a last step, our software can analyze experimental (or simulated) data to rank hypotheses and select the best model. Our software requires simulating the models, and computing and optimizing the likelihood that each model produces the data. We compute likelihoods recursively with a Bayesian filter. By restricting our models to partially observed Markov chains (e.g. a patch clamp making single-channel recordings), we eliminate the need for approximation in the computation of the likelihood, and greatly reduce the amount of computation needed. More general models, planned for future work, require such approximations (e.g. based on the extended Kalman filter, or the particle filter). While our model-based design of experiments can be computationally very costly for such models, the computational expense, when attainable, can pay off, if it justifies or rejects an even more costly experiment.

Disclosures: **S.G. Carver:** A. Employment/Salary (full or part-time):; American University, Yale University. **M. Hines:** A. Employment/Salary (full or part-time):; Yale 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.; NINDS R01NS11613.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Program#/Poster: 597.12/C54

Topic: B.04. Ion Channels

Support: Department of Science and Technology, Government of India

Title: Modulation of acid sensing ion channels by estrogen and quercetin

Authors: *M. MUKHOPADHYAY[1], A. K. BERA[1], S. CHAKRAVARTY[2], P. JHELUM[2]; [1]Biotech., Indian Inst. of Technol., Chennai, India; [2]Indian Inst. of chemical technology, Hyderabad, India

Abstracts: Acid Sensing Ion Channels (ASICs), present on neuronal membranes, are activated by low pH. Activation of ASIC1a during stroke promotes cell death by increasing intracellular calcium. Estrogen has been shown to exhibit neuroprotective properties against several neurological disorders, including stroke. We hypothesize that estrogen mediated neuroprotection involves ASICs. We found that while ASIC1a subtype promotes cell death during ischemia, ASIC2a subtype is neuroprotective. There was significant up-regulation of ASIC2a in female mice, subjected to experimental stroke. This up-regulation was, however, not observed in the ovarectomised mice and male mice, suggesting the possible involvement of estrogen . We also studied the effect of a naturally occurring flavonoid Quercetin on ASIC by performing whole cell patch clamp technique. Quercetin is known to have neuroprotective action. Quercetin inhibits both ASIC1a and ASIC2a mediated currents in sub-micromolar concentration, suggesting that it possibly exerts its activity by targeting ASICs.

Disclosures: M. Mukhopadhyay: None. **A.K. Bera:** None. **S. Chakravarty:** None. **P. Jhelum:** None.

Poster

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Title: Nicotine exposure fragments Golgi and alters the recycling and glycosylation of alpha4beta2-type nicotinic acetylcholine receptors

Authors: *A. P. GOVIND[1], Y. VALLEJO[2], W. GREEN[1,3];
[1]Neurobio., Univ. of Chicago, CHICAGO, IL; [2]Natl. Cancer Inst., Bethesda, MD; [3]Marine Biol. Lab., Woods Hole, MA

Abstracts: Nicotine addiction is initiated by nicotine binding to nicotinic acetylcholine receptors (nAChRs) in brain during chronic exposure. We have characterized how nicotine exposure altered the trafficking and glycosylation of cell-surface alpha4beta2-type nAChRs while new ligand binding sites formed on these nAChRs via the process of nicotine-induced upregulation. Long-term nicotine exposure stimulated surface nAChR endocytosis and recycling and fragmented the Golgi apparatus of the cells and neurons in which they were expressed. Nicotine-induced Golgi fragmentation caused glycosidases to co-localize with recycling nAChRs. As a result, N-linked glycosylation of recycling nAChRs changed from high-mannose to complex-trimmed during nicotine exposure in parallel with their upregulation. During nicotine-induced Golgi fragmentation, nAChR upregulation, surface insertion, recycling and trafficking through the secretory pathway continued in contrast to BrefeldinA-induced Golgi fragmentation where all of these processes were inhibited. Co-exposure of nAChR competitive inhibitors and channel blockers with nicotine did not block the effects of nicotine indicating that nicotine acts independent of surface nAChR activation or desensitization. However, nicotine-induced Golgi fragmentation occurred only in cells and neurons expressing nAChRs. Thus, Golgi fragmentation and likely other nicotine-induced changes are caused by nicotine binding to nAChRs in an intracellular compartment. Altogether, our results suggest novel mechanisms by which membrane-permeable drugs, such as nicotine, act by binding to their intracellular receptors causing Golgi fragmentation and, in turn, the redistribution and modification of receptors at the cell surface.

Disclosures: A.P. Govind: None. **Y. Vallejo:** None. **W. Green:** None.

Poster

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Topic: B.04. Ion Channels

Support: Craig Nielsen Foundation

Albert Einstein College of Medicine

University of Miami School of Medicine

Title: Pannexin1 mediates astrocyte cell death

Authors: *E. SCEMES[1], D. G. JACKSON[2], J. WANG[2], R. W. KEANE[2], G. P. DAHL[2];
[1]Dept of Neurosci, Albert Einstein Col. of Med., BRONX, NY; [2]Physiol. and Biophysics, Univ. of Miami Sch. of Med., Miami, FL

Abstracts: Elevation of the extracellular concentrations of excitatory neurotransmitters (e.g., glutamate, aspartate, ATP) and ions (mainly K^+) resultant from traumatic brain injury and stroke triggers the progressive and long lasting secondary wave of cell death. This secondary bystander damage (necrosis and apoptosis) results from the activation of caspase and phospholipase pathways in nearby cells. Activation of P2 receptors by elevated ATP is one of the initial signals leading to inflammatory responses and pyroptotic cell killing. Pannexin1 (Panx1) forms plasma membrane channels that are activated directly by extracellular K^+ and, through intracellular signaling cascades, by ATP-sensitive P2 receptors. Interestingly, Panx1 is regulated by its permeant, i.e. the permeant ATP inhibits the channel from the extracellular space. Here we tested the interplay between stimulatory and inhibitory factors on the Panx1 channel in mediating cell death. Using pharmacological, electrophysiological, biochemical and fluorescence imaging techniques, we provide evidence that extracellular K^+ attenuates the inhibition of Panx1 channels by ATP and its analogue BzATP. Increased extracellular K^+ not only attenuated the self-inhibition of Panx1 but also affected the potency of other Panx1 channel inhibitors, notably those affecting the putative binding sites for ATP, thus suggesting that the binding sites of K^+ and BzATP on Panx1 channels overlap as well. Astrocyte cell death (LDH release and caspase-3 activation) induced by elevated K^+ was attenuated by BzATP and prevented in astrocytes deficient in Panx1 but not in $P2X_7$-null astrocytes. Similar to the observations made in an expression system and cell cultures, BzATP also attenuated the K^+-induced activation of Panx1 channels (dye uptake) in neurons and astrocytes from hippocampal slices of wild-type and $P2X_7$-null mice; K^+-induced Panx1 activation was absent in hippocampal slices of Panx1 deficient and double $P2X_7$ and Panx1 null mice. In summary, we provide evidence that Panx1 is a key molecule that orchestrates caspase-dependent cell death in response to elevation of extracellular K^+ and ATP concentrations and that the negative feedback control of ATP on Panx1 channels can be abrogated by elevated extracellular K^+ concentration. During acute injuries to the CNS, the loss of ATP-induced inhibition of Panx1 channels in a K^+-rich environment could be a key factor for hyper-stimulation of the apoptosome in neighboring healthy cells resulting in secondary wave of cell death.

Disclosures: E. Scemes: None. **G.P. Dahl:** None. **R.W. Keane:** None. **D.G. Jackson:** None. **J. Wang:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Topic: B.04. Ion Channels

Title: Compound action potential inhibition produced by aroma-oil compounds without TRP activation in the frog sciatic nerve

Authors: S. OHTSUBO, T. FUJITA, A. MATSUSHITA, C.-Y. JIANG, *E. KUMAMOTO; Dept Physiol, Saga Med. Sch., Saga, Japan

Abstracts: Capsaicin, menthol and allyl isothiocyanate, which activate TRPV1, TRPM8 and TRPA1, respectively, inhibited fast-conducting and voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potentials (CAPs) recorded from the frog sciatic nerve without TRP activation. Opioids such as morphine, ethylmorphine and codeine depressed CAPs in a manner dependent on their chemical structures without opioid receptor activation. Similar CAP inhibition was seen between tramadol and mono-*O*-demethyl tramadol and also among various adrenoceptor agonists including dexmedetomidine. We have previously reported that several aroma-oil compounds inhibit CAPs. The present study examined whether the CAP inhibitions are mediated by TRP activation and a detail of how the CAP inhibitions are related to the chemical structures of aroma-oil compounds. The experiment was performed by applying the air-gap method to the frog sciatic nerve. Citral, which activates TRPV1, TRPM8, TRPA1 and TRPV3, attenuated CAP peak amplitudes with the IC_{50} value of 0.48 mM; this action was resistant to a non-selective TRP antagonist ruthenium red. Camphor (TRPV1 and TRPV3 agonist) at 5 mM reduced CAP amplitudes by 33% and (+)-borneol (TRPV3 agonist) inhibited CAPs with the IC_{50} value of 2.0 mM; these actions were insensitive to ruthenium red. Lavender-oil compounds, linalyl acetate and (±)-linalool, reduced CAP amplitudes with the IC_{50} values of 0.49 and 1.7 mM, respectively. -CHO group- (citronellal), -OH group- (citronellol, geraniol, (-)-linalool, (-)-borneol and α-terpineol), -COO- group- (geranyl acetate and bornyl acetate) and oxide group- (rose oxide) containing ones reduced CAP amplitudes with the IC_{50} values of 0.50, 0.38, 0.53, 1.5, 1.8, 1.1, 0.51, 0.65 and 2.0 mM, respectively. On the other hand, myrcene and *p*-cymene at 5 mM reduced CAP amplitudes by 7 and 20%, respectively. Taking into consideration previously-reported data, an efficacy sequence of aroma-oil compounds for the CAP inhibitions was phenols (thymol, carbacrol and eugenol) ≥ aldehydes (citral and citronellal) ≥ esters (bornyl acetate, linalyl acetate and geranyl acetate) > alcohols ((±)-linalool, (-)-linalool, (+)-borneol, (-)-

borneol, α-terpineol, geraniol, citronellol and menthol) > ketones (carbone, menthone and plegone) > oxides (rose oxide and cineole) >> hydrocarbons (*p*-cymene, myrcene and limonene), except for a ketone camphor that was less effective than oxides. These results further confirmed the idea that there is a relationship between nerve conduction inhibitions by aroma-oil compounds and their chemical structures. Such an inhibition was not mediated by TRP activation.

Disclosures: **S. Ohtsubo:** None. **E. Kumamoto:** None. **T. Fujita:** None. **A. Matsushita:** None. **C. Jiang:** None.

Poster

597. Cellular Mechanisms of Neural Excitability: Ion Channels

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Program#/Poster: 597.16/C58

Topic: B.04. Ion Channels

Title: Frog sciatic nerve compound action potential inhibitions by crude medicines contained in daikenchuto and interaction between the inhibitions

Authors: A. MATSUSHITA, *T. FUJITA, S. OHTSUBO, C.-Y. JIANG, E. KUMAMOTO; Dept. Physiol., Fac. Med., Saga Univ., Saga, Japan

Abstracts: We have recently revealed that various plant-derived transient receptor potential channel agonists such as capsaicin, zingerone, menthol, allyl isothiocyanate and cinnamaldehyde inhibit fast-conducting and voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potentials (CAPs) in frog sciatic nerves. A similar inhibitory action was seen by traditional Japanese medicine (Kampo medicine) containing many plant-derived chemicals. Daikenchuto, rikkosan, kikyoto, rikkunshito, shakuyakukanzoto and kakkonto reduced the peak amplitude of the CAP in a concentration-dependent manner. Daikenchuto had a half-maximal inhibitory concentration (IC_{50}) value of 1.1 mg/ml. When compared at a concentration of 2 mg/ml, the extents of the reductions by daikenchuto, rikkosan, kikyoto, rikkunshito, shakuyakukanzoto and kakkonto were 70%, 30%, 25%, 15%, 14% and 12%, respectively. Daikenchuto being the most effective in inhibiting CAPs is composed of three kinds of crude medicine, Japanese pepper, processed ginger and ginseng. The present study examined how the three crude medicines of daikenchuto affect CAPs and whether there is an interaction between the actions of the crude medicines on CAPs. The experiments were performed by applying the air-gap method to the frog sciatic nerve. When each of the crude medicines at 2 mg/ml was

tested, Japanese pepper and processed ginger reduced CAP peak amplitude by 70% and 30%, respectively, while ginseng radix hardly affected CAPs. The inhibitory action of Japanese pepper had the IC_{50} value of 0.77 mg/ml. Ginseng radix (0.6 mg/ml), which had no effect on CAPs, unaffected the inhibitory action on CAPs of processed ginger in a range of 0.2 to 2 mg/ml, but had a tendency to enhance the inhibitions of CAPs by low (< 0.5 mg/ml) but not high (> 0.5 mg/ml) concentrations of Japanese pepper. Processed ginger (1 mg/ml) also had a tendency to increase CAP inhibition by Japanese pepper at low but not high concentrations. These results indicate that two kinds (Japanese pepper and processed ginger) of the crude medicine of daikenchuto have an ability to inhibit CAPs and that there is a positive interaction in nerve conduction inhibition among crude medicines contained in daikenchuto at low but not high concentrations. It is suggested that the pharmacological actions of Kampo medicine may be partly due to nerve conduction inhibition.

Disclosures: A. Matsushita: None. **T. Fujita:** None. **S. Ohtsubo:** None. **C. Jiang:** None. **E. Kumamoto:** None.

Poster

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 597.17/C59

Topic: B.04. Ion Channels

Title: Effects of membrane sealing agents poloxamer-188 and citicoline to cortical spreading depression-induced megachannel opening in the brain

Authors: *S. LULE[1], T. YILDIRIM[2], A. EYLEN[2], S. CANKURTARAN-SAYAR[3], K. SAYAR[4], M. UGUR[3], O. UGUR[5], T. DALKARA[1,6], Y. GURSOY-OZDEMIR[1,6];
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Abstracts: Cortical spreading depression (CSD) is a propagating wave of neuronal and glial depolarization that causes transient neuronal megachannel opening. Poloxamer-188 (P-188) and citicoline are known to seal plasmalemma and protect cells against several types of brain injury, although their mechanism of action is not clear. The aim of this study is to investigate whether P-188 and citicoline could suppress CSD-induced neuronal megachannel opening in the intact male

mouse (Swiss albino) brain and to gain insight to its mechanism of action. Pretreatment with P-188 and citicoline significantly decreased CSD-induced propidium iodide influx to neurons in both cortex (34% and 15%, respectively) and hippocampal dentate gyrus (32% and 22%, respectively), suggesting that these agents can block megachannel opening by either their membrane sealing action or by an unknown mechanism. To gain further insight, we examined whether or not P-188 and citicoline suppress P2X7 megachannel opening in cell cultures. Prolonged stimulation with adenosine tri-phosphate (ATP) induces a non-selective permeability increase due to opening of P2X7-ligand-gated ion channel, allowing molecules as large as 900 Daltons to enter cells. We used human embryonic kidney (HEK) 293 cells transfected with mouse P2X7 receptors (HEK-mP2X7) and a mouse macrophage derived cell line, RAW 264.7 that expresses an endogenous P2X7 receptor. The YO-PRO 1 fluorescence increase was monitored as an indicator of ATP-induced megachannel formation. We have found that neither P-188 nor citicoline had any blocking effect on mP2X7R induced dye uptake. We are currently investigating whether P-188 and citicoline could inhibit dye influx through Pannexin 1 (Panx1) megachannels in cell cultures.

Disclosures: **S. Lule:** A. Employment/Salary (full or part-time):; Hacettepe University. **T. Yildirim:** A. Employment/Salary (full or part-time):; Ankara Atatürk Education and Research Hospital. **A. Eylen:** A. Employment/Salary (full or part-time):; Ankara Atatürk Education and Research Hospital. **S. Cankurtaran-Sayar:** A. Employment/Salary (full or part-time):; Ankara University. **K. Sayar:** A. Employment/Salary (full or part-time):; Ankara University. **M. Ugur:** A. Employment/Salary (full or part-time):; Ankara University. **O. Ugur:** A. Employment/Salary (full or part-time):; Ankara University. **T. Dalkara:** A. Employment/Salary (full or part-time):; Hacettepe University. **Y. Gursoy-Ozdemir:** A. Employment/Salary (full or part-time):; Hacettepe University.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.01/C60

Topic: B.07. Synaptic Transmission

Support: NIH Grant EY019885

Title: Local corticothalamic feedback via presynaptic GABAa receptors on thalamocortical terminals in rat V1

Authors: *L. WANG[1], M. KLOC[1], A. ERISIR[2], A. MAFFEI[1];
[1]Dept. of Neurobio. & Behavior, SUNY At Stony Brook, Stony Brook, NY; [2]Dept. of Psychology, Univ. of Virginia, Charlottesville, VA

Abstracts: Inhibitory GABAergic transmission plays a fundamental role in neocortical circuit function. Recent work showed that in many areas of the brain GABAa receptors can be located presynaptically and directly modulate the efficacy of synaptic transmission at GABAergic and glutamatergic inputs. However, in neocortical circuits the general assumption is that fast phasic or tonic GABA regulates circuit excitability by acting on postsynaptic GABAa receptors. No evidence regarding the possible presence of presynaptic GABAa receptors in neocortex has been reported. Here we tested the possibility that presynaptic GABAa receptors may be present in neocortical circuits and may play an important role in regulating how cortical circuits are activated by incoming inputs. We combined optogenetic, physiological, immunohistochemical and electron microscopy approaches to demonstrate that in rat primary visual cortex (V1) GABAa receptors containing the α4 subunit are specifically located at thalamocortical (TC) presynaptic terminals and modulate the amplitude of evoked TC currents. No evidence for presynaptic GABAa receptors at recurrent excitatory synapses was observed, indicating a connection-specific role for these receptors. Our results compel a thorough reevaluation of current theories about the functional role of inhibition in neocortex, as changes in the level of inhibition can provide connection-specific corticothalamic feedback through fast presynaptic GABA signaling. Therefore, presynaptic GABAa receptors containing the α4 subunit and located at TC terminals may play an important role in regulating neocortical circuit function under healthy and pathological conditions.

Disclosures: **L. Wang:** None. **M. Kloc:** None. **A. Erisir:** None. **A. Maffei:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.02/C61

Topic: B.07. Synaptic Transmission

Support: Seed Grant from the Vice President of Research of the University of Oklahoma HSC

Title: Effects of seizure-inducing munc18-1 mutations on synaptic neurotransmission

Authors: *S. LOGAN[1], A. M. OROCK[2], F. DEAK[1];
[1]Reynolds Oklahoma Ctr. on Aging/Geriatric Med., [2]Oklahoma Ctr. for Neurosci., Univ. of Oklahoma HSC, Oklahoma City, OK

Abstracts: Early infantile epileptic encephalopathy (EIEE), also known as Ohtahara syndrome, is a severe form of epilepsy with pathophysiology manifesting in the first months of life. Patients experience tonic spasms and EEG measurements reveal suppression-burst patterns with poor medical prognosis. Mis-sense point mutations (C180Y, M443R, G544D and V84D) in STXBP1, the gene that encodes the protein munc18-1, have been identified as a probable cause for EIEE. Possible mechanisms for seizure induction by munc18-1 mutants may be through 1) haploinsufficiency whereby reduced stability or expression of the mutants would affect synaptic release; 2) dominant negative effect on wild-type munc18-1; or 3) loss-of-function. The effect of these mutations on synaptic neurotransmission remains to be elucidated. We hypothesized that disease causing munc18-1 point mutants adversely affect synaptic neurotransmission. To address this hypothesis, we virally expressed cerulean-tagged mutants in primary cortical neuronal cultures derived from munc18-1 wild-type (WT; +/+), heterozygotes (HET; +/-) and knock-out (KO; -/-) mouse embryos. Munc18-1 knock-out neurons die within 7 days *in vitro* (DIV) and synaptic transmission is severely impaired. Overexpression of C180Y mutant in KO neurons rescued neuronal survival and synaptic transmission albeit to a lower extent than tagged wild-type overexpression. GFP overexpression did not rescue KO neuronal survival. Using live imaging and FM1-43 staining methodology we measured synaptic release in the transduced neurons. Overexpression of C180Y mutant partially rescued synaptic release in munc18-1 knock-out neurons, albeit to a lower extent than the WT-expressing neurons (~20% of fluorescence lost within 15 seconds). This release rate is comparable to Munc18-1 heterozygote knock-out (20-30%) neurons. Control wild-type neurons have a typical ~40% of fluorescence drop in 15s of destaining. Furthermore, KO neurons expressing C180Y mutant also had a smaller recycling pool (7111 ±492 fluorescence units) compared to WT neurons (9961 ±1973, $p<0.039$). We conclude that the C180Y is a loss of function mutation of munc18 and full restoration of synaptic release is not necessary to prevent neurodegeneration by the munc18 protein. Characterization of the functional consequence of these mutants may lead to therapeutic interventions for patients with munc18 mutations and EIEE pathophysiology.

Disclosures: S. Logan: None. **A.M. Orock:** None. **F. Deak:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.03/C62

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS083127

NIH Grant NS083894

Max Planck Florida Institute

Max Planck Society

Title: Kv3 channel clustering in presynaptic boutons of cerebellar interneurons imparts local control of spike duration between release sites

Authors: *M. J. ROWAN, J. M. CHRISTIE;
Max Planck Florida Inst., Jupiter, FL

Abstracts: Compartmentalized control of electrical excitability including local shaping of action potentials (APs) is a key functional feature in the dendrites and myelinated axons of projection neurons. However, the capacity for local control of APs in the neurites of inhibitory interneurons remains relatively unexplored. We examined for localized control of AP waveform in unmyelinated axons of molecular layer interneurons (MLIs) in the cerebellum, a cell type that drives precisely timed inhibition from *en passant* bouton release sites onto postsynaptic targets. Using two-photon (2P) voltage-sensitive dye imaging to measure APs from multiple axonal locations, we find that the rate of AP repolarization is faster at boutons as compared to connecting axon shafts. In addition, AP repolarization rates of adjacent boutons display considerable variation. Together, these results demonstrate a highly-localized AP topography within small axonal regions (<15μm). Morphological perturbation failed to alter APs in boutons indicating that axon geometry alone does not account for these observed differences. Instead, our results point to Kv channel clustering at boutons as the key determinant of compartmentalized AP shaping within axons. This was experimentally validated using tight-seal patch-clamp recordings from intact boutons and axon shafts (not bleb endings) to measure Kv channel-mediated currents. Peak conductance density was substantially greater in boutons compared to axon shafts. Using targeted 2P photolysis of a caged Kv channel blocker at individual boutons, we ascertained the spatial extent over which Kv-mediated conductances govern AP repolarization. Uncaging was sufficient to locally broaden APs, however, broadening was constrained to the target bouton leaving neighboring boutons largely unaffected. Pharmacological manipulations revealed an essential role for Kv3-type channels in local AP shaping likely owing to their unique biophysical properties. As spike-width directs the magnitude of AP-evoked Ca^{2+} influx, release probability at individual boutons is likely informed by the specific expression and dynamic regulation of the Kv3 channels at independent boutons, thereby tuning neurotransmission at individual synapses.

Disclosures: **M.J. Rowan:** None. **J.M. Christie:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.04/C63

Topic: B.07. Synaptic Transmission

Title: Neurones in the spinal cord of the mouse innervating the external urethral sphincter: Its identification and quantitative analysis of their presynaptic inputs

Authors: ***Y. MERICAN**, R. M. ICHIYAMA, S. A. DEUCHARS, J. DEUCHARS;
Univ. of Leeds, Leeds, United Kingdom

Abstracts: One of the most common and distressing problems in the elderly is poor sphincter control resulting in involuntary passing of urine. The external urethral sphincter, which serves as the muscle of continence, is innervated by the pudendal nerve which carries signal from the Onuf's nucleus in the spinal cord. In ageing, we hypothesise that there is an altered balance of excitatory and inhibitory influences on these motoneurones in the Onuf's nucleus. This is supported by a previous study that showed the urethral sphincter architecture and volume is unaltered in ageing (Russell et. al., 1996). In this study, we investigated the Onuf's nucleus homologue known as the dorsolateral nucleus (DLN) in young mice, followed by analysis of the type and number of presynaptic terminals to the DLN motoneurones. Twelve wild-type mice C57BL6 female mice (age 3 month) were used. For identification of the motoneurones innervating the external urethral sphincter, the sphincteric muscles of 6 mice were injected with cholera-toxin Bchain (2 µl in 1% saline) under Fluothane anaesthesia. 3 days later, the animals were anaesthetised with 80mg/kg pentobarbitone IP, perfused with 4% paraformaldehyde (PFA) and spinal cords were sectioned at 50µm using a vibrating microtome and processed with cholera-toxin immunohistochemistry. Using this retrograde tracing method, the motoneurones innervating the external urethral sphincter were traced and verified to be located in the ventral horn of the sixth lumbar to first sacral segments of the spinal cord. The remaining six mice were perfused and processed as above. The sectioned spinal cords from 3 mice were processed for the choline acethyl transferase (ChAT) using peroxidase immunohistochemistry. The DLN is easily identified based on the location of the ChAT peroxidase immunoreactivity. The mean length of the nucleus was 0.65 ± 0.2 mm, and the mean number of dorsolateral motoneurones was 38.5 ± 1.5 per spinal cord. Sectioned spinal cords from another 3 mice were processed for triple

labelling immunofluorescence with ChAT, glutamic acid decarboxylase (GAD67) and glycine transporter (GlyT2). Images obtained by confocal microscopy were analysed quantitatively using method by Chang & Martin (2009), and revealed that there were 2.0 (± 0.7), 4.4 (± 0.6) and 3.2 ± (0.8) of ChAT-, GAD67- and GlyT2-immunopositive boutons respectively per 100 μm membrane perimeter of motoneurones in the DLN. This initial result are important as baseline findings will be compared to the findings in aged mice to determine if there are age-related changes in the presynaptic inputs to the DLN motoneurones.

Disclosures: Y. Merican: None. **R.M. Ichiyama:** None. **S.A. Deuchars:** None. **J. Deuchars:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.05/C64

Topic: B.07. Synaptic Transmission

Title: Time resolved cryo-electron tomography of stimulated synaptosomes

Authors: *S. ASANO, U. DITTMANN, Z. KOCHOVSKI, V. LUCIC, W. BAUMEISTER; Max Planck Inst. of Biochem., Martinsried, Germany

Abstracts: Within the presynaptic terminal, synaptic vesicles are embedded inside a dense filamentous protein network known as the presynaptic cytomatrix. Earlier cryo-electron tomography studies of fully-hydrated, vitrified synapses showed significant differences in the cytomatrix at the active zone after prolonged stimulation. Here we used a newly developed setup capable of stimulating synapses 10-100 ms before vitrification. Quantitative analysis of stimulated synapses showed differences in synaptic vesicle distribution and cytomatrix organization which depends on stimulation protocol.

Disclosures: S. Asano: None. **U. Dittmann:** None. **Z. Kochovski:** None. **V. Lucic:** None. **W. Baumeister:** None.

Poster

598. Presynaptic Organization and Structure

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Topic: B.07. Synaptic Transmission

Support: NSF Grant 1249546

NIH Grant P41GM103712

Univ. Pitt. CRDF

Title: Presynaptic calcium channel - vesicle release site relationships probed using measurements of synaptic latency

Authors: A. E. HOMAN[1], J. MA[2], M. DITTRICH[2], *S. D. MERINEY[3];
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Pittsburgh Supercomputing Ctr., Carnegie Mellon Univ., Pittsburgh, PA; [3]Univ. Pittsburgh, Pittsburgh, PA

Abstracts: The organization of presynaptic active zones (AZs) can have a considerable impact on neurotransmission at synapses. The spatial relationship between voltage-gated calcium channels (VGCCs) and synaptic vesicle release sites has been demonstrated to influence release dynamics. Synaptic latency, the time delay from the arrival of the presynaptic action potential to the start of the postsynaptic response, provides a measure of the spatial relationship between VGCCs and release sites. We used the frog neuromuscular junction (NMJ) as a model synapse, and experimental manipulations designed to alter VGCC - synaptic vesicle release site relationships, to study variability in synaptic latency between synapses. Frog NMJs contain hundreds of AZs that are highly organized with respect to presynaptic VGCCs and docked synaptic vesicles. Previous studies suggest that there are 20-40 VGCCs in each AZ that open with low probability during a presynaptic action potential. Further, it has been hypothesized that each synaptic vesicle fusion event is triggered predominately by the calcium flux through a single open VGCC, with significant, but minor contributions from neighboring VGCCs. Using a combination of extracellular macropatch recordings positioned on top of nerve terminals and intracellular recordings from postsynaptic cells, we have characterized synaptic latency at these synapses. In control terminals, synaptic latency ranged from 0.29 ms to 0.51 ms with a mean of 0.43 ms, indicating that there is inherent variability between synapses under normal conditions. This distribution was shifted considerably following exposure to low extracellular calcium (0.5mM Ca2+) with synaptic latencies spanning from 0.29 ms to 2.8 ms with a mean of 0.60 ms, an average increase of approximately 100-200µs. Conversely, application of 400 nM conotoxin-GVIA decreased synaptic latency by approximately 100µs. These data support that on average, release at the frog NMJ is largely due to the flux of calcium through a single channel, with some contribution from other nearby channels. However, we also observed that the change in latency

induced by these experimental conditions was not consistent across synapses, suggesting that not all release sites are similarly organized. To aid in the interpretation of these findings we used spatially realistic MCell computer modeling to investigate the effect of VGCC number, location and organization in the AZ on release and facilitation. Our physiological and computer modeling data suggest that different synapses may have variability in their calcium channel-vesicle release site organization, and this has an impact on neurotransmission.

Disclosures: **A.E. Homan:** None. **S.D. Meriney:** None. **J. Ma:** None. **M. Dittrich:** None.

Poster

598. Presynaptic Organization and Structure

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Topic: B.07. Synaptic Transmission

Support: MEC (SAF2011-23711)

Catalan Government (Generalitat) (2009SGR01248)

Title: Adenosine receptors and muscarinic receptors cooperate in acetylcholine release modulation on neuromuscular synapse

Authors: ***N. GARCIA**, M. PRIEGO, M. M. SANTAFÉ, T. OBIS, M. TOMÀS, M. A. LANUZA, N. ORTIZ, E. HURTADO, L. NADAL, J. M. TOMÀS;
Univ. Rovira i Virgili, Reus, Spain

Abstracts: In the neuromuscular synapse, presynaptic muscarinic ACh autoreceptors (mAChR) and purinergic adenosine autoreceptors (AR) directly couple ACh release and adenosine (ADO) secretion, respectively to regulate the nerve ending release mechanism itself. We show by immunocytochemistry that adenosine receptors subtypes (A1R, A2AR, A2BR and A3R) are present in the motor nerve terminals at the neuromuscular junctions (NMJs). Preliminary data from our group show that in resting neuromuscular preparations with fully preserved neurotransmission machinery (μ-CgTx-GIIIB paralyzed muscles), the presence of unselective AR agonist (adenosine 0.3-25μM) or antagonist (8-SPT, 100μM) does not change evoked ACh release. However, A1R can reduce spontaneous quantal leak of ACh and the collaboration of A1R and A2AR may protect synaptic function by reducing depression during repetitive activity. The present study is designed to analyse: 1) the effect of adenosine deaminase (ADA), ADO, 2-

chloroadenosine (CADO) and 8SPT in depression induction during electrically stimulated muscles (40-100Hz) and 2) to detect the AR and mAChR interaction in transmitter release modulation. Result indicates that: i) in basal conditions, endogenous ADO reduces the efficacy of the unsincronised spontaneous ACh release mechanism; ii) some colaborative work between different AR subtypes may reduce synaptic depression at moderate activity level (40Hz); iii) at high activity levels (100Hz), endogenous ADO production in the synaptic cleft could attain sufficient amount to interactuate with A1R receptors to protect for depression; iv) when using the non metabolizable 2-chloroadenosine (CADO) agonist, both quantal content and depression reduction occurs, and A1R seems mainly involved; v) there exist and absolute mutual dependence of AR and muscarinic acetylcholine receptors (mAChR) on the modulation of evoked and spontaneous ACh release in basal conditions and in experimental conditions with CADO stimulation; vi) the purinergic and muscarinic mechanism cooperate in the control of depression by sharing a common pathway though the purinergic control seems more powerful that the muscarinic one because depression can be additionally increased with 8SPT after a first increase in depression by muscarinic block with atropine.

Disclosures: **N. Garcia:** None. **M. Priego:** None. **M.M. Santafé:** None. **T. Obis:** None. **M. Tomàs:** None. **M.A. Lanuza:** None. **N. Ortiz:** None. **E. Hurtado:** None. **L. Nadal:** None. **J.M. Tomàs:** None.

Poster

598. Presynaptic Organization and Structure

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Program#/Poster: 598.08/C67

Topic: B.07. Synaptic Transmission

Support: NHMRC ID# 569680

Title: Loss of laminin-α4 leads to decreased functional capacity in neurotransmission at maturing neuromuscular junctions

Authors: *K. K. CHAND[1], K. M. LEE[1], B. L. PATTON[2], P. G. NOAKES[1,3], N. A. LAVIDIS[1]; [1]Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane, Australia; [2]Sch. of Med. Fac., Oregon Hlth. & Sci. Univ., Portland, OR; [3]Queensland Brain Inst., Brisbane, Australia

Abstracts: Synaptic basal lamina such as laminin-421 (α4β2γ1), play an integral role in the organization of the neuromuscular junction (NMJ). Laminins interact with other synaptic

molecules to ensure precise alignment of pre- and postsynaptic structures. Targeted mutation of the *lama4* gene does not alter formation of active zones (AZs) and junctional folds, though disruptions in the precise alignment of AZs and postsynaptic folds are seen in the NMJs of laminin-α4 deficient mice (*lama4*$^{-/-}$) when compared to wild-type mice (WT). The laminin-α4 chain is suggested to play an instructive role in the placement of postsynaptic specializations such as acetylcholine receptors (AChRs). In the present study we compared neurotransmission at developing (postnatal day 8, P8), mature (postnatal day 18, P18) and young adult (postnatal day 30, P30) NMJs of *lama4*$^{-/-}$ and WT mice. Mice were bred and maintained on a C57BL/6-129SvJ genetic background. We functionally examined the diaphragm, a mixed type I and type IIA fiber muscle; and, *extensor digitorum longus*, a type IIB fiber muscle in *lama4*$^{-/-}$ NMJs (n=6 for each muscle at all ages and both genotypes). Utilizing intracellular electrophysiology we recorded end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs). Early perturbations in neurotransmission were observed at P8, and became more evident by P18 with no significant progression to P30 at *lama4*$^{-/-}$ NMJs. At all ages investigated *lama4*$^{-/-}$ NMJs displayed higher intermittence of transmitter release (P8, $p<0.05$; P18 and P30, $p<0.01$) when compared to WT. MEPP amplitude was significantly increased in *lama4*$^{-/-}$ at P8 ($p<0.05$), P18 ($p<0.01$) and, P30 ($p<0.01$) in comparison to WT. The decay time of MEPPs was increased in *lama4*$^{-/-}$ when compared to WT NMJs for both fiber types. Immunohistochemical studies demonstrated normal distribution of pre- and postsynaptic densities. At the ages investigated we observed no change in the number of AZs at *lama4*$^{-/-}$ NMJs.The present study observed greater intermittence of transmitter release at *lama4*$^{-/-}$ NMJs, as well as decreases in the probability of release and quantal content at *lama4*$^{-/-}$ NMJs. These findings suggest a possible interaction between laminin-α4 and vesicular associated proteins at the AZ, as loss of laminin-α4 has been shown in this study to decrease the probability of transmitter release. Furthermore, the increase in MEPP amplitude and decay time indicates a down regulation of acetylcholine esterase. This is suggested to be a compensatory mechanism for the misalignment of AZs to AChRs seen at *lama4*$^{-/-}$ NMJs.

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Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.09/C68

Topic: B.07. Synaptic Transmission

Support: F31 NS084826-02

Title: Locating synaptic calcium channels

Authors: *S. A. MERRILL, S. WATANABE, E. HUJBER, J. R. RICHARDS, E. M. JORGENSEN;
Biol., Univ. of Utah, Salt Lake City, UT

Abstracts: Neurotransmission occurs when calcium triggers exocytosis of synaptic vesicles primed at release sites. The number, position and activity of nearby calcium channels determine the perdurance of free calcium at a release site. However, calcium entry through multiple sources within a synapse has been studied only indirectly. To understand the synapse we must identify the location of calcium channels in relation to synaptic vesicles, fusion proteins, and subcellular structures such as the dense projection. Mammals contain at least 10 genes that encode thousands of unique calcium channel isoforms. In *C. elegans unc-68* (RyR), *egl-19* (L-type), and *unc-2* (N-type) channels are each encoded by a single gene and contribute the calcium for synaptic vesicle exocytosis at neuromuscular junctions. First, we are transgenically attaching to each channel an enzymatic tag that covalently binds organic fluorophores suitable for correlative imaging by super-resolution fluorescence and electron microscopy (nano-fEM). Additionally, each channel will be imaged by biplane 3D super-resolution fluorescence microscopy to determine the colocalization of calcium channels with other synaptic proteins at nanometer resolution. Finally, we will test the contributions of the vesicle priming proteins *unc-13*, *unc-10* (RIM), and *rimb-1* (RIM-binding protein) to localizing each synaptic calcium channel and its adjoining vesicles.

Disclosures: S.A. Merrill: None. **S. Watanabe:** None. **E. Hujber:** None. **J.R. Richards:** None. **E.M. Jorgensen:** None.

Poster

598. Presynaptic Organization and Structure

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Program#/Poster: 598.10/C69

Topic: B.07. Synaptic Transmission

Support: CIHR

BWF

EJLB

Title: Nanodomain coupling of P/Q-type Ca2+ channels with truncated C-terminus of the alpha1A subunit to the release of synaptic vesicles at a central synapse in the Leaner (tgla/la) mouse

Authors: *Y. YANG[1,2], M. J. FEDCHYSHYN[1,2], T. M. EPPS[1,3], O. C. SNEAD[1,3], L. C. ABBOTT[4], L.-Y. WANG[1,2];
[1]The Hosp. For Sick Children, Toronto, ON, Canada; [2]Physiol., [3]Med. & Biomed. Sci., Univ. of Toronto, Toronto, ON, Canada; [4]Med. & Biomed. Sci., The Texas A&M Univ., College Station, TX

Abstracts: Emerging evidence suggests that the C-terminus of alpha1A or alpha1B subunit of voltage-gated Ca2+ channels (VGCCs) plays a crucial role in interacting with synaptic proteins RIM and RIM-BP and tethering synaptic vesicles (SVs) for efficient fusion at central synapses. However, whether such an interaction is essentially indispensable for fast synchronous release remains elusive. To address this question, we take advantage of the leaner mouse (tgla/la) in which a spontaneous missense mutation proximal to S6 transmembrane domain gives rise to a truncation of the C-terminus of alpha1A subunit, leading to impaired function of P/Q-type VGCCs and severely ataxic and epileptic behavioral phenotypes. By performing single or paired recordings from pre- and post-synaptic compartments of the mature calyx of Held synapse, we find that in contrast to the wild-type nerve terminal where Omega-agatoxin sensitive P/Q-type VGCCs exclusively mediate transmitter release, mutant P/Q-type channels contribute to a fraction of synchronous release in the tgla/la synapse and the loss-of-function mutation is compensated by Omega-conotoxin sensitive N-type VGCCs. After blocking N-type VGCCs, surprisingly we reveal that diffusion of a slow Ca2+ buffer EGTA (10 mM) into the calyx has little effect on transmitter release mediated by mutant P/Q-type channels. Further measurements of the input (presynaptic Ca2+ currents)-output (excitatory postsynaptic currents) relationship demonstrate that the "Ca2+ channel/ domain cooperativity" in the tgla/la synapses is comparable to that in the WT synapses. Collectively, these results indicate that mutant P/Q-type VGCCs lacking its functional C-terminus can form "nanodomain" release modality and effectively engage SVs in neurotransmission. We therefore suggest that the interaction between the C-terminus of alpha1 subunits and RIM/RIM-BP is not necessary for tethering SVs to the vicinity of VGCCs in the active zone to promote nanodomain coupling of Ca2+ influx to release of neurotransmitters.

Disclosures: Y. Yang: None. **M.J. Fedchyshyn:** None. **T.M. Epps:** None. **O.C. Snead:** None. **L.C. Abbott:** None. **L. Wang:** None.

Poster

598. Presynaptic Organization and Structure

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Program#/Poster: 598.11/C70

Topic: B.07. Synaptic Transmission

Support: MEC (SAF2011-23711)

Catalan Government (Generalitat) (2009SGR01248)

Title: The novel protein kinase C epsilon isoform at the adult neuromuscular synapse: Location, synaptic activity-related expression, phosphorylation function and coupling to ACh release

Authors: *M. A. LANUZA, E. HURTADO, N. BESALDUCH, T. OBIS, L. NADAL, N. GARCIA, M. M. SANTAFE, M. PRIEGO, M. TOMAS, J. TOMAS;
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Abstracts: nPKCε is a major isoform in the novel PKC family. Although it has been increasingly implicated in neural functions and associated neurogenic diseases, the role of nPKCε in neurons is still not well understood. Recent studies provide evidence that nPKCε could regulate distinct aspects of neural functions, including neurotransmitter release and signal transduction. Moreover, to date, no reports have been published on the localization and function of the nPKCε at the paradigmatic neuromuscular junction (NMJ). Here, we have examined the distribution of the nPKCε in the adult NMJ cell components, its synaptic activity-related expression changes, its regulation by muscle contraction and its possible involvement in ACh release. Furthermore, we have investigated the relation between skeletal muscle contraction, the tyrosine kinase receptor B (TrkB) signaling and the presynaptic expression of nPKCε at the adult rat NMJ. We use immunohistochemistry and confocal microscopy to demonstrate that the novel isoform nPKCε is exclusively located in the motor nerve terminals on the NMJ. We also report by electrophysiological techniques and using a nPKCε-specific translocation inhibitor peptide (εV1-2) that nPKCε is decisively involved in ACh release potentiation induced by phorbol esters like PMA. Moreover, results also show that synaptic activity-induced muscle contraction enhances nPKCε expression, and its catalytic function in phosphorylating the substrate MARCKS through TrkB activity. Together, these results provide a mechanistic insight into how synaptic activity-induced muscle contraction could regulate the presynaptic action of the PKC isoforms in neurotransmission and suggest that muscle contraction is an important regulatory step in TrkB receptor signaling at the NMJ.

Disclosures: **M.A. Lanuza:** None. **E. Hurtado:** None. **N. Besalduch:** None. **T. Obis:** None. **L. Nadal:** None. **N. Garcia:** None. **M.M. Santafe:** None. **M. Priego:** None. **M. Tomas:** None. **J. Tomas:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

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Swedish Research Council

Title: A novel mechanism controlling reclustering of synaptic vesicles in *Drosophila* synapses

Authors: Å. M. E. WINTHER, K. A. REES, O. VORONTSOVA, E. SOPOVA, A. PECHSTEIN, W. JIAO, G. ARPINO, *O. SHUPLIAKOV;
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Abstracts: Synapsins are synaptic vesicle (SV) associated proteins that modulate neurotransmitter release and control the organization of SVs in adult nerve terminals and during development. Here we demonstrate that at *Drosophila* neuromuscular junctions (NMJs) the scaffolding protein Dap160, the ortholog of mammalian intersectins, controls synapsin localization during the synaptic vesicle cycle. We report that the SH3A-B domain region of Dap160 is essential for the synapsin binding and the remaining SH3 domains facilitate the interaction between the proteins. During synaptic activity synapsin is broadly dispersed throughout the axon in mutants expressing *dap160* lacking the SH3A-B domain region, while in control it is restricted to the nerve terminal. At the subcellular level the nerve terminals in the mutant revealed delayed reclustering of SV at release sites. Numerous small SV clusters were still present within synaptic boutons subjected to intense stimulation whereas control boutons completely recovered their morphology rapidly. Abnormal clustering of vesicles was also observed in NMJs from *synapsin* null mutant. Synapsin expression and delivery to nerve terminals remained unchanged in mutants expressing *dap160* lacking the synapsin-interacting SH3 domains and Dap160 level in *synapsin* null boutons was not altered, implying that for both Dap160 and synapsin additional interactions are involved in their delivery to presynaptic boutons during development. In summary, our results uncover a novel role for Dap160/intersectin interaction in targeting synapsin back to SVs and facilitating reclustering of SVs at the active zone during synaptic activity.

Disclosures: **Å.M.E. Winther:** None. **K.A. Rees:** None. **O. Vorontsova:** None. **E. Sopova:** None. **A. Pechstein:** None. **W. Jiao:** None. **G. Arpino:** None. **O. Shupliakov:** None.

Poster

598. Presynaptic Organization and Structure

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Topic: B.07. Synaptic Transmission

Support: The Hong Kong University of Science and Technology

Research Grants Council (HKUST12/CRF/13G and GRF660813)

Title: The localization of ATR (ataxia telangiectasia and Rad3-related) in synaptic vesicles - A super-resolution study

Authors: *A. CHENG[1], T. ZHAO[2], M. LOY[2], K. HERRUP[1,3];
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Abstracts: ATR (ataxia telangiectasia and Rad3-related), is a PI3-kinase involved in DNA single-strand break repair. Mutations that lower the activity of ATR are responsible for Seckel syndrome, a devastating CNS developmental disorder. In addition to its expected nuclear localization, ATR is found in the neuronal cytoplasm where it physically associates with the homologous PI3-kinase, ATM (ataxia telangiectasia mutated) as well as with VAMP2 (synaptobrevin) and synapsin-I. Cytoplasmic ATM has been localized to vesicular structures in the neuronal cytoplasm; a detailed analysis of ATR localization has not been done. We have now used super-resolution microscopy to gain further insight into the localization and cytoplasmic function of cytoplasmic ATR. We used stochastic optical reconstruction microscopy (STORM), a new technique of optical microscopy that allowed us to achieve two-color labeling of ATR at 20 nm resolution. Antibodies to ATR clearly label vesicular structures in DIV15 neurons. The identity of these structures as vesicles was confirmed by double immunostaining with the CgA vesicular cargo protein. While proving the identity of the labeled structures as vesicles, this finding also emphasizes that ATR is found not only on neurotransmitter containing synaptic vesicles, but also on dense core vesicles, the precursors of nascent synaptic active zones in neurons. Bassoon, an active zone localized protein, is associated with dense core vesicles *in vivo*; and double labeling with ATR and Bassoon showed significant co-localization. Curiously, we

also found ATR co-localized with MAP2 as well as with PSD95, opening the possibility that ATR is located on both sides of the synapse. This is consistent with reports that some potential ATR targets (with [S/T]Q motifs) are localized in dendritic spines on the post-synaptic side of the synapse. We are exploring the hypothesis that ATR is responsible for phosphorylation of [S/T]Q substrates at sites within the dendritic spine itself while its sister kinase, ATM, is localized to and mainly regulates substrates in cytoplasm.

Disclosures: **A. Cheng:** None. **T. Zhao:** None. **M. Loy:** None. **K. Herrup:** None.

Poster

598. Presynaptic Organization and Structure

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MEXT-supported Program for the Strategic Research Foundation at Private Universities

Research Grant from the Takeda Science Foundation

Title: Enrichment of GABARAP relative to LC3 in the axonal initial segments of neurons

Authors: *M. KOIKE[1], I. TANIDA[2], T. NANAO[1], N. TADA[1], J. IWATA[1], T. UENO[1], E. KOMINAMI[1], Y. UCHIYAMA[1];
[1]Juntendo Univ. Sch. Med., Tokyo, Japan; [2]Natl. Inst. of Infectious Dis., Tokyo, Japan

Abstracts: GABAA receptor-associated protein (GABARAP) was initially identified as a protein that interacts with GABAA receptor. Although LC3 (microtubule-associated protein 1 light chain 3), a GABARAP homolog, has been localized in the dendrites and cell bodies of neurons under normal conditions, the subcellular distribution of GABARAP in neurons remains unclear. Subcellular fractionation indicated that endogenous GABARAP was localized to the

microsome-enriched and synaptic vesicle-enriched fractions of mouse brain as GABARAP-I, an unlipidated form. To investigate the distribution of GABARAP in neurons, we generated GFP-GABARAP transgenic mice. Using these transgenic mice, we investigated the intracellular distribution of GFP-GABARAP in hippocampal pyramidal neurons and cerebellar Purkinje cells by comparing its distribution with that of endogenous LC3. Immunohistochemistry in these transgenic mice showed that positive signals for GFP-GABARAP were widely distributed in neurons in various brain regions, including the hippocampus and cerebellum. Interestingly, intense diffuse and/or fibrillary expression of GFP-GABARAP was detected along the axonal initial segments (AIS) of hippocampal pyramidal neurons and cerebellar Purkinje cells, in addition to the cell bodies and dendrites of these neurons. In contrast, only slight amounts of LC3 were detected along the AIS of these neurons, while diffuse and/or fibrillary staining for LC3 was mainly detected in their cell bodies and dendrites. These results indicated that, compared with LC3, GABARAP is enriched in the AIS, in addition to the cell bodies and dendrites, of these hippocampal pyramidal neurons and cerebellar Purkinje cells.

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Poster

598. Presynaptic Organization and Structure

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Topic: B.07. Synaptic Transmission

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NIH Grant NS069629

Title: Comparison of different slice angles in preservation of major hippocampal pathways in the mouse

Authors: ***G. XIONG**[1], B. JOHNSON[1], C. SMITH[1], A. S. COHEN[1,2];
[1]Neurol., Children's Hosp Philadelphia, Philadelphia, PA; [2]Neurosurg., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstracts: The hippocampus plays a critical role in higher cognitive function and has been implicated in numerous neuropathologies. Electrophysiological recording on live slices is one of

the most powerful tools for investigating hippocampal cellular and network activity. In general, three types [frontal, transverse and hippocampal-entorhinal cortex (HEC)] of live slices have been widely used to study the major hippocampal pathways i.e., perforant path (PP), mossy fiber (MF) and Shaffer collateral (SC). In the present study, we focused on intrahippocampal fiber preservation in slices generated by sectioning through different planes. The postmortem neural tract tracer DiI was used to label afferent pathway fibers in slices from fixed mouse brains. Laser scanning confocal microscopy was adopted for imaging DiI-labeled fibers. Our data demonstrate that PP fibers are well preserved in HEC slices, MFs in both HEC and transverse slices, and SCs in all three types of slices. To verify our morphological data, we compared HEC and frontal slices by recording field potentials in stratum lucidum of area CA3 while stimulating in the dentate granule cell (DGC) layer. Using HEC slices, we were consistently able to evoke DGC stimulated mossy fiber responses. By contrast, DGC-evoked potentials in frontal slices required higher stimulation current and could only be recorded in a fraction of slices. The present study provides an anatomical correlate for choosing the most apt slice angle for subsequent physiological experiments.

Disclosures: G. Xiong: None. **B. Johnson:** None. **C. Smith:** None. **A.S. Cohen:** None.

Poster

598. Presynaptic Organization and Structure

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Support: NIH Grant DK080971

Juvenile Diabetes Research Foundation

Title: Pancreatic islet beta cells employ synaptic cleft proteins and synaptogenic mechanisms to establish insulin secretory function

Authors: *S. D. CHESSLER, C. ZHANG, M. R. MIRBOLOOKI;
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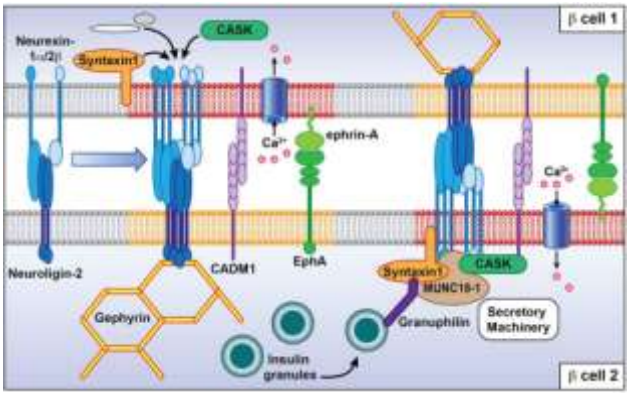
Abstracts: In fruit files and other primitive animals, specialized neurons in the brain function as the equivalent of the mammalian insulin-secreting pancreatic islet beta cells. This and a variety of other evidence suggest that pancreatic beta cells--although endodermal--are evolutionarily

descended from central nervous system neurons. The parallels between the developmental and functional biology of neurons and beta cells are striking. We are interested in the architecture of the beta-cell insulin secretory apparatus, which is remarkably similar to that of the synaptic machinery for neurotransmitter release. We hypothesize that the explosion of new knowledge in the neurobiology field regarding the role of transcellular protein interactions in synaptogenesis and maintenance of synaptic function will yield important insights into beta-cell maturation and function. Our working model of transcellular beta-cell interactions is depicted in the figure. Neurexin-containing pre-synaptic-like microdomains (red) on the beta cell surface are opposed to neuroligin-2-containing post-synaptic-like microdomains (yellow). We have found that neuroligin and neurexin are expressed by beta cells and engage in transcellular interactions promoting insulin secretion. Neurexin, as shown, interacts directly with the submembrane exocytic machinery, promoting its assembly. Here we ask whether interactions involving CADM1 (SynCAM) similarly enhance beta cell function. Co-culture of beta cells with COS-7 cells expressing CADM1 revealed that transcellular interactions involving CADM1 enhance insulin secretion. As with neuroligin, while CADM1 binding interactions in coculture experiments increased insulin secretion, perturbation of levels of the protein in INS-1 beta cells had differing effects. CADM1 overexpression impaired insulin secretion, while CADM1 gene silencing enhanced secretion. CADM transcript levels decreased in response to increased glucose concentrations. These results indicate a significant role for CADM interactions in the establishment of beta-cell function.



Disclosures: **S.D. Chessler:** None. **C. Zhang:** None. **M.R. Mirbolooki:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NSF Grant 1249546

NIH Grant P41GM103712

Title: The importance of the organization of presynaptic calcium channels and synaptic vesicle release sites in plasticity and disease

Authors: J. MA[1], T. TARR[2], S. D. MERINEY[2], *M. DITTRICH[1];
[1]Pittsburgh Supercomputing Ctr., Carnegie Mellon Univ., Pittsburgh, PA; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstracts: Over the last decade, there has been mounting evidence that the function of neuromuscular synapses may be regulated by structural changes at the level of single-vesicle release sites consisting of a synaptic vesicle and a small number of closely associated voltage gated calcium channels (VGCCs). To shed additional light onto the mechanisms behind these structure-function relationships, we have used electrophysiological recordings, calcium imaging, and MCell computer modeling to study how differences in active zone (AZ) structure at the mouse and frog neuromuscular junction (NMJ) can explain their functional properties. We hypothesize that the arrangement of single-vesicle release sites into the different AZ organizations observed in frog and mouse is sufficient to explain major functional differences between the two NMJs (Tarr et al. 2013). Physiologically and structurally, the frog and mouse NMJ differ considerably. The frog NMJ shows significant paired-pulse facilitation (PPF) and tetanic potentiation (TP). On the other hand, mouse NMJs show virtually no PPF and TP. Structurally, the frog NMJ AZs consist of a long linear double row of VGCCs flanked laterally by two rows of 25-30 synaptic vesicles. In contrast, AZs at the mouse NMJ consist of a short row of ~ 2 synaptic vesicles running between two short linear rows of VGCCs. We have previously developed a spatially realistic MCell computer model of the frog NMJ, which predicts experimentally measured vesicle release both under single and multiple stimulus paradigms. Based on this model we were able to show that re-arranging the basic single vesicle release sites from a frog NMJ into a mouse NMJ organization allowed us to predict the observed differences between frog and mouse NMJ physiology. Further, our modeling studies allowed us to gain detailed insight into the underlying mechanisms at the single vesicle release site that relate structure to function in these systems. Our results suggest that facilitation at NMJs can be tuned by defined structural changes at the sub-AZ level. Our approach can also be applied to studies of AZ changes induced by Lambert-Eaton myasthenic syndrome (LEMS), an autoimmune disease characterized by an attack on presynaptic VGCCs that reduces their number and disrupts AZ structural organization. In mice passively transferred with LEMS, there is a dramatic increase in tetanic potentiation. Using experimental comparisons with acute pharmacologic block of

VGCCs, we are using our modeling approach to predict how changes in VGCC number and/or spatial relationships with docked synaptic vesicles impact synaptic function at these synapses.

Disclosures: J. Ma: None. **T. Tarr:** None. **M. Dittrich:** None. **S.D. Meriney:** None.

Poster

598. Presynaptic Organization and Structure

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Support: K12HD073945

R41MH097377

Sloan Foundation

Title: Assayable markers of presynaptic neuronal function suitable for high-throughput screening using fixable FM-dyes

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Abstracts: The development of high-throughput screening (HTS) techniques has advanced the field of drug discovery, particularly in oncology, however these techniques have found only limited success identifying potential therapies for neurological conditions. A significant limiting step is a lack of assays suitable for HTS that measure neuronal function. For example, while many non-neuronal screens utilize experimental endpoints such as inhibition of cellular growth or cell death, neural disease states are often characterized by more subtle changes (e.g. changes in vesicular pool size/distribution and release). In addition, as localized, presynaptic physiology is increasingly implicated in neural disease, functional assays specific to presynaptic effects of compounds are needed. We speculated that fixable styryl (FM) dye use in presynaptic screens would be advantageous as they are employable in all disease-model species, they provide an optical readout, and they can be employed in parallel over a large number of screens. FM dyes are readily loaded into presynaptic vesicles during either chemical or electrical stimulation. This pool of FM-labeled vesicles has been suggested to represent the recycling vesicle pool. Here, we demonstrate the potential for FM dye in assays of presynaptic function that are compatible with

HTS. We also examine disease-induced presynaptic changes in isolation from the postsynaptic compartment using FM dyes. We propose a FM dye-based assay for HTS by chemically inducing endocytosis of fixable FM dyes using a very short loading/fixing protocol. We found a significant decrease in the size of the FM-loaded vesicular pool in Fragile X Disease (FX) in both human and mouse neurons. This difference is consistent with other anatomical studies and could, in part, represent known differences in kinetics of vesicular release (i.e. an increase in "kiss and run" vs. full fusion exocytosis). While we demonstrate assayable differences in vesicle pools labeled with fixable FM-dye in FX neurons, changes in the vesicle pools have been shown in other diseases including epilepsy and schizophrenia, suggesting these protocols could be used in screening for a large variety of neurological disorders. Equally compelling, FM dye loading is altered by certain physiological states (such as LTD) so there exists the potential for even broader usage in functional HTS.

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Poster

598. Presynaptic Organization and Structure

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Program#/Poster: 598.19/D6

Topic: B.07. Synaptic Transmission

Title: Chronic social isolation reduces excitatory synaptic inputs in medial prefrontal cortex

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Abstracts: Social experiences are necessary for the development of forebrain function and the maturation of medial prefrontal cortex (mPFC). Rearing mice in post-weaning social isolation produces behavioral and neurochemical alterations similar to those observed in psychiatric disorders such as schizophrenia and autism. However it is not well-known about electrophysiological changes in neural circuit of mPFC induced by social isolation. In the present study, we examined the change of excitatory synaptic inputs into layer 5 pyramidal cells in

mouse PFC produced by isolation rearing, with whole-cell patch clamp recording. Mice pups were divided into two groups at weaning and reared in isolation (housed one per cage) or reared in a group (housed four or five per cage) for 6 weeks. We found that the spontaneous excitatory postsynaptic current (sEPSC) frequency and miniature excitatory postsynaptic current (mEPSC) frequency were significantly lower in isolated mice than in grouped mice. There was no significant difference in sEPSC amplitude and mEPSC amplitude between both mice. These results show that chronic social isolation reduces excitatory synaptic inputs on layer 5 pyramidal cells of mouse mPFC and suggest that social experience have an impact on neural circuit development of mPFC.

Disclosures: K. Yamamuro: None. **H. Yoshino:** None. **Y. Ogawa:** None. **M. Makinodan:** None. **C. Gabriel:** None. **T. Kishimoto:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NIH Grant NS087601

Title: Analysis of Rab3 structure as related to its function in active zone formation at the *Drosophila* neuromuscular junction

Authors: S. CHEN, H. K. GENDELMAN, J. P. ROCHE, P. ALSHARIF, *E. GRAF;
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Abstracts: The complement of presynaptic release machinery proteins at individual release sites controls the efficacy of vesicle release at each site. The small GTPase Rab3 was previously identified as playing a novel role that controls the localization of release machinery proteins to the release sites of the *Drosophila* neuromuscular junction (NMJ). In the rab3 mutant, key components of the presynaptic release machine, including Bruchpilot (Brp), Ca2+ channels, and T-bars, are enriched at a subset of active zones, leaving the remaining release sites devoid of such proteins and structures. However, the mechanism by which Rab3 performs this function is not understood. To clarify the mechanisms of Rab3 function at the *Drosophila* NMJ, we have employed a structure-function analysis to determine the structural requirements of Rab3 for its localization to the axon terminal and function at the NMJ to regulate active zone development.

Utilizing GTP-binding defective mutations of Rab3, we find that GTP binding is required for Rab3 to localize to NMJs and control Brp distribution across active zones. Although GTP-binding defective mutants are not trafficked appropriately to NMJs, strong expression in a wild type background results in a dominant negative effect that phenocopies the rab3 mutant in a hypomorphic manner. Conversely, mutations that disrupt GTP hydrolysis do not disrupt Rab3 localization or function, resulting in NMJs that are similar to wild type. However, average Brp puncta size is reduced in comparison to wild type when GTP-hydrolysis-defective mutants are expressed in either rab3 mutant or wild type backgrounds, suggesting that the constitutively active mutation may result in a gain-of-function synaptic phenotype. We further show that the protein-binding "switch" regions of Rab3 are required for controlling proper Brp distribution and analyze the requirement of specific residues within the "switch" regions. Mutations associated with the effector-binding N-terminal CDR region also prevent rescue of the rab3 mutant phenotype, whereas mutations in two other CDR regions do not affect Rab3 function. Finally, while mutations that disrupt the membrane association properties of Rab3 do not prevent the transport of Rab3 to the NMJ, we find that membrane association is required for controlling Brp distribution, consistent with a model by which Rab3 may control release machinery localization to active zones via a vesicle docking mechanism.

Disclosures: S. Chen: None. **E. Graf:** None. **H.K. Gendelman:** None. **J.P. Roche:** None. **P. Alsharif:** None.

Poster

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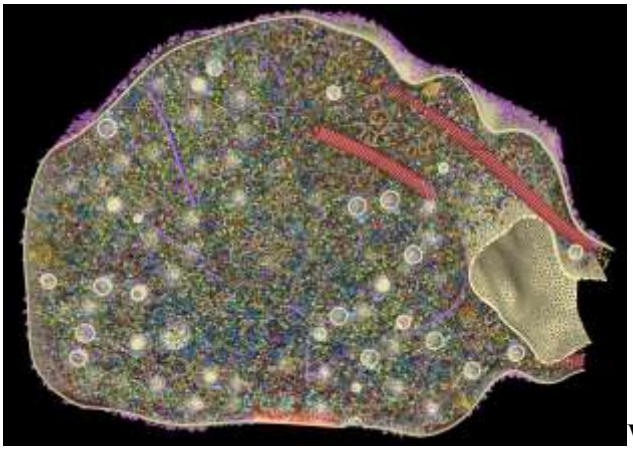
Boehringer Ingelheim Fonds PhD Fellowship

Title: The molecular organization of the synaptic bouton

Authors: *S. TRUCKENBRODT[1], B. G. WILHELM[1], S. MANDAD[2], K. KRÖHNERT[1], C. SCHÄFER[1], B. RAMMNER[1], S. J. KOO[3], G. A. CLAßEN[3], M. KRAUSS[3], V. HAUCKE[3], H. URLAUB[2], S. O. RIZZOLI[1];
[1]Univ. of Göttingen Med. Ctr., Göttingen, Germany; [2]Max-Planck-Institute for Biophysical Chem., Göttingen, Germany; [3]Dept. of Mol. Pharmacol. and Cell Biol., Leibniz-Institute for Mol. Pharmacol., Berlin, Germany

Abstracts:



We present here the 3D model of a synaptic bouton, comprising >300,000 individual proteins as absolute copy numbers of 62 different proteins essential to synaptic vesicle recycling, in their average subcellular localisation. We obtained this model via an integrative approach, using quantitative Western blotting to determine protein copy numbers, electron microscopy to measure organelle numbers, morphology and distribution, and super-resolution fluorescence microscopy to localise the proteins within the synaptic bouton. Using quantitative mass spectrometry, we determined the copy numbers of an additional >1100 different proteins. The model we obtained shows that copy numbers of proteins involved in the same step of synaptic vesicle recycling correlate closely, while copy numbers vary over more than three orders of magnitude between steps. Synaptic vesicle exocytosis is amply provided for, with more than 20,000 copies of each exocytotic SNARE protein per synaptic bouton. Proteins involved in synaptic vesicle endocytosis, on the other hand, are far less abundant (~1000-4000 copies each) and sufficient for simultaneous recycling of only ~7-11% of all vesicles present in the average synaptic bouton. The model thus points to a hitherto unknown level of quantitative regulation in cellular trafficking pathways and provides a reference source for research on synaptic biology.

Disclosures: **S. Truckenbrodt:** None. **B.G. Wilhelm:** None. **S. Mandad:** None. **K. Kröhnert:** None. **C. Schäfer:** None. **B. Rammner:** None. **S.J. Koo:** None. **G.A. Claßen:** None. **M. Krauss:** None. **V. Haucke:** None. **H. Urlaub:** None. **S.O. Rizzoli:** None.

Poster

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Simons Fellowship 177850

AHA Grant 11POST7360078

NIH Grant F32 DA031654

NIMH Grant 1K99MH103531

Title: The differential gene and isoform expression levels contribute to the functional diversity of Neurexin-3

Authors: *J. N. AOTO[1], C. FÖLDY[1], D. MARTINELLI[2], K. TABUCHI[4], R. MALENKA[3], T. SÜDHOF[1,5];
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Abstracts: Neurexins were initially discovered over 25 years ago as the endogenous presynaptic receptor for Black Widow spider venom; however, their functional roles in regulating synaptic transmission remains poorly understood. Neurexins are expressed from three genes (*Nrxn1-3*), each giving rise to a larger alpha and shorter beta mRNA transcript. Moreover each neurexin mRNA transcript can undergo alternative splicing at up to 6 conserved splice sites (SS1-SS6), of which, SS4 has been biochemically demonstrated to regulate binding to most known postsynaptic ligands. The diversity of neurexin gene and isoform expression has posed a

significant hurdle in the synaptic characterization of neurexin function. To systematically address these issues, we here utilize three neurexin-3 mutants - 1.) a constitutive neurexin-3[[Unsupported Character - Symbol Font ]] KO; 2.) a conditional neurexin-3[[Unsupported Character - Symbol Font ]][[Unsupported Character - Symbol Font ]][[Unsupported Character - Symbol Font ]] KO; and 3.) a conditional neurexin-3 SS4 mutant where the SS4 insert is constitutively included, but can be conditionally excluded – to interrogate the synaptic function of neurexin-3. Furthermore, we quantitatively assessed mRNA levels of *Nrxn1*, *Nrxn2* and *Nrxn3* and their SS4 isoforms in single neurons and identified two brain regions – the hippocampus where, antithetical to the other two neurexins, *Nrxn3* primarily lacks an insert at SS4, and the olfactory bulb where GABAergic granule cells express a reduced complement of neurexin gene products. We found that at synapses in the hippocampus, neurexin-3 SS4 isoforms play a central role in transsynaptically regulating basal and activity-dependent postsynaptic AMPA-receptor stability while at GABAergic synapses in olfactory bulb cultures, the neurexin-3 intracellular domain is required to maintain presynaptic release probability. Taken together, our systematic analysis revealed distinct synaptic functions of neurexin-3 and highlights the importance of evaluating region specific and cell-type specific expression patterns of neurexins.

Disclosures: **J.N. Aoto:** None. **C. Földy:** None. **D. Martinelli:** None. **K. Tabuchi:** None. **R. Malenka:** None. **T. Südhof:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.23/D10

Topic: B.07. Synaptic Transmission

Support: NIH Intramural Program

Title: Tomographic analysis of clustering and bridging filaments organized for the release of synaptic vesicles in hippocampal synapses

Authors: *A. A. COLE, X. CHEN, T. S. REESE;
Structural Neurobio. Section, NINDS, Bethesda, MD

Abstracts: Continuous synaptic transmission depends on movement of synaptic vesicles through the presynaptic vesicle cloud to contact the active zone. However, the molecular organization of

the presynaptic terminal is not well understood. Here we used EM tomography of high pressure frozen and freeze substituted dissociated rat hippocampal neurons to render a comprehensive picture of the connections between synaptic vesicles. Long filaments with complex, modular morphology - modular clustering filaments - cluster vesicles throughout the presynaptic vesicle cloud. A smaller bridging filament joins vesicles into pairs and triplets in the cloud. Along the active zone membrane, unique filaments ground vesicles to the active zone membrane. Small clusters of small irregular filaments, presumably representing SNARE complexes, cradle vesicles that contact the active zone membrane. A larger membrane bound modular clustering filament - active zone clustering filament - anchors multiple synaptic vesicles to the active zone membrane. In the synaptic vesicle cloud, a network of modular clustering filaments embraces ~ 80% of synaptic vesicles, including grounded vesicles. Each vesicle in this network has multiple bridging filament associations. Clustering filaments provide a complex network orthogonal to the active zone, favorable for trafficking synaptic vesicles toward the active zone, while bridging filaments do not show preferential orientation but provide a comprehensive network, congealing all vesicles into one cloud. Active zone clustering filaments are distributed throughout the active zone membrane and seem to be a key mechanism binding the vesicle cloud to the active zone. Small irregular active zone bound filaments extend less than 30 nm into the presynaptic terminal. These results provided a mechanical scheme for maintaining vesicle clouds and moving the vesicles to active zones for ultimate release, providing for trafficking of presynaptic vesicles in response to activity.

Disclosures: A.A. Cole: None. **X. Chen:** None. **T.S. Reese:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

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Topic: B.07. Synaptic Transmission

Support: NIH grant EY017836

DFG grant CRC889(B1)

Title: Control of a slow phase of synaptic transmission at a retinal ribbon synapse

Authors: J.-B. KE[1], L. S. MORTENSEN[2], K. REIM[2], J.-S. RHEE[2], N. BROSE[2], *J. H. SINGER[1];

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Abstracts: Signaling between rod bipolar (RB) and AII amacrine cells in the mammalian retina is a critical component of rod-mediated (i.e., night) vision, and the RB->AII synapse has been the subject of much investigation owing both to its central role in night vision and to its accessibility as a model ribbon synapse. The presynaptic ribbon-type active zone (AZ) contains a small pool of readily-releasable vesicles, exocytosis from which can be driven by the opening of single Ca channels. Thus, it was thought that highly localized presynaptic [Ca2+] changes were the primary determinant of transmission at this synapse. Recently, though, it was demonstrated that ribbon AZ-independent release from a unique vesicle pool in RB terminals activated postsynaptic AMPARs on AIIs. This form of release developed slowly, was driven by global changes in intraterminal [Ca2+], and appeared to draw on a unique vesicle pool. Here, we examined this "delayed release" at RB->AII synapses in an *in vitro* mouse retinal slice preparation. First, we demonstrated that delayed release was unaffected by genetic deletion of complexin 3, an accessory AZ protein that binds to the core exocytotic complex and increases the efficiency with which Ca2+ evokes exocytosis. As the initial, transient component of release, which, presumably, is driven by [Ca2+] changes localized to the AZ, was reduced in complexin 3 knockout mice, this finding supports the conclusion that delayed release resulted from an elevated global [Ca2+] acting on a unique vesicle pool. Next, we assessed delayed release without altering intracellular Ca2+ handling in the RB. The light-gated ion channel, channelrhodopsin2, was expressed in RBs, and transmission from unperturbed RBs was evoked by stimulating RBs in with blue-green light. Exocytosis from RB terminals was assessed by recording evoked EPSCs from postsynaptic AIIs. Depolarizing the RB for > 100 ms elicited EPSCs with initial transient and secondary, delayed components. The delayed components were enhanced substantially by blocking inhibition to the RB terminal, indicating that feedback and feedforward inhibition act primarily on slow signaling from RBs. Delayed release was potentiated strongly by the calmodulin (CaM)-dependent phosphodiesterase (PDE) inhibitors IBMX and MPPX, indicating that Ca2+ accumulation in the terminal likely acts through a CaM-PDE to regulate the availability of vesicles in the ribbon-independent pool that contributes to delayed release. In total, our data support the hypothesis that the RB axon terminal contains a pool of vesicles distinct from the readily-releasable pool and modulated independently.

Disclosures: J. Ke: None. **J.H. Singer:** None. **L.S. Mortensen:** None. **N. Brose:** None. **K. Reim:** None. **J. Rhee:** None.

Poster

598. Presynaptic Organization and Structure

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Program#/Poster: 598.25/D12

Topic: B.07. Synaptic Transmission

Support: NHMRC ID# 569680

Title: Loss of laminin-α4 accelerates aging of the neuromuscular junction

Authors: *K. LEE[1], K. K. CHAND[1], B. L. PATTON[3], N. A. LAVIDIS[1], P. G. NOAKES[1,2];
[1]Sch. of Biomed. Sci., [2]Queensland Brain Inst., The Univ. of Queensland, Brisbane, Australia;
[3]Sch. of Med. Fac., Oregon Hlth. and Sci. Univ., Portland, OR

Abstracts: Laminin-α4 is involved in the alignment of pre- and postsynaptic apparatuses at the neuromuscular junction (NMJ). Laminins-α4, -β2 and -γ1 form the laminin-421 heterotrimer, a key component of the synaptic basal lamina. Loss of critical basal lamina components may result in structural and functional changes at the NMJ. Mutation of laminin-α4 gene results in morphological abnormalities that resemble changes seen in aged wild-type mice. Here, we compared neurotransmission properties of wild-type (WT) and laminin-α4 deficient (*lama4*$^{-/-}$) NMJs at 3 months (3mnth), 6 months (6mnth) and 12 months (12mnth) of age. Mice were bred and maintained on a C57BL/6-129SvJ genetic background. We functionally examined the diaphragm muscle using intra- and extracellular electrophysiological recordings of evoked and spontaneous transmitter release. The diaphragm and innervating nerve were dissected from WT and *lama4*$^{-/-}$ mice at each age group (n=6 for 3mnth and 6mnth; n=4 for 12mnth for each genotype). Immunohistochemistry studies were conducted to investigate the colocalization and distribution of active zone markers to postsynaptic acetylcholine receptors. Our results demonstrated aberrations in neurotransmission at *lama4*$^{-/-}$ NMJs that persist throughout aging. The NMJs of *lama4*$^{-/-}$ displayed a higher number of stimuli that failed to evoke neurotransmitter release at 3mnth and 6mnth in comparison to age-matched WT NMJs ($p<0.01$ for 3mnth; $p<0.05$ for 6mnth; $p>0.05$ for 12mnth). Analysis of spontaneous transmitter release displayed a decrease in frequency ($p<0.0001$ for 3mnth; $p<0.001$ for 6mnth and 12mnth) and slower rise time ($p<0.0001$ for 3mnth; $p<0.01$ for 6mnth and 12mnth) at *lama4*$^{-/-}$ NMJs. Spontaneous amplitude also increased in *lama4*$^{-/-}$ NMJs at 3mnth ($p<0.001$) and 6mnth ($p<0.01$). Evoked transmitter release displayed slower rise time ($p<0.001$ for 3mnth; $p<0.0001$ for 6mnth) at *lama4*$^{-/-}$ NMJs. Immunohistochemical studies demonstrated morphological changes at *lama4*$^{-/-}$ NMJs that are characteristic of aged NMJs. Motor function in these *lama4*$^{-/-}$ mice is not compromised despite the abnormalities observed in our present studies due to the NMJ possessing a large safety factor. Our findings demonstrate that loss of laminin-α4 results in early perturbations in neurotransmission normally observed at aged NMJs. These findings suggest that laminin-α4 may play a role in the maintenance of the NMJ during aging.

Disclosures: **K. Lee:** None. **K.K. Chand:** None. **B.L. Patton:** None. **N.A. Lavidis:** None. **P.G. Noakes:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 598.26/D13

Topic: B.07. Synaptic Transmission

Title: Microtubule-dependent trafficking of synaptic vesicle "superpool" in cultured giant synapse

Authors: *L. GUILLAUD, D. DIMITROV, T. TAKAHASHI;
Okinawa Inst. of Sci. and Technol. Grad. Univ., Onna-Son, Okinawa, Japan

Abstracts: We previously visualized and analyzed synaptic vesicle movements in cultured calyx of Held synapses and demonstrated that, within this large presynaptic terminal, synaptic vesicles show different motions that could be categorized into 3 pools with respect to their dynamic properties. It has already been reported at various synapses that a small fraction of synaptic vesicles (synaptic vesicle "superpool") can move between adjacent synaptic boutons (inter-synaptic trafficking). Here we report that 10-15% of synaptic vesicles in the calyx-type presynaptic terminals likewise move between synaptic swellings (inter-swelling trafficking) with a high velocity and directional movements, and that inter-swelling trafficking is significantly disrupted by the microtubule depolymerizing drug nocodazole. We also found that the presynaptic terminal was enriched with de-tyrosinated microtubules that invaded into and interconnected presynaptic swellings. The kinesin KIF1A also co-localized with a subset of synaptic vesicles both within the pre-synaptic swellings and digit-like processes of calyceal terminals. These results suggest that the fast moving synaptic vesicle "superpool" in the calyceal terminal depends on microtubule/kinesin based transport between presynaptic swellings.

Disclosures: **L. Guillaud:** None. **D. Dimitrov:** None. **T. Takahashi:** None.

Poster

598. Presynaptic Organization and Structure

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Program#/Poster: 598.27/D14

Topic: B.07. Synaptic Transmission

Support: NINDS R01 NS052664

Title: Control of antero- and retrograde mitochondrial transport in *Drosophila* motor axons

Authors: *M. BABIC[1], B. W. HUNTER[2], G. J. RUSSO[3], A. J. WELLINGTON[2], K. E. ZINSMAIER[2];
[1]Grad. Interdisciplinary Program in Neurosci., [2]Dept. of Neurosci., [3]Dept. of Mol. and Cell. Biol., Univ. of Arizona, TUCSON, AZ

Abstracts: Microtubule-based transport of mitochondria into dendrites and axons is critical for normal synaptic function. In axons, kinesin motors drive anterograde transport of mitochondria while retrograde transport is driven by dynein. However, the mechanisms controlling the selective use of these motors remain poorly understood. The mitochondrial GTPase Miro is required for mitochondrial transport in axons and dendrites. Here, we show that the activity of *Drosophila* Miro's N-terminal GTPase domain controls antero- and retrograde transport of mitochondria in axons. Rendering the domain inactive by introducing the mutation T25N inhibited anterograde transport and depleted larval *Drosophila* motor axons of mitochondria. Conversely, rendering the domain constitutively active by introducing the mutation A20V caused a pronounced accumulation of mitochondria at axon terminals, and facilitated anterograde transport while suppressing retrograde transport. In mammals, kinesin and dynein are linked to mitochondrial Miro through the adaptor proteins TRAK1 and 2. We show that the only *Drosophila* homolog of TRAK1/2, Milton, facilitates not only kinesin- but also dynein-driven transport in axons. Milton expresses four different protein isoforms (A-D) generated by alternative mRNA splicing. Acute overexpression of Milton-A accumulates mitochondria at axon terminals within a few hours, while Milton-B depletes axons of mitochondria and accumulates them in the cell body. We are currently testing how Miro's N-terminal GTPase may control the selective use of Milton-A and -B and thereby antero- and retrograde axonal transport of mitochondria.

Disclosures: M. Babic: None. **B.W. Hunter:** None. **G.J. Russo:** None. **A.J. Wellington:** None. **K.E. Zinsmaier:** None.

Poster

598. Presynaptic Organization and Structure

Location: Halls A-C

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Program#/Poster: 598.28/D15

Topic: B.07. Synaptic Transmission

Support: National Institutes of Health (NIH) R01 NS061914

Title: The trade-off between the capacity to sustain neurotransmitter release and presynaptic energy efficiency

Authors: *Z. LU[1], A. CHOUHAN[2], A. ROSSANO[3], G. MACLEOD[1];
[1]Biol. Sci., Florida Atlantic Univ., Jupiter, FL; [2]Baylor Col. of Med., Houston, TX; [3]Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstracts: Synapses, building blocks for neural information processing, spend most of the energy in the brain. Given that large synaptic energy demand contrast with relative limited energy supply, it is advantageous for the synapse to adopt energy-efficient mechanisms in neurotransmission. We proposed that optimization of energy efficiency is widespread among different nerve terminals. Therefore, we further hypothesize that energy efficiency is the same amongst synapses. Here, we estimated energy efficiency in two *Drosophila* glutamatergic nerve terminals using electrophysiology recordings and calcium imaging. Surprisingly, presynaptic energy efficiencies are not the same. Energy efficiencies differ between these functionally differentiated nerves terminals innervating the same postsynaptic target. The nerve terminal with larger capacity for neurotransmitter release is less efficient. Furthermore, our simulation results indicate that calcium entry per active zone plays an important role in coordinating neurotransmitter release and energy use. In conclusion, our findings suggest a trade-off between the capacity to sustain neurotransmitter release and presynaptic energy efficiency.

Disclosures: Z. Lu: None. **A. Chouhan:** None. **A. Rossano:** None. **G. Macleod:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.01/D16

Topic: B.07. Synaptic Transmission

Support: DA010355

MH061469

Title: Dynamic phosphorylation of synaptic and extrasynaptic AMPA receptors in the rat striatum and prefrontal cortex in response to amphetamine

Authors: *B. XUE, L. MAO, J. WANG;
Dept. of Basic Med. Sci., UMKC Sch. of Med., Kansas City, MO

Abstracts: The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is sensitive to psychostimulants. Chronic stimulant administration induces plastic changes in AMPA receptors in the mesolimbic and mesocortical circuitry, which contributes to the enduring remodeling of excitatory transmission in relation to the addictive properties of drugs of abuse. Phosphorylation of AMPA receptors has been shown to be regulated by stimulants, although the specific AMPA receptor population at synaptic sites is less studied for their responses to stimulants. In this study, we used a pre-validated fractionation procedure to enrich AMPA receptors from synaptic and extrasynaptic pools to investigate the effect of the stimulant amphetamine on phosphorylation of AMPA receptors at these distinctive subsynaptic sites in the adult rat striatum and prefrontal cortex (PFC) *in vivo*. We found that amphetamine at a single, behaviorally active dose induced a marked increase in GluA1 phosphorylation at serine 845 (S845) in the striatum. The amphetamine-stimulated S845 phosphorylation occurred at both synaptic and extrasynaptic sites and was a time-related event as the response returned to the normal level by 4 h after amphetamine injection. In contrast to S845, phosphorylation of GluA1 at serine 831 (S831) remained stable in response to amphetamine at both synaptic and extrasynaptic sites. Similar results were observed in the PFC. No significant change in total levels of GluA1 was seen after amphetamine administration. These results demonstrate a dynamic and serine residue-specific upregulation of GluA1 AMPA receptor phosphorylation in the synaptic and extrasynaptic compartments of striatal and cortical neurons in response to stimulant exposure.

Disclosures: B. Xue: None. **L. Mao:** None. **J. Wang:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

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Program#/Poster: 599.02/D17

Topic: B.07. Synaptic Transmission

Support: ANR-2011-tVTA VTA

ANR-2012- Darling

Title: Neural bases for the excitatory control of VTA dopamine neurons by the ventral subiculum

Authors: *C. GLANGETAS[1], G. R. FOIS[2], M. JALABERT[3], C. HERRY[4], M. DIANA[2], S. CAILLÉ[5], F. GEORGES[1];
[1]IINS UMR CNRS 5297, Bordeaux, France; [2]Dept. of Chem. Pharmacol., Sassari, Italy; [3]INMED, Marseille, France; [4]INSERM CRI U862, Bordeaux, France; [5]CNRS UMR 5287, Bordeaux, France

Abstracts: The hippocampal formation including the ventral subiculum (vSUB) and the ventral CA1 area is a brain region that is involved in context-dependent processes, and also is considered as a regulator of emotion. The ventral tegmental area (VTA) plays a role in the acquisition of learned appetitive behaviors and in the development of drug addiction. It is now well accepted that the vSUB activates the dopamine (DA) system, however, the long term effect of the vSUB stimulation on VTA DA neurons activity is still unclear. First, we used electrophysiological approaches in anesthetized rats to demonstrate that Delta-bursts stimulation (DBS) protocols in the vSUB induces *in vivo* potentiation of VTA DA neurons activity 24 h after. This potentiation was still present 5 days after DBS protocols in the vSUB. To better understand the mechanism by which the vSUB activates VTA DA neurons, we decided then to use low frequency stimulation (that do not elicit plasticity) in the vSUB and observed the impact on VTA DA neurons activity. This low stimulation of vSUB has an excitatory effect on the tonic activity of VTA DA neurons. Futhermore, this electrical stimulation of the vSUB evoked both excitatory and inhibitory responses of VTA DA neurons. Moreover, we have previously shown that the bed nucleus of the stria terminalis (BNST) stimulation increases VTA dopamine neuron activity and that the BNST receives excitatory inputs from the vSUB. Interestingly, we demonstrated that DBS protocols in the vSUB induced *in vivo* NMDA long-term potentiation (LTP) in BNST neurons. By infusing locally in the BNST glutamatergic receptors antagonist, we showed that the BNST relays the excitatory drive between the vSUB and VTA. All together, these results strongly suggest that the NMDA long-term potentiation that occurs in the BNST induced by the DBS protocols in the vSUB is crucial for the hyperactivity of VTA DA neurons.

Disclosures: **C. Glangetas:** None. **G.R. Fois:** None. **M. Jalabert:** None. **C. Herry:** None. **M. Diana:** None. **S. Caillé:** None. **F. Georges:** None.

Poster

599. Synaptic Transmission: Modulation III

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Program#/Poster: 599.03/D18

Topic: B.07. Synaptic Transmission

Support: ANR-2011-tVTA-VTA

ANR-2012-DARLING

Title: *In vivo* homeostatic plasticity in BNST neurons

Authors: *F. E. GEORGES[1], G. FOIS[2], M. JALABERT[3], D. GIRARD[1], M. DIANA[2], C. GLANGETAS[1];
[1]IINS-CNRS 5297, BORDEAUX, France; [2]Dept of Chem. & Pharm., Sassari, Italy; [3]INMED, Marseille, France

Abstracts: The anteroposterior part of the bed nucleus of the stria terminalis (BNST) plays a critical role in anxiety and learning reward related behaviors. It is clearly established that the BNST projects to the paraventricular nucleus of the hypothalamus, important regulatory center of the HPA axis but also to the ventral tegmental area, key structure in motivational rewarding related behaviors. The BNST receives massive glutamatergic afferents from the ventral subiculum (vSUB) and the infralimbic cortex (ILCx) which are strategic areas that integrate/encode contextual and emotional informations. We have previously demonstrated that ILCx and the vSUB exert a strong excitatory influence on BNST neurons. However, it is unknown how synaptic transmission and plasticity evoked by the stimulation of the vSUB will affect the responses of the ILCx inputs at the level of individual BNST neurons. We first combine anatomical and *in vivo* electrophysiological approaches to demonstrate that the majority of the BNST neurons were under monosynaptic excitatory influence of both, ILCx and vSUB inputs. Using Delta-bursts stimulation (DBS) protocols in the vSUB, we were able to induce *in vivo* NMDA long-term potentiation (LTP) at vSUB-BNST synapses and long-term depression (LTD) at ILCx-BNST synapses. In conclusion, we demonstrated that an individual BNST neuron: 1) integrate both informations coming from ILCx and vSUB and 2) displays homeostatic plasticity after DBS. Together, these data show that synaptic scaling occur *in vivo* in the BNST suggesting that the BNST is uniquely positioned to process both emotional- and context-dependent informations arising from the ILCx and the vSUB.

Disclosures: **F.E. Georges:** None. **G. Fois:** None. **M. Jalabert:** None. **D. Girard:** None. **M. Diana:** None. **C. Glangetas:** None.

Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: Engineering and Physical Sciences Research Council (EPSRC) studentship from the Complexity Science Doctoral Training Centre, University of Warwick.

Title: Modelling the spatiotemporal dynamics of adenosine in neural tissue

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

Abstracts: The neuromodulator adenosine is involved in both physiological and pathological activity, such as sleep, epilepsy and stroke. However, the complex processes underlying the release, transport and clearance of adenosine from tissue, and their interactions, are poorly quantified. Using parameters taken from an extensive search of the literature, we present the first detailed model of the spatiotemporal dynamics of adenosine in tissue. The mechanisms included in our model are: diffusion and breakdown by adenosine deaminase (ADA) in the extracellular space, transport between the extracellular and intracellular space by equilibrative nucleoside transporters (ENT), neuronal breakdown by ADA and glial metabolism, involving both 5'-nucleotidase and adenosine kinase. The literature search allowed us to estimate the range of the available parameters as well as identifying aspects of the purine transport and metabolism that have yet to be experimentally quantified. These unknown parameters comprise: the kinetics of adenylate kinase and the affinity of ENTs for inosine. A hierarchy of models is considered, starting with extracellular metabolism only, then including neurons, or glia, or both. Analysis of these models determines that the concentration following local adenosine release is primarily determined by diffusion through the tortuous extracellular space and that parameters suggest that neuronal uptake should be the dominant clearance mechanism. We apply the model to the rat neocortex and compare our model to the experimentally measured response of purine biosensors, which measure both adenosine and its breakdown products inosine and hypoxanthine. The model predicts the range of influence of an isolated adenosine source, characterised as the maximum distance where the amplitude of an EPSP would be halved due to the activation of A1 receptors, the most abundant class of adenosine receptors in the neocortex. The greatest influences on this

effective range are: the tortuous diffusion coefficient, extracellular volume fraction and the basal adenosine tone.

Disclosures: A. Newton: None. **M.J. Wall:** None. **M.J.E. Richardson:** None.

Poster

599. Synaptic Transmission: Modulation III

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Program#/Poster: 599.05/D20

Topic: B.07. Synaptic Transmission

Support: NSF Grant 0951549

Title: Rapid PACAP-induced plasticity at autonomic synapses requires nAChR-dependent NOS1 activation and AKAP-mediated PKA targeting

Authors: *J. F. MARGIOTTA[1], S. S. JAYAKAR[2], P. C. PUGH[3], E. R. STARR[1]; [1]Neurosciences, UT Col. of Med. & Life Sci., TOLEDO, OH; [2]Neurobio., Harvard Med. Sch., Boston, MA; [3]Psychiatry & Behav Neurobio., Univ. of Alabama, Birmingham, AL

Abstracts: Pituitary adenylate cyclase activating polypeptide (PACAP) engages a selective G-protein coupled receptor (PAC_1R) to enhance quantal acetylcholine (ACh) release from ciliary ganglion neuron terminals within minutes *via* neuronal nitric oxide synthase (NOS1) and cyclic AMP/protein kinase A (PKA) dependent processes. Here, we examined how PACAP stimulates NOS1-dependent NO production and targets resultant PKA-dependent outcomes to synapses. Scavenging extracellular NO blocked PACAP-induced plasticity supporting a retrograde (post- to presynaptic) NO action on transmitter release. Live-cell NO imaging revealed that PACAP stimulates NO production by mechanisms requiring NOS1, PKA and Ca^{2+} influx. Ca^{2+}-permeable nicotinic ACh receptors composed of α7 subunits (α7-nAChRs) that are potentiated by PKA-dependent PACAP/PAC_1R signaling, are required for PACAP-induced NOS1-dependent NO production and synaptic plasticity since both outcomes were blocked following their selective inhibition. Co-precipitation studies revealed that NOS1 associates with α7-nAChRs, many of which are perisynaptic, as well as with heteromeric α3*-nAChRs that generate the bulk of synaptic activity. Such an arrangement would facilitate NO production at perisynaptic and adjacent postsynaptic sites to regulate focal ACh release from juxtaposed presynaptic terminals. These PKA-dependent outcomes of PACAP/PAC_1R signaling are localized to synaptic membrane components by PKA anchoring proteins (AKAPs). PKA regulatory-subunit

overlay assays identified five AKAPs in CG lysates, including the prominent neuronal subtype, AKAP5 (AKAP79/150). Moreover PACAP-induced synaptic plasticity was selectively blocked by inhibiting PKA regulatory-subunit binding to AKAPs. Taken together, our findings indicate that PACAP/PAC_1R signaling rapidly coordinates nAChR, NOS1 and AKAP activities to induce targeted, retrograde plasticity at autonomic synapses. Such coordination has broad relevance for understanding the control of autonomic synapses and consequent visceral functions.

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Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: NIH Grant 5R01MH092926-04

Title: Direct evidence of altered neuronal excitability due to electric field stimulation

Authors: ***B. LAFON**, A. RAHMAN, M. BIKSON, L. C. PARRA;
City Col. of New York, New York, NY

Abstracts: Transcranial direct current stimulation (tDCS) is gaining importance both in clinical as well as basic neuroscience research. tDCS generates weak electric fields in the brain of ~1 mV/mm per mA of current applied. This causes an incremental passive membrane polarization in pyramidal cells of up to 0.1mV. This incremental polarization has been shown to acutely affect spike timing, increase firing rate, and synaptic efficacy. Moreover, plastic changes elicited by electric fields have been assessed in terms of synaptic efficacy. However, neuronal excitability for a constant synaptic input has not been concurrently documented yet in-vitro. We hypothesized that due to membrane polarization the synaptic input required for firing will be affected, therefore modulating the neuronal input-output (I/O) function. Simultaneous recording of the fEPSP (synaptic input) and the population spike (neuronal output) were performed in the CA1 region of rat hippocampal slices. Measurements were taken in three conditions: control, positive and negative fields. Our results indicate that DC stimulation which depolarizes the soma advances and amplifies neuronal firing for a given synaptic input. The latter is seen as a leftward shift of the threshold of the I/O curve. We built computational models to test two alternative hypotheses: 1) single cell effect - increased output results from an increased drive within a cell

due to the potential gradient induced by the electric fields, 2) network effect - increase output results from a differential effect of fields on the timing of excitatory and inhibitory neurons. Irrespective of the precise mechanism, the increased spiking activity of a neuronal population observed here can have important implications for the effect of fields during acute stimulation as well as plastic mechanisms.

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Poster

599. Synaptic Transmission: Modulation III

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Title: Robust and novel modulation of synaptic excitability in the Hippocampus via endogenous free iron

Authors: ***R. S. WHITE**, A. BHATTACHARYA, Y. CHEN, G. CARLSON, S. KIM; Psychiatry, Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstracts: We identified a novel signaling cascade in neurons whereby stimulation of glutamate-NMDA receptors activates Dexras1, inducing iron movement into the neurons via an iron channel, DMT1. This led us to demonstrate that Dexras1-mediated iron influx plays a crucial role in NMDA excitotoxicity. This suggested that free iron may also have a role in normal neurophysiological processes. We investigated whether chelating iron with membrane permeable iron specific chelator, pyridoxal isonicotinoyl hydrazone (PIH), affects hippocampal activity and excitability. PIH application induced an increase in the frequency of spontaneous events (Control=0.9 +/- 0.09; 100μM PIH= 1.6 +/- 0.19Hz) and produced a 47% increase in evoked synaptic excitability following PIH treatment. To investigate if this generated differences in circuit activity we utilized voltage sensitive dye imaging of hippocampal slices and found an increase in both amplitude and tau of decay of the schaffer evoked EPSP following PIH treatment. When NMDA receptor activity is blocked by AP5 this increase in VSDi response and

sEPSCs frequency in PIH is abolished. However when AMPA receptor activity is blocked with DNQX, PIH still causes an increase in evoked VSDi response in CA1 (+35.2% n=5). To identify the localization of free iron, hippocampal slices were imaged using an iron sensitive fluorescent dye Calcien-AM. After PIH application, CA1 had the greatest change in fluorescence showing that chelatable iron was present and at higher concentration than in the dentate gyrus or CA3. To test if the Dexras1/DMT1 pathway was active in mediating this iron dependent synaptic modulation, we investigated the role of dexras1 in the PIH dependent increase in synaptic excitability. Recording and imaging from the hippocampi of dexras1 KO mice showed a complete lack of all PIH-dependent response. Similar results were found in the DMT1 blocker ebselen. The lack of iron in the ACSF bathing the slices indicated that there must be an internal source of iron in the neurons. We found that collapsing the lysosomal proton gradient with NH4Cl significantly reduced free iron, suggesting that lysosomes are releasing iron into the intracellular space causing changes in NMDA mediated excitability. Linking the dexras1/DMT1 pathway to the lysosome, immunoblotting different subcellular fractions for our showed Dexras1 in the lysosomal fraction along with the lysosomal protein, LAMP-2, along with ACBD3, a scaffolding protein, which forms a complex of Dexras1 and the DMT1 iron channel. These data reveal a novel mechanism that demonstrates an active role of iron in modulating synaptic excitability.

Disclosures: R.S. White: None. **A. Bhattacharya:** None. **Y. Chen:** None. **G. Carlson:** None. **S. Kim:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.08/D23

Topic: B.07. Synaptic Transmission

Title: Waves of noradrenaline and corticosterone after stress differentially affect glutamatergic transmission in the mouse basolateral amygdala

Authors: *H. KARST, M. JOËLS;
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Abstracts: stress-related information and restoring homeostasis. The amygdala, including the basolateral amygdala (BLA), is the main brain structure for emotional and fear regulation. It was

shown1 that both After stress, the brain is exposed to waves of stress hormones which are important for encoding of Isoproterenol (ISO), a β-adrenoceptor agonist, and corticosterone (Cort) are necessary to strengthen the consolidation of fear-related information after stress. In the BLA, mineralocorticoid (MR) and glucocorticoid receptors (GR) are expressed. MRs and GRs generally affect properties of neurons via genomic pathways, but more recently rapid non-genomic actions through membrane-located MRs and GRs were demonstrated2. Application of 100 nM corticosterone was reported to cause an increase of the BLA mEPSC frequency, reflecting an increased release probability of glutamate. The mEPSC amplitude was not affected. Remarkably, a second surge of corticosterone 1-4 hours later resulted in a rapid reduction of the mEPSC frequency. We now show that this metaplastic mode of action requires a delay of ~60 minutes between the first and second corticosterone application. We furthermore show that ISO also rapidly increases BLA glutamatergic transmission. Surprisingly however, this gradually reverses in a decrease in mEPSC frequency, indicating that ISO also affects transmission via a genomic pathway. Co-application of ISO and Cort has a mixed synergistic and additional effect on the mEPSC frequency. Under normal physiological circumstances the brain first experiences a wave of NA after a stressful event, followed by a gradual increase in the Cort level. When Cort was administered after ISO, we found that Cort decreases the mEPSC frequency. Our final step was to mimic the physiological situation by exposing brain slices to consecutive waves of ISO and Cort. We mimicked an arousing event, a mildly stressful and a severely stressful event, by varying the ISO and Cort concentrations. We observed that low to moderate concetrations cause a brief increase in mEPSC frequency followed by a prolonged suppression. With very high concentrations -mimicking severe stress- BLA glutamatergic transmission remained at a very high level for hours. The latter may relate to the inappropriately strong encoding of emotional aspects after traumatic events. 1 Roozendaal et al. (2006) Proc Natl Acad Sci USA 103, 6741-6 2 Karst et al. (2010) Proc Natl Acad Sci USA 107, 14449-54

Disclosures: **H. Karst:** None. **M. Joëls:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.09/D24

Topic: B.07. Synaptic Transmission

Support: NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

NIH Grant DA017392

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Title: Compartment-specific modulation of GABAergic inputs by TRPV1 channels in the dentate gyrus

Authors: *A. E. CHAVEZ[1], V. M. HERNANDEZ[2], A. RODENAS-RUANO[1], S. CHAN[2], P. E. CASTILLO[1];
[1]Dominick P Purpura Dept Neurosci., Albert Einstein Col. Medici, BRONX, NY; [2]Dept. of Physiology, Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL

Abstracts: The transient receptor potential TRPV1 or vanilloid receptor is a nonselective cation channel mainly found in nociceptive neurons of the peripheral nervous system. TRPV1 has also been reported in different brain regions, where its activation depresses excitatory synaptic transmission. However, whether TRPV1 could regulate inhibitory synapses in the brain remains unknown. Here, using a combination of pharmacology, electrophysiology and *in vivo* knockdown strategies, we show that activation of TRPV1 channels by capsaicin (2 μM) or by the endocannabinoid anandamide (30 μM) depresses somatic but not dendritic inhibitory transmission in both rat and mouse dentate gyrus. These effects on somatic GABAergic transmission were completely abolished in TRPV1 knockout mice and were also eliminated by two different TRPV1 shRNAs expressed in dentate granule cells (DGCs), strongly supporting a functional role for TRPV1 in modulating GABAergic synaptic function in the dentate gyrus. Moreover, the TRPV1-mediated depression of inhibition was not associated with changes in paired-pulse ratio and coefficient of variation, suggesting that TRPV1 does not affect GABA release but modulates postsynaptic function. To directly test this possibility, we monitored GABAergic responses elicited by brief puffs of GABA, a manipulation that shortcuts transmitter release. GABA-evoked responses elicited in the soma (i.e. stratum granulosum moleculare), but not in dendrites (i.e. middle third of the molecular layer) of DGCs, were significantly depressed by capsaicin (2 μM). In addition, we found that TRPV1-mediated effects require postsynaptic Ca2+ rise, activation of calcineurin, and are likely mediated by a clathrin-dependent internalization of GABAA receptors. Altogether our findings reveal a novel form of compartment-specific regulation of synaptic transmission, whereby activation of TRPV1 channels depresses inhibitory synaptic inputs presumably by targeting postsynaptic GABAA receptor number. Such specific regulation of GABAergic transmission along the somato-dendritic axis may have important functional consequences in controlling DGC output.

Disclosures: A.E. Chavez: None. **V.M. Hernandez:** None. **A. Rodenas-Ruano:** None. **S. Chan:** None. **P.E. Castillo:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.10/D25

Topic: B.07. Synaptic Transmission

Support: HSFC

CFI

SHRF

Title: Protein phosphatase 2A mediates adenosine A1 receptor-induced GluA1 AMPA receptor internalization and persistent synaptic depression in rat hippocampus

Authors: *F. S. CAYABYAB, J. STOCKWELL, Z. CHEN, Z. MING, A. GARGOUM; Dept. of Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstracts: The adenosine A1 receptor (A1R) plays an essential role in synaptic depression induced by increased extracellular adenosine surges seen in neuronal insults such as hypoxia or ischemia. We recently showed that A1 receptor-induced synaptic depression was mediated postsynaptically by inducing GluA1- and GluA2-containing AMPA receptor internalization. The GluA1 AMPA receptor subunit contains two major regulatory C-terminal serine phosphorylation sites, Ser831 and Ser845, which are known to be involved in GluA1 surface expression and synaptic trafficking. We have shown that dephosphorylation of these two regulatory sites accompanies GluA1 internalization, and that it involves A1R-induced protein phosphatase activity through protein phosphatase 2A (PP2A). Previous data shows pharmacological inhibition of PP2A reduces APSD and GluA1 internalization, but the drugs used are not easily translated into an *in vivo* stroke model. We further explored this A1 receptor - PP2A - GluA1 signaling complex using a Tat peptide interference strategy. We designed a new peptide, called Tat-YD peptide, which is expected to prevent PP2A activation. We hypothesized that GluA1 internalization would be inhibited after Tat-YD treatment. Using fEPSP electrophysiology experiments on rat hippocampal slices, we found that Tat-YD peptide perfusion onto naive hippocampal slices increased fEPSP slope by approximately 20%, indicating that PP2A inhibition increases the surface levels of GluA1. Accordingly, our biochemistry data showed that Tat-YD incubation increased surface GluA1 in hippocampal slices. Adenosine A1 receptor activation by a selective agonist CPA induced both AMPA receptor internalization and adenosine-induced persistent synaptic depression (APSD), which are both reduced by pre-incubation of slices in Tat-YD peptide. The levels of phosphorylation of GluA1-Ser831 and

GluA1-Ser845 were increased by Tat-YD alone, and were not reduced below baseline levels after prolonged CPA treatment. Together, these results indicate that adenosine A1 receptors recruit PP2A to downregulate AMPA receptor trafficking, thereby enhancing APSD. Targeting PP2A to prevent excessive APSD represents a novel approach to promote neuroprotection during cerebral ischemic damage.

Disclosures: F.S. Cayabyab: None. **J. Stockwell:** None. **Z. Chen:** None. **Z. Ming:** None. **A. Gargoum:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.11/D26

Topic: B.07. Synaptic Transmission

Support: ANR blanc tVTA VTA 2011

Title: Species differences in the BNST-VTA pathway: Comparative anatomical and electrophysiological analysis

Authors: *J. KAUFLING[1], D. GIRARD[1], M. MAITRE[2], F. GEORGES[1];
[1]Dept. of Neurosciences, Interdisciplinary Inst. for Neurosci., Bordeaux, France; [2]Ctr. de Recherche Inserm U862, Borbeaux, France

Abstracts: The Bed Nucleus of the Stria Terminalis (BNST) is a forebrain region mostly GABAergic (GABA) with a significant component of glutamatergic (GLU) neurons. Recent optogenetic findings from experiments performed in mice confirm the main conclusion of our original work performed in rats: the BNST exert an excitatory influence onto VTA dopaminergic (DA) neurons activity. However, the neuronal circuit implicated in this excitatory influence remains a matter of debate. Our hypothesis is to test if there are any differences between mice and rats in evoked responses of VTA DA neurons induce by stimulation of monosynaptic inputs of the BNST. Using a combination of *in vivo* electrophysiological, neuroanatomical tracing and laser capture approaches we reevaluate in rats and mice the BNST influence on VTA DA neurons activity. First, we characterize in rat the molecular phenotype of the BNST neurons projecting to the VTA. We found that this projection is multiple and complex in terms of neurotransmitter: expression of GABA markers and GLU markers (vGlut2 and vGlut3). Then VTA injections of a classic retrograde tracer, the B sub-unit of the choleric toxin (CTB) reveal a

stronger BNST VTA projection in mice than in rats. Finally, electrical stimulations of the BNST during VTA DA neurons recording in rats and mice demonstrate that an excitatory monosynaptic BNST VTA connection is more important in rats than in mice. All this data, illustrate anatomically but also functionally, a significant difference between rats and mice in the BNST VTA DA pathway. More generally, this study correlating previous research highlights the awareness of the animal model use for the interpretation and the generalization of research data.

Disclosures: J. Kaufling: None. **D. Girard:** None. **M. Maitre:** None. **F. Georges:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.12/D27

Topic: B.07. Synaptic Transmission

Support: NIH R01 MH56838-16

Title: Platelet activating factor enhances presynaptic vesicle release

Authors: *J. W. HAMMOND, S.-M. LU, H. A. GELBARD;
Ctr. for Neural Develop. and Dis., Univ. of Rochester, Rochester, NY

Abstracts: Platelet activating factor (PAF) is a potent, inflammatory phospholipid that can also alter synaptic strength. During neuroinflammation, PAF levels are chronically high and PAF directly contributes to neuronal injury as PAF receptor (PAFR) antagonism has been shown to be neuroprotective in mouse models of HIV-1 associated neurocognitive disorders, seizure, trauma, stroke, and multiple sclerosis. Yet, there is much about the mechanisms of PAF signaling at the synapse that we do not understand. We show that the PAF receptor is peri-synaptically localized in mixed cultures of primary hippocampal neurons. Using FM dye or pHluorin tagged synaptophysin to label synaptic vesicle recycling, we show that PAF treatment enhances presynaptic vesicle release. Specifically PAF increases the size of the primed, readily releasable pool (FM dye = 42% increase; pHluorin = 32% increase). PAF also activates previously silent presynaptic terminals. During neuroinflammation, inflammatory leukocytes infiltrate synapses in response to chemotactic stimuli and are capable of secreting PAF in supra-physiologic levels. Thus, our data suggest that PAF-enhanced neurotransmitter release in the presence of physiologic levels of stimulation likely places synapses in a high activity state where they are vulnerable to excitotoxic injury and energy failure.

Disclosures: **J.W. Hammond:** None. **S. Lu:** None. **H.A. Gelbard:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.13/D28

Topic: B.07. Synaptic Transmission

Support: AFRL Section 219 Venture Award

AFOSR LRIR

Title: tDCS current intensity on immediate early gene induction *in vivo*

Authors: ***J. WAGNER**[1,2], R. JANKARD[2];
[1]Wright State Univ., Dayton, OH; [2]Wright Patterson Air Force Base, Fairborn, OH

Abstracts: Transcranial direct current stimulation (tDCS) sends a weak polarizing current across the brain that can modulate human behavior. It is unknown how tDCS modulates the brain and affects behavior, therefore we examined some of the underlying biological processes that could be induced in response to tDCS. In this study, we utilize a rodent tDCS model to discern which gene transcripts are modulated by tDCS. Immediate early genes (IEGs) are induced in response to neuronal activating stimuli, so we chose two IEGs, cFos and zif268, as candidates to assess the effects of tDCS. Our results show that tDCS induces neuronal activation, as indicated by the induction of cFos and zif268. Of interest, the magnitude of IEG induction was dependent upon the current intensity used during stimulation with the highest current intensity yielding the largest IEG induction being observed. These results indicate that the scale of neuronal activation is dependent upon the current intensity utilized during stimulation. Also, the fold change of the IEG's reached its greatest under the stimulation site and decreases as the area become more distal, suggesting a gradient of IEG induction across brain regions. Our study has shown tDCS modulates the biological activity of the brain and that the impact of tDCS on immediate early gene induction is dependent on the current intensity dose. Thus, the induction of immediate early genes by tDCS is a mechanism by which tDCS can affect neuronal activity and may thereby modulate behavior.

Disclosures: **J. Wagner:** None. **R. Jankard:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.14/D29

Topic: B.07. Synaptic Transmission

Support: NSF Grant 0951549

Title: PACAP induces acute and sustained synaptic plasticity by distinct cellular mechanisms

Authors: *E. R. STARR, J. F. MARGIOTTA;
Neurosciences, UT Col. of Med. & Life Sci., Toledo, OH

Abstracts: Neuropeptide generated signals alter synaptic components and properties. Pituitary adenylate cyclase activating polypeptide (PACAP) is found at synapses throughout the nervous system. In autonomic ciliary ganglion (CG) neurons, PACAP engages a selective G-protein coupled receptor (PAC_1R), activating signal cascades that rapidly influence synaptic components. Minutes after PACAP application, PAC_1R signaling enhances cholinergic synaptic output, increasing the frequency and amplitude of spontaneous nAChR-mediated excitatory postsynaptic currents (sEPSCs). We previously found that this acute plasticity results from enhanced ACh release from presynaptic terminals without affecting postsynaptic quantal size, and show at an accompanying poster that it arises from coordinated, membrane-localized activation of neuronal nitric oxide synthase (NOS1), and consequent retrograde NO-enhanced ACh release. In addition to these actions, brief PACAP exposure activates transcription and alters expression of genes relevant to synaptic function. Consistent with these actions, PACAP induces synaptic plasticity that persists long after the brief exposure. Specifically, 2 d after 15 min treatment with PACAP, sEPSC frequencies and amplitudes remain elevated compared to controls. While this sustained plasticity resembles that immediately following acute PACAP exposure, we now report that its correlates reflect different cellular mechanisms. First, while sEPSC frequencies 2 d after 15 min PACAP treatment are similar to those immediately after PACAP exposure, sEPSC amplitudes are increased even further. Second, the sustained changes are accompanied by increased postsynaptic quantal size as determined from elevated miniature nAChR-mediated EPSC (mEPSC) amplitudes, and elevated mEPSC frequencies, neither of which are seen immediately after PACAP exposure. Third, the sustained synaptic plasticity is independent of NOS1 since it is unaffected by blocking NOS1 and subsequent production of NO with L-NAME during the initial 15 min PACAP treatment. These findings indicate that PACAP has short- and long-term consequences that, respectively, produce acute and sustained synaptic plasticity by different mechanisms. Experiments are underway to determine how the sustained

plasticity arises by probing its dependence on processes downstream from local membrane signaling such as PAC_1R trafficking and gene transcription. Since PACAP is widely distributed and can also modulate GABAergic and glutamatergic synaptic transmission, uncovering the basis for its ability to induce sustained plasticity could have broad relevance throughout the nervous system.

Disclosures: E.R. Starr: None. **J.F. Margiotta:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.15/D30

Topic: B.07. Synaptic Transmission

Title: Calcium channel subtypes on glutamatergic presynaptic terminal projecting to rat hippocampal CA3 neurons

Authors: *M.-C. SHIN, K. NONAKA, M. YOSHIMURA, N. AKAIKE;
Kumamoto Hlth. Sci. Univ., Kumamoto City, Kumamoto Pref., Japan

Abstracts: High-voltage-activated (HVA) Ca2+ channels on presynaptic nerve terminals (boutons) are known to play an important role in neurotransmitter release. Ca2+ flows into the presynaptic terminals through the HVA Ca2+channels bind to various Ca2+-binding proteins at presynaptic transmitter release sites which then triggers exocytosis of neurotransmitter. HVA Ca2+ channel subtypes triggering glutamate release from nerve terminals projecting to rat hippocampal CA3 pyramidal neurons were studied. Single CA3 neurons were mechanically isolated with adherent nerve terminals, namely the ‘synaptic bouton preparation’, and spontaneous glutamatergic excitatory synaptic potentials (sEPSCs) and EPSCs evoked by focal electrical stimuli of a single presynaptic glutamatergic bouton (eEPSCs) were recorded using conventional whole-cell patch recordings. In the synaptic bouton preparation, 30μM nifedipine (L-type selective antagonist), 100nM ω-cg-GVIA (N-type selective antagonist), and 100nM ω-Ag-IVA (P/Q-type selective antagonist) reduced the sEPSC frequency, without altering the mean sEPSC amplitude. However, 100nM SNX-482 (R-type selective antagonist) and 300μM efonidipine (T-type selective antagonist) had no significant effect on the frequency and amplitude of glutamatergic sEPSCs. In the single bouton focal stimulation recordings, not only 30μM nifedipine, 100nM ω-cg-GVIA, 100nM ω-Ag-IVA, but also 100nM SNX-482 increased failure rate (Rf) of glutamatergic eEPSCs, without altering the mean eEPSC amplitude. 300μM

efonidipine had no significant effect on the Rf and amplitude of glutamatergic eEPSCs. Overall, our results suggest that the dominant control of glutamate release depends on Ca2+ entry through L-, N-, P/Q- and R-type Ca2+ channels that ubiquitously populate hippocampal CA3 glutamate release sites.

Disclosures: **M. Shin:** None. **K. Nonaka:** None. **M. Yoshimura:** None. **N. Akaike:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.16/D31

Topic: B.07. Synaptic Transmission

Title: Melanin-Concentrating Hormone modulates excitatory transmission at the hippocampal synapses by acting on astrocytes

Authors: *L. LE BARILLIER, M. ROSIER, G. MALLERET, P.-A. SALIN;
CRNL Univ. Claude Bernard, LYON, France

Abstracts: The hypothalamic neuropeptide Melanin Concentrating Hormone (MCH) plays an important role in several homeostatic functions as sleep regulation and enhances memory retention in hippocampus-dependent learning tasks in rodents. As we showed that glutamatergic transmission was impaired in mice lacking MCH-receptor-1 gene, we investigated how MCH could modulate excitatory transmission at Schaffer collaterals-CA1 synapse in mice hippocampal slices. We found that application of MCH induced a depression of the synaptic response. Changes in paired-pulse facilitation ratio as well as in $1/CV^2$ suggested that MCH modulates glutamatergic transmission by acting on neurotransmitter release. MCH induced depression was suppressed by adenosine receptor A1 antagonist and inhibitors of ectonucleotidases which degrades ATP in adenosine in extracellular space. As astrocytes are the major source of ATP in the brain, we hypothesized that MCH modulating effects on glutamatergic transmission could be relayed by astrocytes. It has been shown that P2Y1 and mGlur5 play a critical role in ATP release by astrocytes. In agreement with our hypothesis, the selective P2Y1 and mGluR5 antagonists inhibited the MCH induced depression. Moreover, in presence fluoroacetate which selectively inactivates astrocytes, the MCH induced depression was totally blocked. All this results suggest that MCH decreases hippocampal glutamatergic transmission by activating astrocytes which triggers release of ATP. Since recent studies pointed out that astrocytes are

essential to synaptic plasticity, our work suggests a new cellular mechanism by which MCH could facilitate memory consolidation.

Disclosures: **L. Le Barillier:** None. **M. Rosier:** None. **G. Malleret:** None. **P. Salin:** None.

Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: RFBR Grant 13-04-00413-a

Title: Presynaptic adenosine A2A-receptor mediated pathway acts as a counteragent of calcineurin-based downregulation of L-type calcium channel activity in mouse neuromuscular junctions

Authors: ***E. TARASOVA**, A. GAYDUKOV, O. BALEZINA;
Dept. of Human and Animal Physiol., Fac. of Biology, Moscow State Lomonosov Univers, Moscow, Russian Federation

Abstracts: It is well known that L-type voltage-gated calcium channels are present in mouse neuromuscular junctions (NMJs) but do not contribute to acetylcholine (ACh) release. In our previous work we demonstrated that in mouse NMJs the activity of L-type calcium channels is hindered by calcineurin which acts presynaptically as a downregulator of evoked neurotransmitter release. Calcineurin inhibitor cyclosporine A (CsA) increased evoked synaptic transmission by 30%. The goal of our present study was to reveal a possible pathway of L-type channel upregulation despite of the presence of functionally active calcineurin. In central neurons the activation of adenosine A2A-receptors (A2AR) leads to phosphorylation of L-type calcium channels by PKA and thereby enhances calcium influx. Presynaptic adenosine A2AR are also present in NMJs, but their involvement in secretion regulation is rather under question. Therefore we decided to focus on the interaction of calcineurin and the molecular cascade triggered by A2AR. Experiments were conducted on «dissected» mouse diaphragm preparations using standard microelectrode technique of biopotential registration. We registered both spontaneous and evoked activity of NMJs in form of miniature end plate potentials (mEPP) and end plate potentials (EPP) respectively. PKA inhibitor H-89 (1μM) had no effect on evoked synaptic transmission. Nevertheless, preliminary H-89 application fully prevented the increase of

EPP quantal content caused by following CsA (1 μM) treatment. So, PKA seems to be involved in L-type channel regulation reciprocally to calcineurin. Application of A2AR blocker ZM241385 (1 nM) did not alter neither spontaneous nor evoked ACh secretion. But in the presence of ZM241385(1 nM) CsA (1 μM) failed to enhance evoked transmission, similarly to the effects of H-89(1 μM) . Presynaptic A2AR agonist CGS-21680(1 μM) produced a rise of EPP quantal content by nearly 20%. The data obtained suggests that the cascade of molecular events starting from A2AR activation and calcineurin act antagonistically and have the same target - L-type calcium channels. Interestingly, when L-type calcium channels were already activated by calcineurin inhibition and transmission was facilitated, applying CGS-21680 (1 μM) did not lead to any additional changes in the EPP quantal content. Thus, we showed that the presynaptic pathway involving A2A-receptors and PKA is able to counteract with calcineurin in its influence on L-type calcium channels.

Disclosures: **E. Tarasova:** None. **A. Gaydukov:** None. **O. Balezina:** None.

Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: NIH Grant MH609197

NIH Grant T32NS007101

NIH Grant T32GM065823-11

Title: Regulation of spontaneous synaptic transmission by extracellular calcium at excitatory synapses onto hippocampal CA1 pyramidal cells

Authors: W. E. BABIEC[1], R. GUGLIETTA[2], S. A. JAMI[3], *T. J. O'DELL[1];
[1]Dept Physiol, David Geffen Sch. Med. UCLA, LOS ANGELES, CA; [2]Interdepartmental Ph.D. Program for Neurosci. at UCLA, [3]Molecular, Cellular, and Integrative Physiol. Ph.D. Program, UCLA, Los Angeles, CA

Abstracts: Schaffer collateral (SC) fiber synapses onto pyramidal cells (PCs) in the CA1 region of the dorsal and ventral hippocampus exhibit striking differences in both short-term and long-term plasticity. Importantly, evoked and spontaneous synaptic transmission may involve distinct

molecular mechanisms, pools of presynaptic vesicles, postsynaptic receptors. Thus, we examined evoked and spontaneous transmission at excitatory synapses onto CA1 PCs in the dorsal and ventral hippocampus to determine whether the properties of spontaneous transmitter release also differ along the septotemporal axis of the hippocampus. Paired-pulse facilitation of SC fEPSPs was significantly smaller and the block of NMDA receptor-mediated excitatory posesynaptic currents (EPSCs) by the use-dependent blocker MK-801 was significantly faster in ventral PCs, suggesting that the probability of transmitter release is higher at SC fiber synapses in the ventral hippocampus. In contrast, the frequency and amplitude of spontaneous miniature EPSCs (mEPSCs) were identical in dorsal and ventral PCs, suggesting that spontaneous excitatory transmission is similar in these two regions. Blocking voltage-gated calcium channels (VGCCs) with Cd^{2+} had no effect on mEPSC frequency in dorsal and ventral PCs, suggesting that spontaneous release in both regions is not due to stochastic openings of presynaptic VGCCs. However, increasing extracellular Ca^{2+} (from 2 to 4 mM) significantly enhanced mEPSC frequency in dorsal PCs ($p < 0.001$) but had no effect on mEPSC frequency in ventral PCs ($p = 0.996$). Elevating extracellular Mg^{2+} (from 2 to 4 mM) also enhanced mEPSC frequency in dorsal PCs ($p < 0.05$) with no effect on mEPSC frequency in ventral PCs ($p = 0.52$). Both Ca^{2+} and Mg^{2+} are agonists of the calcium-sensing receptor (CaSR), a G protein coupled receptor that regulates spontaneous transmission at synapses onto cortical neurons (PMC3097128). Consistent with a role for the CaSR in regulating spontaneous release, bath application of the CaSR agonist spermine induced a 5-fold increase in mEPSC frequency in dorsal PCs but increased mEPSC frequency only 2-fold in ventral PCs. Together our results suggest that the CaSR may have a key role in regulating spontaneous synaptic transmission. Moreover, alterations in CaSR expression and/or signaling may provide a mechanism that maintains a constant level of spontaneous transmitter release at excitatory synapses onto CA1 PCs along the septotemporal axis of the hippocampus at physiological levels of Ca^{2+} and Mg^{2+} despite different probabilities of evoked transmitter release at these synapses.

Disclosures: **W.E. Babiec:** None. **R. Guglietta:** None. **S.A. Jami:** None. **T.J. O'Dell:** None.

Poster

599. Synaptic Transmission: Modulation III

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.19/D34

Topic: B.07. Synaptic Transmission

Support: MIUR,Grant 2009R7WCZS_003 to MM

Title: *In vitro* exposure to nicotinic agonists modulates the function of NMDA receptors present on glutamatergic and GABAergic nerve endings in rat nucleus accumbens

Authors: ***M. MARCHI**[1], S. ZAPPETTINI[2], M. GRILLI[1], G. OLIVERO[1], J. CHEN[1], C. PADOLECCHIA[1], A. PITTALUGA[1];
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Abstracts: We provide functional evidence supporting the presence on rat Nucleus Accumbens (NAc) glutamatergic terminals of N-methyl-D-aspartic acid (NMDA) receptors co-localized with nicotinic acetylcholine receptors (nAChRs). We have previously shown that brief pretreatment *in vitro* with nicotine for few minutes decreased the NMDA-induced dopamine release from NAc nerve terminals, indicating that the recruitment of nAChRs dynamically and negatively regulates NMDA receptors through the selective internalization of glutamate N2B-NMDA receptors. This reduction was completely counteracted when dopaminergic synaptosomes were pretreated with nicotine plus mecamylamine or in presence of the selective antagonist DHβE indicating that the changes of the NMDA-dependent dopamine release reported was dependent to the activation of a β2* nAChR subtype. Conversely we here show that the *in vitro* short-term pre-exposure of glutamatergic synaptosomes to α7 nAChR agonists caused a significant potentiation of the 100 μM NMDA-evoked [3H]D-Aspartate overflow possibly throughout the selective externalization of glutamate N2A-NMDA receptors . The brief pretreatment *in vitro* with β2* nAChR agonists did not modify the NMDA-evoked [3H]D-Aspartate overflow. The pre-exposure of GABAergic synaptosomes to α7 nAChR or β2* nAChR agonists did not modify the NMDA-evoked [3H]GABA overflow. Our result show that at the nerve terminal level, nicotinic agents may not only induce neurotransmitter release but, throughout the interaction with other co-existing receptors may also exert functional modulatory role on the functions of co-localized receptors. These mechanisms of adaptation should be relevant to understand the interplay between nAChR and NMDAR in the processes of neuronal plasticity as well as in the mechanisms of learning and memory

Disclosures: **M. Marchi:** None. **M. Grilli:** None. **G. Olivero:** None. **J. Chen:** None. **C. Padolecchia:** None. **A. Pittaluga:** None. **S. Zappettini:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.20/D35

Topic: B.07. Synaptic Transmission

Support: KAKENHI 26350498

KAKENHI 23500516

Health Labour Sciences Research Grant

Title: Fetal application of HDAC inhibitors facilitates the elongation of Purkinje cell dendrites and the network formation in rat cerebellar cortex

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Abstracts: Valproate (VPA), the popular anticonvulsant and mood stabilizer, is known as an inducer of autism. It has many kinds of physiological properties, including the inhibition of histone deacetylase (HDAC). Recently we reported VPA application to rat fetus caused developmental changes in cerebellar cortex. In treated cerebellum, the dendrites of Purkinje cells were elongated earlier than in vehicles. Using the enzyme-linked photo assay device, it was observed that some neurotransmitter releases were changed. However it was unclear whether theses effects derived from HDAC inhibition or not. In this study, we report the effects of two other HDAC inhibitors on cerebellar development and compare them with the effect of VPA. The HDACs are classified into three main groups, Class I to III. VPA is possible to inhibit Class I HDACs. Suberoylanilide hydroxamic acid, SAHA, is major anticancer drug, and possible to inhibit Class I and II. Trichostatin A, TSA, is also Class I inhibitor. VPA application to embryonic day 16 p.o. increased GABA release even from early developing periods, and changed its spatial pattern of release. Furthermore, purinergic stimulation of the inhibitory input to Purkinje cells primarily mediated by activation of P_2X receptors, and the inhibitor of ATP-degrading enzymes suggested endogenous ATP would be released 2 weeks later after birth in developing cerebellar cortex. Recently, we developed a new ATP imaging system with ATP requiring redox enzyme, and observed distribution and transition of ATP release in the developing cerebellar cortex. ATP was released to 100 μM glutamate stimulation and observed in the lower molecular layer and granular layer. The ATP release was increased drastically at postnatal day 10 (P10), and decreased gradually to P14. Both VPA and SAHA facilitated these ATP release earlier than vehicle. Purkinje cells in the VPA-applied rat elongated their dendrites all over the molecular layer even in P12. SAHA-applied rat also elongated the dendrites of Purkinje cells earlier, however, the effects was less than VPA-applied ones. The effects of TSA application were observable but slight. These three HDAC inhibitors were categorized as same class, but have different organic structures. VPA belongs to the aliphatic acid compounds, while SAHA and TSA belong to hydroxamic acids. Their physiological spectrum would be different,

however, all of them promoted the corresponding neuronal differentiation. We suggest that some factors induced by the function of HDAC inhibitors would be conditioned the developmental schedule of Purkinje cells.

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Poster

599. Synaptic Transmission: Modulation III

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Program#/Poster: 599.21/D36

Topic: B.07. Synaptic Transmission

Support: Swedish Research Council Grant 13482

Title: Regulation of histone H3 phosphorylation at K27me3S28 in response to amphetamine and haloperidol

Authors: ***G. FISONE**[1], A. BONITO-OLIVA[1], E. SÖDERSTEN[1], X. HU[1], G. SPIGOLON[1], J. CABOCHE[2], K. HANSEN[3];
[1]Karolinska Inst., Stockholm, Sweden; [2]Univ. Pierre et Marie Curie, Paris, France; [3]BRIC, Copenhagen, Denmark

Abstracts: Psychostimulant and antipsychotic drugs act on striatal GABAergic medium spiny neurons (MSNs), promoting adenylyl cyclase activity and cAMP/PKA signaling, and leading to phosphorylation of the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32). Psychostimulant drugs (e.g. amphetamine) mainly target the striatonigral MSNs of the direct pathway, expressing dopamine D1 receptors. In contrast, antipsychotic drugs (e.g. haloperidol) preferentially bind to dopamine D2 receptors, expressed in the striatopallidal MSNs of the indirect pathway. Administration of these drugs is associated to chromatin modifications, which ultimately lead to changes in gene transcriptions implicated in synaptic plasticity and long-term behavioral responses. Both amphetamine and haloperidol have been shown to increase histone H3 phosphorylation at Ser10, whereas nothing is known about their effects on Ser28. Recent evidence indicates that histone H3 phosphorylation at Ser28 (H3S28p) in the context of K27 trimethylation (H3K27me3) leads to displacement of gene repressor complexes containing Polycomb group proteins, followed by transcriptional activation. Here, we investigated changes in H3K27me3S28 phosphorylation using an antibody recognizing the occurrence of the two

concomitant modifications on histone H3. We found that amphetamine and haloperidol increase H3K27me3S28p levels specifically in the MSNs of the direct and indirect pathway, respectively, and that these effects depend on DARPP-32. Moreover, we show that the H3K27me3S28p increase induced by amphetamine requires the mitogen-and stress-activated kinase 1 (MSK1). In contrast, MSK1 does not appear to be necessary for haloperidol-mediated H3K27me3 phosphorylation. These results unveil a novel mechanism potentially implicated in the ability of psychostimulants and antipsychotics to modify gene expression in distinct neuronal populations.

Disclosures: **G. Fisone:** None. **A. Bonito-Oliva:** None. **E. Södersten:** None. **X. Hu:** None. **G. Spigolon:** None. **J. Caboche:** None. **K. Hansen:** None.

Poster

599. Synaptic Transmission: Modulation III

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Program#/Poster: 599.22/D37

Topic: B.07. Synaptic Transmission

Support: NIH Grant DA025143

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Title: Distinct populations of synaptic vesicles in dopamine neurons

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Abstracts: Dopamine (DA) neurons in midbrain are essential for motor control, reward learning and cognition. However, it became clear that DA is not the only neurotransmitter release from axonal terminals of DA neurons. Some of those neurons reportedly co-release glutamate (Glu) and/or GABA alongside DA. First, vesicular Glu transporter 2 (VGluT2) has been found in those neurons and vesicular monoamine transporter (VMAT) reportedly transports GABA into Synaptic vesicles (SV). Second, EPSCs and IPSCs directly triggered by the activation of DA terminals have been observed in neurons having monosynaptic contact with DA neurons. Third,

conditional knockout of VGluT2 only in DA neurons lead to altered locomotor response and enhanced reward learning. Given DA neurons ability to release different neurotransmitters, it becomes important to address if their release is executed distinctly or not and how so. One way to achieve differentiated control of release is spatial segregation, i.e. release from different synapses or different SV. Existing evidence suggested that the former is unlikely as the synaptic location and kinetics of the co-release were quite similar. The latter is also unlikely for GABA given VMAT co-transports GABA and DA into the same SV. However, the limitations of optical or electronic microscopy make it hard to separate different vesicular transporters and same SV with different contents. Using single vesicle tracking enabled by quantum dots (Qdots), we observed some intriguing features of SV in DA neurons. First, there was a subpopulation of Qdot-labeled vesicles located close to but not within the presynaptic boutons marked by FM dyes. Second, those vesicles exhibited an activity-triggered motion towards presynaptic terminals prior to release. Third, the probability and kinetics of release for those vesicles resembled reserve pool vesicles. Furthermore, we utilized physiological and pharmacological interventions to probe the mechanisms governing the traffic of this sub-population of vesicles. We observed that their mobility was associated with stimulation strength, basal calcium concentration and actin but not microtubule. Hypothesizing that this subpopulation of vesicles may be associated with Glu or GABA transmission from DA neurons, we have been performing super-resolution imaging to investigate if there were distinct vesicular transporters and neurotransmitters in those vesicles and how that correlated with the localization of those vesicles. This line of study will be beneficial to dissect out the molecular regulators that contribute to the diversity of synaptic transmission unique to DA neurons in CNS.

Disclosures: **H. Gu:** None. **L. Iacovitti:** None. **Q. Zhang:** None.

Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: NIH Grant AG041360

Title: Estrogen treatment offsets IPSC frequency reduction and calcium buffering elevation in basal forebrain neurons of reproductively senescent F344 female rats

Authors: *D. A. MURCHISON, A. FINCHER, S. BAKE, D. W. DUBOIS, J. C. DAMBORSKY, W. H. GRIFFITH;
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Abstracts: Despite evidence of the beneficial effects of estrogens in animal models, outcomes of estrogen treatments in aged humans have been equivocal. Our lab has been investigating neuronal estrogenic signaling in a rat model of reproductive aging, with the goal of identifying mechanisms that could be targeted therapeutically. We examined the properties of spontaneous inhibitory post-synaptic currents (sIPSCs), cellular Ca2+ buffering and mRNA expression in acutely dissociated neurons from the medial septum/diagonal band of F344 rats in the following age groups: young (2-6 mo) males and females (diestrus), aged males (20-21 mo), reproductively senescent (RS, acyclic) females (15-21 mo), and RS females that were ovariectomized (OVX), including those that received 3-week estrogen treatment (OVX+E). Previously, we used whole-cell voltage-clamp in a reduced synaptic preparation to show a significant decrease in frequency of sIPSCs onto basal forebrain (BF) cholinergic neurons in aged males that could be mimicked in young males by adding exogenous Ca2+ buffer to the presynaptic terminals (J Neurophysiol 2014, 111:273). We have now found a parallel reduction in the IPSC frequency of RS OVX compared to young females and this reduction is eliminated in RS OVX+E rats. Because we hypothesize that reduced synaptic transmission in aged males is mediated by increased Ca2+ buffering, we assessed the buffering in the somatic compartment of BF cholinergic neurons by loading the cells with fura-2 AM and evoking Ca2+ transients by focal stimulation with an elevated K+ solution (20 mM). Different stimulus intensities were obtained by varying the duration and these were graphed against the amplitude of the evoked transients to create a buffering curve, the slope of which represents relative Ca2+ buffering. We confirmed the previously reported increase in Ca2+ buffering with age for males with the buffering slope changing significantly ($p<0.01$) from a mean (± SE) of 22.6 ± 2.3 (n=25) in young to 14.5 ± 1.7 (n=26) in aged (less slope indicates greater buffering). A similar significant ($p< 0.03$) change occurs in RS and OVX females and is alleviated by estrogen treatment. The slopes in females were: 25.0 ± 3.3 (n=36) for young, 13.6 ± 1.0 (n=16) for RS, 13.1 ± 1.8 (n=16) for RS OVX and 39.2 ± 5.9 (n=19) for RS OVX+E. Also, a single-cell RT-PCR survey showed reduced frequency of detection for choline acetyltransferase and G-protein coupled estrogen receptor sequences in RS OVX relative to young and RS OVX+E females. These results are consistent with the hypothesis that age and hormonal status alter Ca2+ buffering, synaptic transmission and gene expression.

Disclosures: D.A. Murchison: None. **A. Fincher:** None. **S. Bake:** None. **D.W. DuBois:** None. **J.C. Damborsky:** None. **W.H. Griffith:** None.

Poster

599. Synaptic Transmission: Modulation III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 599.24/D39

Topic: B.07. Synaptic Transmission

Support: Pontificia Universidad Javeriana

Title: BDNF-TrkB signaling regulation of GABAergic neurotransmission

Authors: M. P. LOZANO[1], M. MARTA[2], J. R. DIAZ[1], Z. CASAS[2], S. L. ALBARRACIN[1], *J. J. SUTACHAN-RUBIO[3];
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Abstracts: The mammalian central nervous system is driven by a delicate balance between excitatory and inhibitory neurotransmission that is established during embryonic development and the first years of life. Impair to establish this balance can lead to the development of neurological diseases such as epilepsy, schizophrenia, and autism. Neurotrophins, especially the Brain-derived neurotrophic factor (BDNF), have been shown to play an important role in the establishment of this balance by regulating the migration, maturation and synaptic transmission of GABAergic system. However, little is known about how BDNF is able to regulate such variety of processes in the GABAergic system. To gain insight into the molecular mechanisms by which BDNF induces enhancement of GABAergic neurotransmission, initially we evaluated the role of TrkB postsynaptically by silencing the receptor on glutamatergic neurons. The results obtained showed that a reduction in the expression of TrkB receptor postsynaptically induces changes in miniature inhibitory synaptic currents (mIPSCs) in addition to modify the expression of Gephyrin and the enzyme GAD65. These results suggest that TrkB postsynaptically is necessary not only for maintaining the inhibitory postsynaptic density but also for regulating the expression of enzymes involve in the synthesis of GABA.

Disclosures: **M.P. Lozano:** None. **M. Marta:** None. **J.R. Diaz:** None. **Z. Casas:** None. **S.L. Albarracin:** None. **J.J. Sutachan-Rubio:** None.

Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: China NSF Grant 31271155 (WMY)

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Title: Apparent receptor kinetics analyses of EPSPs evoked by ipsilateral pericentral canal stimulation in spinal cord motoneurons *in vitro*

Authors: W. QIN, C. ZHENG, B.-A. WANG, *M.-Y. WANG;
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Abstracts: Our previous study has shown that apparent receptor kinetics is suitable to analyze the transmitter-receptor binding kinetics of excitatory postsynaptic potentials (EPSPs) evoked by the ipsilateral ventrolateral funiculus (iVLF) in spinal cord motoneurons (MNs) *in vitro* (*Acta Physiol Sin* 2014, 66: 129). To explore the apparent receptor kinetics properties of synaptic transmission from ipsilateral pericentral canal (iPCC) to MNs, the intracellular recordings were performed in MNs of spinal cord slices isolated from neonatal rats (8-14 days old). Electrical stimulation at threshold intensity (T) of iPCC induced mainly EPSPs (iPCC-EPSPs) in 18 MNs and their apparent receptor kinetics parameters, apparent association and dissociation rate constant (K_1 and K_2) and apparent equilibrium dissociation constant (K_T), were estimated to be 0.790±0.613 $T^{-1}\cdot ms^{-1}$ (Mean±SD), 0.078±0.055 ms^{-1}, 0.249±0.464 T, which were not significantly different from those parameters of iVLF-EPSPs recorded in the same MNs ($P>0.05$). However, as compared to a negative linear correlation of K_2 ($r=-0.972$, $P<0.05$) and K_T (-0.953, $P<0.05$) of iVLF-EPSPs in 5 MNs with the stimulation intensity at 1T-1.3T, K_2 and K_T of iPCC-EPSPs in 6 MNs presented dramatic decrease with stimulation intensity from 1T to 1.1T, and were negatively correlated with the intensity of stimulation at 1.1T-1.5T, with linear correlation coefficients of -0.952 ($P<0.05$) and -0.970 ($P<0.05$), respectively. Although the amplitude and area under curve of iPCC-EPSPs and iVLF-EPSPs were both increased by pretreatment of bicuculline (30 μmol/L) and strychnine (1 μmol/L) each for 15 min in 6 MNs ($P<0.01$ or $P<0.05$), APV (30 μmol/L) and DNQX (1 μmol/L) almost completely nullified iVLF-EPSPs ($P<0.01$ or $P<0.05$) and only partially inhibited iPCC-EPSPs ($P<0.01$). These preliminary results suggest that iPCC-EPSPs may be mediated by more heterogeneous transmitter-receptor

mechanisms in addition to glutamate-NMDA and AMPA receptors than iVLF-EPSPs in spinal cord MNs.

Disclosures: **W. Qin:** None. **C. Zheng:** None. **M. Wang:** None. **B. Wang:** None.

Poster

599. Synaptic Transmission: Modulation III

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Topic: B.07. Synaptic Transmission

Support: RFBR grant 13-04-00413a

Title: Potentiation of synaptic transmission in mouse neuromuscular junctions by ATP

Authors: ***P. O. BOGATCHEVA**, O. P. BALEZINA;
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Abstracts: In neuromuscular junctions ATP is known to be a co-mediator of acetylcholine (ACh). It is released from peripheral motor nerve endings along with ACh and then undergoes fast hydrolysis by ectonucleotidases in synaptic cleft. ATP acts both on its pre- and postsynaptic receptors of P2X and P2Y types and its most well-known effect is autoregulatory decrease of synaptic transmission via metabotropic P2Y receptors. Whether this effect is preserved during prolonged presence of ATP in synaptic cleft is unknown. In this study possible pre- and postsynaptic effects of ATP accumulation in synaptic cleft of neuromuscular junctions due to ectonucleotidases inhibition and tonic application of exogenous ATP were investigated. Data presented were obtained from the fast-twitch extensor digitorum longus muscle of adult mice using standard single microelectrode technique. Spontaneous miniature endplate potentials (MEPPs) and evoked endplate potentials (EPPs) were recorded; amplitude, time course and quantal content were analyzed. In mouse neuromuscular junctions, wide range purine receptor blocker suramin (10 and 100 μM) enhanced quantal content of low frequency (0,3 Hz) EPPs by 19 and 37%, respectively, but did not influence the time course of MEPPs or EPPs. The data obtained corresponds well with the literature showing that the primary effect of endogenous ATP is downregulation of evoked transmitter release. Ectonucleotidase inhibitor ARL 67156 (50 μM) did not alter the amplitude of MEPPs and the quantal content of low frequency EPPs, but significantly prolonged their time course by 25%. During long time high frequency (50 Hz, 1 s) rhythmic nerve stimulation, ARL 67156 caused an increase in the amplitude of EPPs by 33% and

time course of MEPPs and EPPs by 20%. The quantal content of EPPs remained unchanged, suggesting postsynaptic mechanisms of neurotransmission potentiation. Non-hydrolyzable ATP analogue γ-S-ATP (10 μM) significantly increased the amplitude and prolonged the time course of synaptic potentials (both MEPPs and EPPs) by 28% and 25%, respectively. Hence, exogenous non-hydrolyzable ATP analogue shows pronounced postsynaptic potentiating effects in motor synapses. These could be due to increased opening frequency of muscle nicotinic ACh receptors and/or increased input resistance of muscle fibers caused by blockade of chloride leak channels. Based on the data obtained we propose that regulatory effects of ATP on neuromuscular transmission strongly depend on its accumulation level in synaptic cleft which differs with patterns of synaptic activity.

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Poster

599. Synaptic Transmission: Modulation III

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Program#/Poster: 599.27/D42

Topic: B.07. Synaptic Transmission

Support: NIH NS076312

Title: O-GlcNAcylation dampens hyperexcitability in hippocampus during acute epileptoform activity

Authors: L. T. STEWART[1], K. WANG[1], J. C. CHATHAM[2], *L. L. MCMAHON[1];
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Abstracts: Epilepsy and associated seizure disorders are characterized by the spontaneous induction of hypersynchronous neuronal activity in various circuits in the brain. These periods of increased excitability have detrimental effects on both acute and long-term synaptic function. O-GlcNAcylation is a dynamic posttranslational modification involving O-linked addition of β-N-acetylglucosamine (GlcNAc) to serine/threonine residues on nucleocytoplasmic and membrane proteins. Hippocampal neurons express high levels of OGT and OGA, enzymes that add and remove O-GlcNAc moieties, respectively. Our lab recently reported that acutely increasing O-GlcNAcylation using Thiamet-G (TMG) to block OGA, or glucosamine (GlcN) to provide increased substrate to OGT, induces LTD at CA3-CA1 hippocampal synapses. Here, we

investigated the impact of hyperexcitability on hippocampal basal protein O-GlcNAc levels and tested the hypothesis that increasing O-GlcNAcylation can dampen circuit hyperexcitability using *in vitro* and *in vivo* models of seizure activity. Using acute hippocampal slices from adult rats, hyperexcitability induced via pharmacological blockade of GABAARs with picrotoxin (100 μM) significantly decreased global protein O-GlcNAcylation in area CA1. In pilocarpine treated rats experiencing recurrent seizures 30 days post-injection, basal protein O-GlcNAc levels were also significantly decreased compared to sham. Moreover, in resected human brain tissue from epileptic patients, O-GlcNAc levels were decreased in epileptic tissue compared to marginally resected tissue. Given the findings that hyperexcitability decreases protein O-GlcNAcylation, we next tested whether pharmacologically increasing O-GlcNAc levels could depress hyperexciability. Using hippocampal slice electrophysiology at CA3-CA1 synapses, we found that hyperexcitability induced by picrotoxin could be significantly reduced by increasing O-GlcNAcylation levels in hippocampus using a combination of GlcN and TMG. Finally, EEG recordings were carried out in adult mice to examine the anticonvulsant effects of increasing O-GlcNAcylation using the pentylenetetrazole (PTZ) seizure model. We found a significant reduction in total spike number in mice pretreated with GlcN/TMG compared to vehicle. Collectively these findings suggest that increasing protein O-GlcNAcylation is a viable therapeutic strategy to treat epilepsy and seizure disorders.

Disclosures: L.T. Stewart: None. **K. Wang:** None. **J.C. Chatham:** None. **L.L. McMahon:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 600.01/D43

Topic: B.08. Synaptic Plasticity

Title: Expression of long-term memory after training with inedible food in *Aplysia*: Modification of fast synaptic connections from buccal ganglia mechanoafferents to B4, but not to B31/B32

Authors: *S. TAM[1,2];
[1]BAR ILAN UNIVERSITY, MOSHAV BEIT ARIF, Israel; [2]Gonda Multidisciplinary Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel

Abstracts: Training *Aplysia* with an inedible food causes changes in behavior, which animals remember 24 hours later. Long-term memory is expressed by changes in motor patterning, as

well as by a decrease in time to stop responding to the inedible food. Molecular correlates oflong-term memory have been found in the buccal ganglia, which have primarily a motor function in organizing consummatory feeding behaviors. The molecular changes are expressed in the S1 and S2 mechanoafferent clusters that innervate the interior of the buccal mass. These neurons monosynaptically and polysynaptically contact additional buccal ganglia neurons, including the B31/B32 neurons, which decide on the initiation of a consummatory feeding movement, and the B4/B5 neurons, whose firing biases motor activity toward rejection-like patterns. We examined whether monosynaptic connections from the mechanoafferents to B31/B32 and B4/B5 are changed after training leading to long-term memory. Connections were sampled in buccal ganglia removed from *Aplysia* 24 hrs after 2 training sessions with inedible foods, which were separated by 24 hrs. We found no significant changes in synaptic connections to B31/B32, indicating that the decrease in the likelihood to respond to food when memory is expressed is unlikely to arise from a change in the properties of synapses from mechanoafferents to B31/B32. However, there were significant changes in the synaptic connections from the mechanoafferents to B4.Fast monosynaptic connections from S1 neurons to B4 were increased, whereas fast monosynaptic connections from S2 neurons to B4 were decreased. These findings indicate that plasticity from mechanoafferents to B4 is likely to contribute to the changes in motor activity that are expressed as part of long-term memory. The data suggest that different aspects of memory are expressed at different neural sites, with changes in motor activity being expressed in the buccal ganglia. Changes in the likelihood to respond to food are likely to be expressed elsewhere, perhaps in cerebral ganglion neurons that recruit B31/B32.

Disclosures: S. Tam: None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

Location: Halls A-C

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Program#/Poster: 600.02/D44

Topic: B.08. Synaptic Plasticity

Support: Swedish research council 3050

Swedish research council 2862

Linkoping university

Title: Vesicular glutamate transporters at corticothalamic synapses contribute to short-term plasticity and visual attention

Authors: *B. GRANSETH[1], S. H. LINDSTROM[2];
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Abstracts: Vesicular glutamate transporters (VGluTs) are essential for most excitatory signaling in the central nervous system (CNS), and evidence suggests that the presynaptic function(s) of the VGluTs extend beyond loading glutamate into presynaptic vesicles. Two isoforms are used by glutamatergic synapses, VGluT1 and VGluT2. VGluT1 tends to be expressed at synapses with low release probability (Pr) and short-term facilitation, while VGluT2 expressing synapses tend to have high Pr and short-term depression. Relay cells in the lateral geniculate nucleus (LGN) receive glutamatergic input from two sources. The driving input, from retinal ganglion cells, has been shown to have high Pr and short-term depression. In contrast, modulatory input from the corticothalamic (CT) neurons has low Pr and strong short-term facilitation. Anatomical data suggests that CT neurons express VGluT1 while retinal ganglion cells express VGlut2; however, this dichotomy has not been fully established. In this study we aim to determine how much of the CT input to relay cells is mediated by VGluT1, examine the effect of reduced VGluT1 expression on CT short-term facilitation, and determine if these functional changes are reiterated in a behavioral measure of visual attention. To examine the role of VGluT1 at CT synapses, we performed whole-cell patch-clamp recordings of LGN relay cells in brain slices from wild-type (WT), VGluT1+/-, and VGluT1-/- mice. Visual learning and attention were assessed in WT and VGluT1+/- mice using a paired visual discrimination task and the 5-Choice Serial Reaction Time Task (5CSRTT). Our results showed that relay cells in VGluT1-/- mice lack CT input, while retinal input is largely unchanged. We conclude that CT input uses exclusively VGluT1, while retinal input is primarily mediated by VGluT2. Previous studies with VGluT1+/- mice have demonstrated that VGluT1 protein levels are half that observed in WT mice and this reduced expression alters the distribution of synaptic vesicles. In agreement with this finding, our results suggest that short-term facilitation is reduced at CT synapses in VGluT1+/- mice. Behavioral testing showed that VGluT1+/- mice had no deficit in learning a complex visual discrimination. Thus their ability to process visual information seems intact. However, the 5CSRTT revealed deficits in visual attention and inhibitory control, suggesting that the alterations in CT short-term facilitation produced by reduced VGluT1 expression may be important in these processes.

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Poster

600. Synaptic Plasticity: Short-Term Plasticity

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NSERC PhD Fellowship

CTRN PhD Fellowship

Title: Compartmentalized calcium microdomains in large mossy fiber terminals gates short-term facilitation at mossy fiber to CA3 pyramidal cell synapses

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[1]Cell. and Mol. Neurosci., CRULRG, Quebec, QC, Canada; [2]IUSMQ, Quebec, QC, Canada

Abstracts: Presynaptic terminals can be composed of several active zones, each supported by assigned pools of vesicles. The properties of synaptic transmission strongly depend on the location of the calcium sensors in relation to the source of calcium influx in the terminals. Spatial dynamics of calcium in individual presynaptic terminals will dictate the release of vesicles at specific release sites. However, the spatial compartmentalization of calcium signaling and its consequences on short-term synaptic facilitation remain largely unexplored. We performed whole-cell patch-clamp recordings from CA3 pyramidal cells in acute mouse hippocampal slices. Mossy fiber (MF)-mediated EPSCs were evoked by electrical minimal stimulation. The spatial dynamics of calcium elevations in mossy fiber boutons was probed using random-access two-photon microscopy (RAMP) in combination with single granule cell recordings. RAMP calcium imaging in individual large mossy fiber terminals revealed spatially-heterogeneous calcium elevations associated with one action potential evoked at the soma of granule cells in 2.5 mM [Ca2+]. Accordingly, a significant increase in the standard error of calcium elevations recorded simultaneously at multiple sites within the same bouton was observed. In this condition, coefficient of variation analysis suggested that short-term facilitation at MF-CA3 was supported by an increase in the number of release sites (N). Variance-mean analysis showed that a significant increase in N contributes to synaptic transmission in 2.5 mM [Ca2+]. Therefore, the increase in N could be promoted by heterogeneous calcium microdomains observed in single mossy fiber boutons. Two-photon glutamate uncaging confirmed that short-term facilitation was of presynaptic origin. Contrasting with the increase in N observed in 2.5 mM [Ca2+], synchronization of multivesicular release was observed during trains of facilitating EPSCs recorded in 1.2 mM [Ca2+]. Indeed, covariance analysis revealed a gradual augmentation in quantal size (Q) during trains of EPSCs and the low-affinity glutamate receptor antagonist γ-DGG showed an increase in cleft glutamate concentration. The synchronization of multivesicular

release was partially dependent on intracellular calcium stores. Our findings show that the compartmentalized spatial profile of calcium elevations in large mossy fiber gates the recruitment of additional release sites. Thus, synchronization of multivesicular release acts in combination with the recruitment of additional release sites to increase glutamate release during burst activity and expands the dynamic range of mossy fibers information transfer.

Disclosures: S. Chamberland: None. **A. Evstratova:** None. **K. Tóth:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R56 MH065328

UAB CCTS Pilot Grant

Evelyn F. McKnight Brain Institute

Civitan International Research Center

Title: Differential synaptically-evoked spiking of two subgroups of NPY interneurons in the stratum radiatum of hippocampal CA1

Authors: *Q. LI, A. F. BARTLEY, L. E. DOBRUNZ;
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Abstracts: Neuropeptide Y (NPY) is a widely expressed peptide in the brain that has anti-epileptic and anti-anxiety properties. NPY is released by a subset of GABAergic interneurons that also express nitric oxide, enabling these cells to regulate brain circuits through multiple means. It is therefore important to understand the mechanisms that regulate firing of NPY interneurons, including their excitatory inputs. In the CA1 region of hippocampus, NPY interneurons are very abundant, even more so than parvalbumin basket cells. NPY interneurons are widely distributed in stratum radiatum (SR) of CA1, where they could potentially receive feed-forward inputs from Schaffer collateral (SC) or temporoammonic (TA) afferents. However, little is known about synaptically-evoked firing of SR NPY cells in response to feed-forward inputs. In this study, we examined physiological properties, morphology and biochemical

properties of NPY interneurons in the SR of CA1. Our data shows that excitatory inputs from the SC pathway can drive firing of NPY interneurons in SR. SC stimulation onto NPY cells elicits two forms of short-term plasticity: most NPY cells express paired-pulse facilitation (SC-PPF) while a subset has paired-pulse depression (SC-PPD). The SC-PPF cells and SC-PPD cells also show differences in their intrinsic excitability and synaptically evoked spiking. The firing probability of SC-PPF cells increases during high frequency SC stimulation, while that of SC-PPD cells decreases. Both types of NPY cells receive facilitating input from the TA pathway in stratum lacunosum-moleculare (SLM) that also drives spiking, suggesting that the two major inputs to CA1 are both important for NPY cell firing. Morphological reconstruction of NPY cells in SR revealed that SC-PPD cells are more abundant close to the pyramidal layer, whereas SC-PPF cells are evenly distributed across SR. SC-PPD cells have axons primarily in SR, while SC-PPF interneurons also target their axons to SLM. Together, these data suggest that these two subgroups may represent two distinct classes of NPY cells in SR. Two main classifications of NPY interneurons have been previously described: ivy cells located in stratum pyramidale and SR, and neurogliaform cells in SLM. Based on location and morphology, the SC-PPD cells show similarities to ivy cells. Single cell PCR will be used to test whether the SC-PPD and SC-PPF cells correspond to ivy and neurogliaform cells or instead depict new NPY subtypes in SR. Our data indicate that NPY cells in SR are heterogeneous, and that they may be differentially recruited by feed-forward stimulation of the SC pathway, enabling them to have different effects on CA1 circuit function.

Disclosures: **Q. Li:** None. **A.F. Bartley:** None. **L.E. Dobrunz:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: FP7/2007-2013 n 238608

Title: *In vivo* analysis of synaptic function in CA3 microcircuits using optogenetics

Authors: ***S. ZUCCA**[1,2], M. GRIGUOLI[2], C. MULLE[2];
[1]Wickens Unit, Okinawa Inst. of Sci. and Technol., Kunigamigun, Japan; [2]Interdisciplinary Inst. for Neuroscience, Univ. of Bordeaux, CNRS UMR 5297, Bordeaux, France

Abstracts: In the hippocampus synaptic transmission between the dentate gyrus and CA3 region is mediated by the axons of granule cells (GCs), the so-called mossy fibers (MFs). MFs to CA3 pyramidal cell (CA3-PCs) synapses are particularly distinctive for their low basal release probability and prominent activity-dependent short-term synaptic plasticity, including pronounced paired-pulse facilitation, and frequency facilitation. The reliability of monosynaptic glutamatergic transmission at MFs-CA3 synapses is highly sensitive to the firing frequency of GCs, and this indicates that MFs synapses are "conditional detonators", making short term plasticity a crucial component for GCs-CA3 spike transfer. Despite a large body of information from computational studies and *in vitro* preparations, the functional consequences of short term plasticity on the operation of CA3 circuits in the intact brain are still elusive. To address this question we used an optogenetic approach *in vivo* to selectively target and control the activity of GCs while performing electrophysiological recordings in anesthetized mice. We injected a Cre-inducible recombinant AAV vector containing a mutated variant of ChR2 (AAV-DIO-ChR2(H134R)-EYFP) in mice expressing Cre-recombinase in POMC neurons in the dentate gyrus of the hippocampus. To test the functionality of ChR2 we first performed whole cell recordings from ChR2+ granule cells in acute brain slices. Brief pulses of blue light (0.5-1 ms; 470 nm) reliably induce action potentials at different frequencies of stimulation (up to 40 Hz), and short term plasticity of excitatory postsynaptic currents in CA3-PCs. To examine the functional properties of MFs-CA3 synapses *in vivo* in the anesthetized mice, we implanted an optical fiber in the dentate gyrus to deliver optical stimulation at 0.05 Hz (1-5 ms, 470 nm) and record fEPSP responses in the CA3 region. Gradual increase of frequency of stimulation over a range of frequencies (1, 3, 10 Hz) produced a clear potentiation of fEPSP slope compared to baseline values. To better understand the impact of frequency facilitation relative to the output of CA3 targets we performed juxta-cellular recordings from CA3 neurons. We found that light stimulation of ChR2+ GCs *in vivo* reliably discharged CA3 interneurons but only rarely discharged CA3-PCs, suggesting that a fine tuning of inhibition/excitation balance may be critical for the transfer of information between GCs to CA3. Further studies at the single cell level will allow us to better characterize the mechanisms of short term facilitation occurring at MFs-CA3 synapses *in vivo* and gain new insights on the role of conditional detonators of MFs in the CA3 network.

Disclosures: **S. Zucca:** None. **M. Griguoli:** None. **C. Mulle:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

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Support: NIH NS032405

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T32 NS007484

Title: PKC is a calcium sensor for short-term synaptic plasticity

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Abstracts: In presynaptic boutons, calcium (Ca^{2+}) triggers both neurotransmitter release and short-term synaptic plasticity. Whereas synaptotagmins are known to mediate vesicle fusion through binding of high local Ca^{2+} to their C2 domains, the proteins that sense smaller global Ca^{2+} increases to produce short-term plasticity have remained elusive. Here we identify a Ca^{2+} sensor for post-tetanic potentiation (PTP), a form of plasticity thought to underlie short-term memory. We find that at the functionally mature calyx of Held synapse the Ca^{2+}-dependent protein kinase C isoforms α and β are necessary for PTP, and expression of PKCβ in PKCαβ double knockout mice rescues PTP. Disruption of Ca^{2+} binding to the PKCβ C2 domain specifically prevents PTP without impairing other PKCβ-dependent forms of synaptic enhancement. We conclude that different C2-domain-containing presynaptic proteins are engaged by different Ca^{2+} signals, and that Ca^{2+} increases evoked by tetanic stimulation are sensed by PKCβ to produce PTP.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: ANR PreAD

France Alzheimer

Fondation Plan Alzheimer

Title: The role of presenilin in presynaptic plasticity at hippocampal mossy fiber synapses revealed by an optogenetic approach

Authors: *C. MULLE, J. RUMI, G. BARTHET;
Interdisciplinary Inst. For Neuroscience, Univ. Bordeaux, Bordeaux, France

Abstracts: Presenilin (PS), the catalytic subunit of the intramembrane protease gamma-secretase, cleaves various synaptic proteins including APP and N-cadherin but the role of these processings in synaptic function is not known. Importantly, more than 150 missense mutations have been found on PS1 gene which cause the early-onset form of familial Alzheimer's disease (FAD) and decrease the proteolytic activity of PS. PS regulates presynaptic plasticity by an unknown mechanism. Here we provide new understanding to the role of PS in presynaptic forms of short-term plasticity. We have examined the role of PS1 and PS2 at synapses between dentate gyrus (DG) granule cells and CA3 pyramidal neurons of the hippocampus which are characterized by short-term presynaptic plasticity with a high dynamic range, endowing these synapses with detonator properties essential for information transfer and memory encoding. We have developed new optogenetic tools which specifically target the DG, combining specific expression of a channelrhodopsin with cell-specific genetic manipulation. We have generated a bicistronic lentiviral tool to co-express the variant of channelrhodopsin ChIEF together with a transgene of interest separated by a 2A-peptide specifically targeted to DG cells via the use of a DG specific promoter cloned for the first time. Stereotaxic injection of this virus lead to co-expression of ChIEF and Cre recombinase to knock-down PS1 gene in PS1-floxed mice. We have performed electrophysiological recordings (field EPSP and whole-cell patch clamp) in a slice preparation. Light activation of DG cells triggered synaptic responses in CA3 pyramidal cells which only originated from DG cells lacking PS1. This approach has allowed to characterize the role of PS1 in different forms of presynaptic short term plasticity. We have also shown that PS-dependent control of presynaptic function and plasticity depends on the fragment CTFbeta of APP, a main substrate of gamma-secretase, which accumulates in absence of PS1. This optogenetic approach combined with cell-specific genetic manipulation of presynaptic DG cells offers great potential for understanding the presynaptic mechanisms of action of PS and its consequences in terms of hippocampal circuit acitivity and memory encoding, in the context of Alzheimer's disease.

Disclosures: **C. Mulle:** None. **G. Barthet:** None. **J. Rumi:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NSF Grant IOS1048556

HHMI Grant 52005120

Title: Characterization of the heterosynaptic interaction of the septal and crossed entorhinal projections to the dentate gyrus after unilateral entorhinal cortex lesion in rats: A time course study

Authors: N. A. UPRIGHT, E. L. KRAUSE, G. R. SMITH, M. K. MOSES-HAMPTON, P. G. LAKHMANI, *J. J. RAMIREZ;
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Abstracts: Axonal sprouting has been investigated following various forms of damage to the CNS, including Alzheimer's disease, stroke, and traumatic brain injury. Alzheimer's disease is known to involve a marked degeneration of the entorhinal cortex (EC). A significant number of entorhinal projections to the hippocampus terminate in the ipsilateral dentate gyrus (DG); however, a small number of fibers project to the contralateral DG forming the crossed temporodentate (CTD) pathway. Following a unilateral EC lesion in the rat, the glutamatergic CTD and the acetylcholinesterase-containing, cholinergic septal input to the DG, termed the septodentate (SD) pathway, have been shown to undergo axonal sprouting. Lesion-induced, CTD sprouting results in greater synaptic efficacy as early as 6 days postlesion relative to the normal CTD. The present study explored whether, relative to CTD stimulation alone, paired stimulation of the SD pathway followed by CTD stimulation would affect the response of the DG at 6, 8, 12, or 90 days postlesion. Male, Sprague-Dawley rats were given either unilateral entorhinal lesions or sham operations, which consisted of a craniotomy over the entorhinal area. Stimulating electrodes were placed in the medial septum and in the intact, contralateral EC 6, 8, 12, or 90 days after a unilateral entorhinal lesion or sham operation. Evoked, field excitatory postsynaptic potentials (fEPSPs) were recorded in the DG ipsilateral to the lesioned EC. The paired-pulse paradigm involves stimulation of an initial input, termed the "conditioning pulse," followed by a

second input, termed the "test pulse." In the heterosynaptic paired-pulse stimulation portion of this study, SD stimulation preceded CTD stimulation at a range of interpulse intervals (IPIs; from 30 to 500 ms). Relative to control cases, unilateral EC lesions significantly increased the fEPSPs produced by CTD stimulation alone at all the time points we examined. In contrast, compared to CTD stimulation alone, pairing SD with CTD stimulation significantly depressed the amplitude of the fEPSPs across IPIs at 8, 12, and 90 days postlesion. Stimulation of the CTD alone rarely produced granule cell discharge (i.e., population spikes) at the time points we explored; however, paired stimulation of the SD and CTD produced granule cell discharge in a significant number of cases at the 90-day time point with a response profile similar to that observed after paired stimulation of the SD and the perforant path in intact cases. Thus, heterosynaptic stimulation in which the septal input and crossed entorhinal input are paired following entorhinal lesions significantly affects DG responsivity.

Disclosures: **N.A. Upright:** None. **G.R. Smith:** None. **E.L. Krause:** None. **M.K. Moses-Hampton:** None. **J.J. Ramirez:** None. **P.G. Lakhmani:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

Location: Halls A-C

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Program#/Poster: 600.09/D51

Topic: B.08. Synaptic Plasticity

Support: NIH R01 Grant NS040723

Title: Prolonged ketamine exposure increases synaptic plasticity in the anterior cingulate cortex of neonatal rats

Authors: S. KOKANE[1], J. PERISH[1], R. WANG[1], A. WOMACK[1], R. STEVENS[1], X. ZOU[1], *Q. LIN[2];
[1]Psychology, Univ. of Texas at Arlington, Arlington, TX; [2]Psychology, Univ. of Texas At Arlington, Arlington, TX

Abstracts: Prolonged exposure to ketamine, a commonly used pediatric anesthetic, has been reported in animal studies to induce widespread neuroapoptosis in many parts of the neonatal brain. The hippocampus and anterior cingulate cortex (ACC), which are the two main brain areas involved in learning and memory, show maximal neuronal loss due to ketamine exposure. Increasing evidence shows that neuroapoptosis eventually leads to cognitive and memory

deficits. Ketamine is a non-competitive N-methyl D-aspartate receptor (NMDAR) antagonist. One of the molecular mechanisms, by which ketamine induces neurotoxic insult, suggests that a glutamate storm caused by the compensatory up-regulation of NMDARs after ketamine withdrawal results in increased Ca^{2+} influx that triggers downstream pro-apoptotic cascades. We hypothesize that this is mediated by alterations in the synaptic strength. To examine this hypothesis, we performed whole-cell patch-clamp recordings from ACC neurons of neonatal Sprague-Dawley rat brain slices after ketamine withdrawal. Ketamine was administered at postnatal days 4-7 (subcutaneous injections, 20 mg/kg given 6 times at 2 hour intervals). Both evoked and spontaneous postsynaptic current activities mediated by NMDARs were sampled at 2, 4, 6, and 8 h after ketamine withdrawal by recording evoked and miniature excitatory postsynaptic currents (eEPSCs and mEPSCs). We observed that both eEPSCs and mEPSCs were significantly increased beginning at 4 h until 8 h after ketamine withdrawal. This trend of altered current activity was significantly different than that seen in the saline administered group where the current activity remained unchanged at basal levels over the time course. Thus, these data provide strong pathophysiological evidence to indicate that ketamine exposure-induced neurotoxic insult is initiated by increasing enhancement of excitatory synaptic plasticity.

Disclosures: **S. Kokane:** None. **J. Perish:** None. **R. Wang:** None. **A. Womack:** None. **R. Stevens:** None. **X. Zou:** None. **Q. Lin:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 600.10/D52

Topic: B.08. Synaptic Plasticity

Support: NSERC Grant

Title: Effect of synaptic activity on the replenishment of resting pool

Authors: ***M. I. GLAVINOVIC**, L. BUI;
Physiol., McGill Univ., Montreal, QC, Canada

Abstracts: The synaptic activity at room temperature renders the replenishment of the readily releasable pool (RRP) slower (Bui & Glavinovic, Cogn. Neurodyn. 7, 105, 2013). Vesicular dynamics of the excitatory synapses of rat hippocampus is thus time dependent. Given that the replenishment rate of the RRP is also Ca++-independent and is largely unaffected by compounds

that speed-up or slow down vesicular trafficking, the replenishment of the RRP does not appear to be associated with a significant vesicular movement (Bui & Glavinovic, Cogn. Neurodyn. 8, 99, 2014). The replenishment dynamics of the resting pool (RP) is far less known and its biophysical mechanism is far less understood. To start with we do not know: a) how large the RP is, b) how fast its replenishment is, whether it changes with synaptic activity and if so how fast, c) whether it is Ca++-dependent, and d) whether vesicular trafficking plays a significant role in its replenishment. We characterize the dynamics of the vesicular storage and release system and its changes during stimulation using model-fitting. Experiments were done on rat hippocampal pyramidal neurons in slices at room temperature using standard whole cell clamp recording, and patterned stimulation of Schaffer collaterals. The optimal values of all parameters of the storage and release system of presynaptic cells were calculated by minimizing the difference between the measured output (the amplitudes of the evoked excitatory post-synaptic currents) and the estimated output (calculated from the estimated parameters). The replenishment of the RP is much slower (>100 times) than that of the RRP. Interestingly, the replenishment coupling into the RP surpasses the replenishment coupling into RRP, but because the RP size is >1000 times that of the RRP, this renders the replenishment of the RP 'only' ~100 times slower than that of the RRP. During prolonged high frequency stimulation the replenishment rate of the RP decreases, and though it is slow, its decrease during stimulation is fast. This study provides much needed information about the replenishment dynamics of the resting pool, and the findings suggest that the replenishment is probably not associated with a significant vesicular movement.

Disclosures: M.I. Glavinovic: None. **L. Bui:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 600.11/D53

Topic: B.08. Synaptic Plasticity

Support: CIHR

Title: Presynaptic Ca2+ dynamics in the mature calyx of Held synapse with morphological heterogeneity

Authors: *A. FEKETE, L.-Y. WANG;
Program in Neurosci. and Mental Hlth., The Hosp. for Sick Children, Toronto, ON, Canada

Abstracts: The morphology of the mature calyx of Held synapses becomes highly heterogeneous with different levels of segmentation, namely varying number of stems, stalks and swellings. The morphological variability at maturity strongly correlates with the differences in quantal parameters, forms of STP and fidelity of spiking (Grande and Wang, 2011). In this study, we have examined Ca2+ dynamics in different types of mature calyces and distinct compartments of the same calyx (P16-23). We applied two-photon laser scanning microscopy of high- and low-affinity Ca2+-indicators (50 μM Fluo-4 / Fluo-4FF) and the morphological tracer Alexa 594 (15-30 μM). Action potentials are evoked by stimulating axons either with a bipolar electrode after removing the loading pipette or directly by a patch electrode in current-clamp mode. We delivered trains of APs with different frequencies (2x100/200/300 Hz, Δt=500 ms) or single APs to determine the spatiotemporal profile of Ca2+ transients mediated solely by P/Q-type Ca2+ channels in the presynaptic terminal in slices from both WT and Cacna1aCitrine knock-in mice (citrine-tagged α1A subunit encoding P/Q-Ca2+ channel). We found Ca2+ transients in every morphological subtype of the calyces and throughout the entire calyceal arborization including the swellings, stems and stalks. The size of the Ca2+ transients varied with the size of the compartment, being the most robust in the smallest compartments (swellings). Application of potassium channel blocker (TEA,1 mM) increased the Ca2+ transients in the different calyx subtypes and compartments, suggesting that APs can successfully propagate into distinct compartments and recruit increasing number of calcium channels to boost Ca2+ transients. Our data implicate the heterogeneity of presynaptic Ca2+ transients as a mechanistic link underpinning different quantal properties and short-term synaptic plasticity at the mature calyx of Held synapses with distinct morphological complexity.

Disclosures: **A. Fekete:** None. **L. Wang:** None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: 2R15DA021683

5SC1DA029329

Title: The NMDA receptor-mediated spontaneous EPSCs are sensitive to constitutively- active ghrelin receptors in the hippocampus

Authors: *M. ISOKAWA;
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Abstracts: Increasing evidence points a stomach hormone, ghrelin, may have numerous physiological functions in the brain in addition to its primary role in stimulating hypothalamic orexigenic neurons. Intra-hippocampal ghrelin improved memory retention, increased dendritic spines, and facilitated spine synapse formation, suggesting that ghrelin appears to modulate molecular and cellular signaling events involved in memory acquisition and consolidation. The ghrelin receptor, GHSR1a, is expressed highly in the hippocampus, which further supports the involvement of ghrelin signaling in synaptic transmission and plasticity in the hippocampus. We previously reported that a low concentration of ghrelin (25-100 nM) enhanced the NMDA receptor-mediated EPSCs (NMDAR-EPSCs) in the CA1 pyramidal cells that were isolated with a whole-cell patch electrode containing Cs methanesulfonate in the bath-presence of picrotoxin ($GABA_A$ receptor antagonist) and NBQX (AMPA receptor antagonist). Involvement of GHSR1a was characterized using the receptor antagonists L-Dys3-GHSR-6 (1 microM) and Substance-P analogue (1 microM), which negated the effect of ghrelin in a reversible manner. GHSR1a displays a high degree of ligand-independent signaling activity. In the present study, we tested if constitutive activation of GHSR1a was involved in the NMDA receptor-mediated synaptic transmission in the hippocampus. In the absence of exogenous ghrelin in the hippocampal slice culture, we found that the antagonist of GHSR1a reduced the peak amplitude of NMDAR-EPSCs suggesting that constitutively-active GHSR1a may determine the magnitude of the NMDAR-mediated synaptic transmission. In addition, the amplitude of spontaneously occurring EPSCs (sEPSCs mediated by the NMDAR), in particular, those with the amplitude greater than 100 pA were sensitive to the application of GHSR1a antagonists. The generation of NMDA spikes, which are spontaneously-generated local electrical signals at dendritic branches where NMDA receptors are highly localized, was also reduced by the GHSR1a antagonists. These findings suggest that hippocampal CA1 pyramidal neurons are likely to be under constant modulation by GHSR1a signaling in synaptic activities, and this modulation occurs, to some extent, independent of the availability of the endogenous ligand. Supported by NIH grants 2R15DA021683 and 5SC1DA029329.

Disclosures: M. Isokawa: None.

Poster

600. Synaptic Plasticity: Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: 1R21NS082680-01A1

Title: Differences in short-term modulation of excitatory synaptic responses in stratum radiatum and stratum lacunosum moleculare in mouse hippocampus

Authors: D. PEKALA, *M. RAASTAD;
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Abstracts: We investigated short-term modulation of synaptic strength in two layers of the CA1 field of hippocampi from 8-9 weeks old mice. We used four stimuli with 25 ms intervals and an additional stimulus with 100 ms interval, giving synaptic peak amplitudes A1 - A5. Experiments were made with 300 um transverse slices, 36 °C, standard Ringer's solution with 2 mM Ca2+. APV (50 uM) was added to reduce postsynaptic modulation of synaptic strength. When the five-pulse trains were repeated once a minute facilitation (A2/A1) was larger in str. lacunosum moleculare (SLM, 2.22 ± 0.13%) than in str. radiatum (SR, 1.52 ± 0.04%). When the test-trains were repeated at higher rate (1 Hz) A1 increased and approached A2, i.e facilitation was reduced, to 0.92 ± 0.03 and 1.12 ± 0.02 in SLM and SR respectively. A1 increased relatively similarly in SLM and SR during these 1 Hz repetitions to 243 ± 0.22% and 209 ± 18% in SR and SLM, respectively. However, a striking difference between SLM and SR appeared in the change of the other synaptic amplitudes in the five-pulse train (A2 - A5): A2 - A5 were slightly reduced in SLM (to 87 ± 3%) but increased in SR (to 136 ± 9%) of their original value. By subtracting from A1 these changes, that seemed to be general to all the five synaptic responses in the train, even larger differences in the change of A1 were detected: A1 in SR grew to 173 ± 0.09% while A1 in SLM grew to 274 ± 0.19% of their original amplitudes. These differences between short-term changes in synaptic responses in SLM and SR are likely to reflect different mechanisms for control of transmitter release. Blockers of GABAa receptors did not eliminate the difference between SR and SLM synaptic responses, but other modulating receptors are not ruled out.

Disclosures: D. Pekala: None. **M. Raastad:** None.

Poster

601. Long-Term Depression

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Topic: B.08. Synaptic Plasticity

Support: Boston Children's Hospital Translational Research Program

Title: Mechanism of metabotropic glutamate receptor 5-dependent long-term depression induced by cathodal direct-current stimulation in mouse primary motor cortex

Authors: *Y. SUN[1,2], J. LIPTON[1,3], M. SAHIN[1], A. ROTENBERG[1];
[1]Dept. of Neurol. and the F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA;
[2]Program in Neurosci., [3]Div. of Sleep Med., Harvard Med. Sch., Boston, MA

Abstracts: Transcranial direct-current stimulation (tDCS) is a method for focal noninvasive cortical stimulation where low-amplitude direct current is conducted to the brain via scalp electrodes. Extensive data indicate that anodal tDCS leads to enhanced cortical excitation and cathodal tDCS leads to depressed cortical excitation, similar to long-term potentiation (LTP) and long-term depression (LTD), respectively. However, the mechanisms underlying the tDCS effect are poorly understood. A recent study reported that anodal tDCS induced brain-derived neurotrophic factor (BDNF)-dependent LTP in mouse primary motor cortex (M1) and improves BDNF-dependent motor skill learning in humans. In the present study, we tested whether and to what extent a range of DCS settings modulates cortical field excitatory postsynaptic potential. We demonstrate that cathodal DCS applied to mouse M1 slices induced a LTD (DCS-LTD), which depends on the intensity, duration, and relative orientation of the DC current vector and the vector of the neuronal structures. Partial attenuation of the magnitude of DCS-LTD was observed by applying the antagonists of NMDA (D-2-amino-5-phosphonopentanoate, D-AP5) and $GABA_A$ receptors ([R-(*R**,*S**)]-6-(5,6,7,8-Tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)furo[3,4-*e*]-1,3benzodioxol-8(6*H*)-one, Bicuculline). However, bath application of 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4yl)ethynyl)pyridine (CTEP), a potent inhibitor of mGluR5, blocked the DCS-LTD. Meanwhile, the positive allosteric modulator of mGluR5, 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) enhanced the transient effect by short DCS application which otherwise did not lead to a durable depression of M1 excitability. Given that mGluR-dependent LTD requires mTOR pathway-dependent translation of dendritic mRNA, we queried the expression ratio of phosphorylated ribosomal protein S6 (S6)/total S6 (P-S6/S6) as a gauge of relative mTOR pathway activity in M1 slices during DCS-LTD. We observed a significant increase in P-S6/S6 ratio in stimulated compared to unstimulated controls. Our findings suggest that mGluR5 activation is critical for the DCS-LTD and mTOR pathway activation is involved during this after-effect in M1 slices. We will further investigate the signaling pathways that couple mGluR5 activation to translation initiation pathways (such as mTOR signaling) during DCS-LTD. Our study may implicate cathodal DCS as a potential treatment of neurologic conditions in which mGluR5 signaling and/or mGluR-mediated synaptic plasticity are impaired, such as tuberous sclerosis complex.

Disclosures: **Y. Sun:** None. **J. Lipton:** None. **M. Sahin:** None. **A. Rotenberg:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.02/D57

Topic: B.08. Synaptic Plasticity

Support: NIH grant EY12782

Title: The roles of IP_3R in LTD induction in visual cortex

Authors: ***D. KALIKULOV**, M. J. FRIEDLANDER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstracts: We evaluated the contributions of inositol trisphosphate receptors (IP_3R) to the induction of long-term synaptic depression (LTD) in layer 2/3 pyramidal neurons in guinea pig (ages P6-P12) visual cortex in response to three induction paradigms: i) low frequency afferent stimulation (LFS), ii) bath application of (S)-3,5-Dihydroxypenylglycine hydrate (DHPG - agonist of mGluR), and iii) pairing of presynaptic activity induced by stimulation of afferent fibers and postsynaptic spiking activity induced by current injection into the postsynaptic neuron at a fixed temporal delay. In the LFS group, a net LTD was induced (-16±4%; n=17), while individual cells either underwent statistically significant LTD or no significant change (NC). Inhibition of IP_3Rs with xestospongin C (1.0μM, -17±6%, n=11) in the LFS group did not alter the likelihood or magnitude of LTD, while inhibition of NMDARs with bath applied D-AP5 (DL-2-Amino-5-phosphonopentanoic acid) prevented the induction of LFS induced LTD (50 μM d-AP5; +0.3±3%; n=14). Bath application of the mGluR agonist DHPG (100 uM) also resulted in a net LTD (-24±5%; n=6). In contrast to the LFS group, inhibition of IP_3Rs with xestospongin C (1.0μM) reduced the magnitude of LTD induced (from -24%± 5%; n=6 to -11±5%; n=4). Pre-postsynaptic pairing resulted in a range of plasticity outcomes at the individual cell level from LTD to NC to LTP with a net LTP (+9±5%; n=30). Inhibition of IP_3Rs (1.0μM xestospongin C) in the pre- postsynaptic pairing group resulted in a significant change in the plasticity outcome ($p \leq 0.05$; t-test) with a net LTP (+31±9%, n=25) with individual cells undergoing only LTP or NC and no cells in this group undergoing LTD. These findings demonstrate that IP_3R activation is not necessary for LFS induced LTD, while both chemically and pairing induced LTD require IP_3R activation.

Disclosures: **D. Kalikulov:** None. **M.J. Friedlander:** None.

Poster

601. Long-Term Depression

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Program#/Poster: 601.03/D58

Topic: B.08. Synaptic Plasticity

Support: Ministerio de Ciencia e Innovación (SAF2011-30281)

CIBERNED (CB06/05/0042)

Generalitat de Catalunya (SGR2009-1231)

Title: Chemical LTD mediated changes in synaptic AMPA receptors is associated to changes in postsynaptic density scaffold proteins

Authors: *J. RODRIGUEZ-ALVAREZ[1], W. CHEN[2], D. SIEDLECKI[2], V. JIMENEZ[2], A. OTXOA DE AMEZAGA[2], C. A. SAURA[2], A. J. MINANO-MOLINA[2];
[1]Inst. De Neurociencies/ UAB, Barcelona, Spain; [2]Inst. de Neurociencies and Dpt. Bioquímica i Biología Molecular, Univ. Autònoma de Barcelona, Cerdanyola del Vallès, Spain

Abstracts: Excitatory synaptic transmission is tightly regulated by total number and activation of AMPA receptors (AMPAR) present at the synapse. Synaptic AMPARs localization is closely related with NMDA receptor (NMDAR) activity. Current evidences suggests that AMPARs are inserted into the postsynaptic membrane during LTP and could be removed from the membrane during LTD. Dephosphorylation of GluA1 at Ser845 and enhanced endocytosis could be critical events in the modulation of LTD. Moreover, changes in scaffold proteins from the postsynaptic density (PSD) could be also related to AMPAR regulation in LTD. In the present study we have analyzed the effect of chemical LTD (cLTD) on AMPAR and AKAP150 levels in cultured cortical neurons. cLTD reduces surface expression of GluA1 and GluA2 AMPAR subunits and GluA1 phosphorylation at Ser845. Moreover cLTD induces concomitant changes in AKAP79/150 and PSD95 protein levels that are dependent on calcineurin (CaN) and proteosome activation since pharmacological inhibition of CaN or proteosome activity revert cLTD-mediated changes in AKAP79/150 and PSD95. Since PSD95 and AKAP79/150 are synaptic proteins that has been proposed to function as a signaling scaffold that regulates phosphorylation, channel

activity, and endosomal trafficking of AMPAR, the cLTD-mediated changes in these proteins could be related to a deregulation of synaptic AMPA receptors in LTD.

Disclosures: J. Rodriguez-Alvarez: None. **W. Chen:** None. **D. Siedlecki:** None. **V. Jimenez:** None. **A. Otxoa de Amezaga:** None. **C.A. Saura:** None. **A.J. Minano-Molina:** None.

Poster

601. Long-Term Depression

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.04/D59

Topic: B.08. Synaptic Plasticity

Support: CREST, JST

Title: The BDNF pro-peptide is a novel facilitator of hippocampal LTD, and its biological action is altered by the common BDNF polymorphism Val66Met

Authors: *M. KOJIMA[1,3], T. MIZUI[2,4], H. KUMANOGOH[5,4], Y. ISHIKAWA[6];
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Abstracts: Most growth factors are initially synthesized as precursors and subsequently processed into their mature form by proteolytic cleavage, resulting in removal of a pro-peptide. These events take place in the secretory pathway and in extracellular spaces. The biological roles of pro-peptides are not as well understood as those of the mature forms. Given that mature and pro-peptide forms are produced simultaneously, pro-peptides may physiologically serve important functions. In this study, we aimed to elucidate the biological roles of the pro-peptide of brain-derived neurotrophic factor (BDNF). Mature BDNF promotes neuronal growth of developing neurons, and also modulates synaptic plasticity in the adult brain. Here, we demonstrate that the BDNF pro-peptide directly facilitates hippocampal long-term depression (LTD) in a manner that requires the activation of N-methyl-D-aspartate (NMDA) receptor

subunit 2 (NR2B)-containing NMDARs and p75NTR activity. Moreover, NMDA-induced alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor endocytosis is enhanced by the BDNF pro-peptide, and this mechanism of AMPA receptor (AMPAR) trafficking is elicited by the pro-peptide itself. Thus, the BDNF pro-peptide, a previously functionally unknown ligand, is involved in synaptic plasticity that controls a mechanism responsible for promoting LTD. In a common polymorphism in the BDNF gene, Val66Met, methionine replaces valine at position 66 of the BDNF pro-domain and it was reported that this BDNF polymorphism affects human memory function. This genetic variant affects the biological activity of the BDNF pro-peptide. In contrast to the wild-type pro-peptide, the Met-BDNF pro-peptide markedly inhibits LTD and NMDA-induced AMPAR trafficking. Moreover, the binding of the mutant pro-peptide to p75NTR is much weaker than that of the wild-type. Thus, the Val66Met polymorphism has a functional impact on the BDNF pro-peptide. Finally, we show that the BDNF pro-peptide can bind mature BDNF but not other neurotrophins, and that this heterodimer complex is stabilized by the amino-acid substitution in the Val66Met variant. Our findings thus provide new insight into the effects of a naturally occurred human BDNF polymorphism, Val66Met.

Disclosures: **M. Kojima:** None. **T. Mizui:** None. **H. Kumanogoh:** None. **Y. Ishikawa:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

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Program#/Poster: 601.05/D60

Topic: B.08. Synaptic Plasticity

Support: NARSAD (2006YI)

NSF (IOS-0824393)

NIH (R01NS060879)

LSU Research Enhancement Fund (REF)

Title: Distinct roles of neurabin and spinophilin in the targeting and regulation of protein phosphatase 1 in AMPA receptor trafficking and LTD induction

Authors: *H. YANG, V, J. GAO, X. HU, H. XIA;
Neurosci., LSU Hlth. Sci. Ctr., New Orleans, LA

Abstracts: Protein phosphatase-1 (PP1) plays an important role in the induction threshold of long term potentiation (LTP) and depression (LTD) which contributes to PP1's constraint role in learning and memory. PP1's promoting function in LTD induction requires both PP1's enzymatic activity and its proper anchoring to synaptic spines. We have shown previously that neurabin, a major synaptic scaffolding protein, targets PP1 to synapses for LTD induction. We now discovered that PP1 targeted by spinophilin, a close homologue of neurabin and another major synaptic PP1 anchoring protein, does not play a role in LTD induction, suggesting the privileged role of neurabin in nano-domain targeting of PP1 in spines for LTD induction. Moreover, we found that protein kinase A (PKA) can significantly weaken neurabin-PP1 interaction in neurons, via phosphorylation of neurabin at serine 461, a phosphorylation site which is immediately adjacent to PP1 binding motif and not conserved in spinophilin. Finally, we found that expression of neurabin phosphorylation mimicking mutant, neurabin (S461E), blocked AMPA receptor endocytosis and LTD induction. Our study thus elucidated the critical importance of nano-domain targeting of PP1 within synaptic spines and its regulation in LTD induction.

Disclosures: **H. Yang:** None. **J. Gao:** None. **X. Hu:** None. **H. Xia:** None.

Poster

601. Long-Term Depression

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Topic: B.08. Synaptic Plasticity

Support: Wellcome Trust grant 087855/Z/08/Z

Title: Control of hippocampal to medial prefrontal cortex signalling by long-term depression of NMDA receptor mediated transmission

Authors: ***P. J. BANKS**, A. BURROUGHS, Z. I. BASHIR;
Physiol. & Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstracts: The hippocampus is connected to the medial prefrontal cortex (mPFC) by a long-range monosynaptic glutamatergic projection which is known to undergo activity-dependent modification. This connection has been shown to be involved in numerous cognitive processes including learning and memory. It has been shown in mouse that this synaptic connection can be studied in modified coronal mPFC slices *in vitro* using conventional electrophysiological means.

Here we demonstrate that this slice preparation is possible in rat brain and study plasticity of this synapse, focussing on NMDAR mediated transmission. We show that activation of G-protein coupled receptors induces long-term depression (LTD) of pharmacologically isolated NMDAR-mediated transmission in the hippocampal-prefrontal pathway: bath application of D2-like dopamine receptor agonist quinpirole potently reduces NMDAR transmission for at least 1 hour. Similar effects were observed following application of DHPG, carbachol and mAChR1 agonist AF102B. Interestingly, we discovered that delivery of 300 stimuli at 5 Hz induces LTD selectively of NMDAR-, but not of AMPAR-mediated transmission. However, this activity-dependent LTD was not blocked by antagonists of muscarinic or mGlu receptors or by inhibition of NMDA receptors during the 5 Hz stimulation (by hyperpolarisation to -100 mV or by temporary wash in of AP5). Given the prominent role of dopamine in synaptic plasticity and learning and memory in mPFC we then investigated whether this transmitter may be involved in activity-dependent NMDAR-LTD. Interestingly we show that the antagonism of D2-like receptors, but not D1-like receptors, blocked induction of LTD. NMDARs have been shown to play a highly significant role in the temporal summation of short bursts of synaptic stimuli. We demonstrate that this is also the case in the hippocampal-mPFC connection: NMDAR attenuation by bath application of AP5 or induction of NMDAR-LTD considerably reduced temporal summation of synaptic stimuli in a frequency-dependent manner, reducing or abolishing spiking in those cells which had previously fired action potentials. These results demonstrate that dopamine-dependent plasticity of NMDA receptors can profoundly alter input-output characteristics of hippocampal to prefrontal transmission - this may be important for learning and memory and other cognitive processes such as working memory in which this synapse is involved.

Disclosures: **P.J. Banks:** None. **A. Burroughs:** None. **Z.I. Bashir:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

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Program#/Poster: 601.07/D62

Topic: B.08. Synaptic Plasticity

Title: A myosin va mouse mutation acting as a dominant negative disrupts glutamate scaffolding in the hippocampus and abnormal behavior

Authors: *S. PANDIAN, Y. MURATA, J.-P. ZHAO, M. CONSTANTINE-PATON;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstracts: MyosinVa (MyoVA), a vesicular cargo-binding actin motor, is abundant in vertebrates and critical for survival. Elimination of *myo5a* causes early death in mice. Human *myo5a* mutations cause neurological dysfunction, mental retardation and hypomelanation and death in infancy or childhood (Griscelli et al., 1978 and Elejalde et al., 1979). The mouse line Flailer (Flr) expresses, only in brain, a truncated cargo-binding domain of MyoVA. When present in a 1:1 ratio *flr* and *myo5a* genes, cause early seizures, mild ataxia, (Jones et al., 2000) and, in visual neurons (Yoshii et al., 2013), defective long-term synaptic depression (LTD). We now show defective LTD in hippocampal CA1, reductions in hippocampal synaptosome proteins necessary for scaffolding glutamate receptors, and abnormal pup vocalizations and, as adults, repetitive grooming, anxiety, asocial, and defective memory behaviors. Thus, Flr is similar to Tsc1 and PTEN mouse mutants, where LTD loss has been associated with similar neuropsychiatric disorders (Bateup et al., 2011 Takeuchi et al., 2013). However, *flr* is an additional gene whose site-specific removal may identify specific pathways involved in abnormal behaviors in mice, and perhaps, in humans as well.

Disclosures: S. Pandian: None. **Y. Murata:** None. **J. Zhao:** None. **M. Constantine-Paton:** None.

Poster

601. Long-Term Depression

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Program#/Poster: 601.08/D63

Topic: B.08. Synaptic Plasticity

Support: ANR-12-BSV4-0021-01

Title: How plasticity of inhibitory transmission in area CA2 modulates CA1 activity

Authors: *K. NASRALLAH, R. A. PISKOROWSKI, V. CHEVALEYRE;
CNRS UMR8118, Paris, France

Abstracts: The hippocampus is a region of critical importance for memory formation and learning. For decades, studies have focused on a linear model of information transfer to explain hippocampal function: the classical trisynaptic pathway which successively recruits the

enthorinal cortex (EC), the dentate gyrus (DG), CA3 pyramidal neurons (PNs) and CA1 PNs. However, this trisynaptic cortico-hippocampal loop in which area CA2 is excluded is not sufficient to explain hippocampal function. Furthermore, very recent studies have revealed a role of CA2 area in certain forms of memory. However, the understanding of CA2 function is just emerging. CA2 PNs are connected by CA3 input, and like CA3 PNs, they project to area CA1. In area CA2, a strong feed-forward inhibition (FFI) controls the Schaffer collateral (SC) synaptic drive onto CA2 PNs and prevents their activation by CA3 PNs. Furthermore, inhibitory transmission from parvalbumin-positive interneurons onto CA2 PNs expresses a delta-opioid-mediated long-term depression (I-LTD). Therefore, we examined whether I-LTD in area CA2 can increase the SC synaptic drive onto CA2 PNs and permit the recruitment of CA2 PNs by CA3 PNs. Our results show that the inhibitory synapses that express the delta-opioid-mediated I-LTD participate to the control of the SC synaptic drive onto CA2 PNs. This I-LTD allows a long-lasting increase in the SC synaptic drive onto CA2 PNs which is sufficient to allow CA2 PNs to fire action potentials in response to CA3 inputs activation, even though synaptic transmission at the SC-CA2 excitatory synapses do not express long-term potentiation. Because area CA1 is connected by both CA2 and CA3, we examine how the SC synaptic drive onto CA1 PNs could be changed by the recruitment of CA2 PNs by CA3 PNs after induction of I-LTD in area CA2. By combining pharmacology, optogenetics and electrophysiology, we show how the delta-opioid-mediated I-LTD in area CA2 directly contributes to CA1 activity.

Disclosures: **K. Nasrallah:** None. **R.A. Piskorowski:** None. **V. Chevaleyre:** None.

Poster

601. Long-Term Depression

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Topic: B.08. Synaptic Plasticity

Support: FCT Grant

CONACYT Fellowship

Title: Bidirectional structural changes driven by protein synthesis-dependent activity at individual spines

Authors: ***Y. RAMIRO**, I. ISRAELY;
Champalimaud Neurosci. Programme, Champalimaud Ctr. For the Unknown, Lisbon, Portugal

Abstracts: Dendritic spines are highly dynamic structures whose morphology and lifespan are modified as a response to synaptic efficacy changes. Structural modifications in response to different forms of activity may underlie the long term encoding of information within neural circuits. Long lasting changes in synaptic efficacy require new protein synthesis, and during synaptic potentiation, lead to long lasting growth of spines. Synaptic tagging and capture between individual inputs allows for the cooperative expression of long lasting plasticity and growth, by sharing new proteins. However, when multiple spines are stimulated closely together in time, they compete for the expression of plasticity, and individual spines oscillate between growth and shrinkage. While the relationship between spine volume and current amplitude are directly correlated, little is known about whether this spine shrinkage represents a true decrease in synaptic efficacy. Here, we determine what are the structural correlates of synaptic depression when new proteins are available. LTD that is mediated by metabotropic glutamate receptors, mGluR-LTD, is known to be long lasting and requires new protein synthesis. We showed that the global induction of mGluR-LTD induces robust shrinkage and elimination of spines, requires new protein synthesis and synaptic activity, occurs at spines of various sizes, and lasts for up to 24 hours. However, it is unknown whether this form of depression can occur at individual synapses, and if there are structural correlates. Using 2-photon imaging and glutamate uncaging in hippocampal neurons, we induce mGluR-LTD at individual inputs, and examine the functional and structural consequences. We test if depression induced at multiple spines within a domain leads to competition that depends on protein availability. Finally, we see how different forms of plasticity interact between spines, to determine the learning rules for bidirectional changes in structure and function. We extend these findings by the use of mouse genetics to manipulate signaling pathways, such as mGluR5 KOs, or levels of available proteins, such as in Fragile X models. Thus, we hope to gain an understanding of how activity can effect long lasting structural remodeling of neural circuits.

Disclosures: **Y. Ramiro:** None. **I. Israely:** None.

Poster

601. Long-Term Depression

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Topic: B.08. Synaptic Plasticity

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Ministerio de Ciencia e Innovación SAF2009-05558-E

Ministerio de Ciencia e Innovación SAF2008-04616

Ministerio de Ciencia e Innovación CSD2010-00045

Fundación Ramón Areces

Title: Role of microtubule-dependent transport in synaptic plasticity

Authors: *A. LARIO[1], J. D. PETERESEN[2,3], D. CHOQUET[2,3], J. A. ESTEBAN[1];
[1]Ctr. De Biología Mol. Severo Ochoa. CSIC - UAM, Madrid, Spain; [2]Univ. Bordeaux, Inst. Interdisciplinaire de Neurosciences, UMR 5297, F-33000, Bordeaux, France; [3]CNRS, Inst. Interdisciplinaire de Neurosciences, UMR 5297, F-33000, Bordeaux, France

Abstracts: The majority of excitatory synapses are located at dendritic spines. Consequently, these are considered key compartments where the machinery for synaptic plasticity will operate. During synaptic plasticity expression, spines alter their morphology and structure, and neurotransmitter receptor trafficking events take place. These phenomena have been related to actin cytoskeleton changes, but recently, microtubules (MT) have been shown to enter the spine in an activity-dependent manner. Therefore, in this study we have evaluated the relevance of the MTcytoskeleton during specific forms of synaptic plasticity. Specifically, we have investigated whether molecular transport along MT participates in neurotransmitter receptor trafficking associated to synaptic plasticity. To address this question, we carried out a series of electrophysiological, molecular and imaging experiments on CA1 excitatory synapses from hippocampal slices. We will present our recent findings suggesting that there is a bidirectional influence between synaptic plasticity and MT dynamics. More precisely, we found that NMDA-receptor dependent LTD is decreased when MT associated transport is impaired. In conclusion, this work pretends to shed some light into the interplay between cytoskeletal elements and the regulation of synaptic strength.

Disclosures: **A. Lario:** None. **J.D. Peteresen:** None. **D. Choquet:** None. **J.A. Esteban:** None.

Poster

601. Long-Term Depression

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Program#/Poster: 601.11/D66

Topic: B.08. Synaptic Plasticity

Support: NIH NRSA 1F32NS067896

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NIH grant R01NS077906

Title: Rab11Fip5, a Rab11 adaptor, is selectively required for hippocampal LTD

Authors: *T. BACAJ[1], M. AHMAD[4,2], S. JURADO[5,2], R. C. MALENKA[2], T. C. SUDHOF[3]; [1]Mol. and Cell. Physiol., [2]Dept. of Psychiatry and Behavioral Sci., [3]HHMI & Mol. and Cell. Physiol., Stanford Univ., Stanford, CA; [4]Dept. of Cell Biol., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [5]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstracts: Postsynaptic AMPA-type glutamate receptors (AMPARs) are among the major determinants of synaptic strength, and can be trafficked into and out of synapses. Neuronal activity regulates AMPAR trafficking during synaptic plasticity to induce long-term changes in synaptic strength, including long-term potentiation (LTP) and long-term depression (LTD). Rab family GTPases regulate most membrane trafficking in eukaryotic cells and, particularly, Rab11 and its effectors are implicated in mediating postsynaptic AMPAR insertion during LTP. To explore the synaptic function of Rab11Fip5, a major neuronal Rab11 effector and a candidate autism-spectrum disorder gene, we performed shRNA-mediated knock-down and genetic knock-out (KO) studies. Surprisingly, we observed robust shRNA-induced synaptic phenotypes that were rescued by a Rab11Fip5 cDNA, but that were nevertheless not observed in conditional KO neurons. Both in cultured neurons and acute slices, KO of Rab11Fip5 had no significant effect on basic parameters of synaptic transmission, indicating that Rab11Fip5 is not required for fundamental synaptic operations such as neurotransmitter release or postsynaptic AMPAR insertion. KO of Rab11Fip5 did, however, abolish hippocampal LTD as measured both in acute slices or using a chemical LTD protocol in cultured neurons, but did not affect hippocampal LTP. The Rab11Fip5 KO mice performed normally in several behavioral tasks, including fear conditioning, but showed enhanced contextual fear extinction. These are the first findings to suggest a requirement for Rab11Fip5, and presumably Rab11, during LTD.

Disclosures: T. Bacaj: None. **M. Ahmad:** None. **S. Jurado:** None. **R.C. Malenka:** None. **T.C. Sudhof:** None.

Poster

601. Long-Term Depression

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Program#/Poster: 601.12/D67

Topic: B.08. Synaptic Plasticity

Support: NIH Grant RO1 NIAG032320

Title: Effects of BRAG1 X-Linked Intellectual Disability mutations on synaptic function

Authors: *A. N. PETERSEN, J. C. BROWN, N. Z. GERGES;
Cell Biology, Neurobio. & Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstracts: Brefeldin-A-Resistant Arf-GEF 1 (BRAG1) is a post-synaptic protein with a critical role in regulating synaptic transmission and plasticity. BRAG1 is involved in the insertion of recycling GluA2-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) as well as the activity-dependent removal of AMPARs underlying long-term depression (LTD). Mutations of BRAG1 have been identified which result in X-linked Intellectual Disability (XLID). Several of these mutations (R758Q, Q801P, and R863W) are located within the Sec7 domain, which is responsible for BRAG1's guanine nucleotide exchange factor (GEF) activity. An additional mutation (R359C) is found in the IQ motif, a calmodulin binding site. Mutations of both the Sec7 and IQ regions have been shown to reduce the GEF activity of BRAG1, which was found to be required for LTD. However, the effect of these mutations on synaptic function is unknown. In this study we have expressed mutations of BRAG1 in CA1 pyramidal neurons to determine their effects on synaptic transmission and plasticity. We find that the XLID-causing mutations disrupt synaptic function and differentially impact the roles of BRAG1 in AMPAR regulation.

Disclosures: **A.N. Petersen:** None. **J.C. Brown:** None. **N.Z. Gerges:** None.

Poster

601. Long-Term Depression

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS062771

Title: Calcium threshold shift enables frequency-independent control of plasticity by an instructive signal

Authors: *H. TITLEY[1], C. PIOCHON[1], Y. ELGERSMA[2], C. HANSEL[1];
[1]Dept. of Neurobio., Univ. of Chicago, Chicago, IL; [2]Dept. of Neurosci., Erasmus Med. Ctr., Rotterdam, Netherlands

Abstracts: At glutamatergic synapses both long-term potentiation (LTP) and depression (LTD) can be induced at the same synaptic activation frequency, under control of instructive signals that modulate local calcium transients. Synapses maintain the ability to potentiate or depress over a wide frequency range, but it remains unknown how threshold-controlled synaptic plasticity operates when variations of frequency alone cause differences in calcium transient profiles. We addressed this problem at cerebellar parallel fiber - Purkinje cell synapses, which can undergo LTD or LTP in response to 1Hz- as well as 100Hz-stimulation. High-frequency stimulation elicits dramatically larger spine calcium transients than low-frequency stimulation but, regardless of activation frequency, climbing fiber co-activation provides an instructive signal that further enhances calcium transients and promotes LTD. This frequency-independence of the instructive signal requires a threshold shift that results from inhibitory autophosphorylation of calcium/calmodulin-dependent kinase II (CaMKII) at Thr305/306, enabling plasticity control based on relative, not absolute, calcium thresholds.

Disclosures: H. Titley: None. **C. Piochon:** None. **C. Hansel:** None. **Y. Elgersma:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.14/D69

Topic: B.08. Synaptic Plasticity

Support: NSERC Discovery Program 385732-2012

Title: Co-regulation of synaptic plasticity, learning and memory in the mouse hippocampus by D2 dopamine receptor and dopamine transporter

Authors: *J. ROCCHETTI, C. FASANO, E. GUMA, G. DAL BO, E. ISINGRINI, S. EL MESTIKAWY, T. WONG, B. GIROS;
Neurosci., Douglas Res. Ctr., Verdun, QC, Canada

Abstracts: Converging evidences link a dysfunction in mesocorticolimbic dopamine (DA) signaling in cognitive processes to the pathophysiology of mental disorders like schizophrenia. Endogenous levels of DA modulate synaptic plasticity and memory in the rodent hippocampus (HP) but the function of the mesohippocampal DA pathway is not fully understood. We reported that genetic deletion of DA D2 receptors (D2R) or D2R blockade by antipsychotics in the mouse HP induce profound impairments in learning and memory and long-term depression (LTD) in CA1 due to a lack of presynaptic D2R activity (Rocchetti et al, Biol. Psychiatry, 2014, PMID: 24742619). However, antipsychotics action on positive symptoms of schizophrenic individuals is based on the hypothesis that the DA system and D2R are overactivated. A clarification of the antipsychotic effects in mouse models with preexisting DA hyperactivity in the HP is therefore needed. In DAT KO mice, a model of persistent endogenous hyperdopaminergia, LTD is absent in CA1 and mice exhibit impaired behavioral flexibility in the Morris watermaze (MWM). Both deficits are rescued by the antipsychotic haloperidol. To precise the impact of endogenous DA elevation on HP synaptic plasticity, we studied the effect of GBR12935, a DAT blocker on CA1 long-term potentiation (LTP) and LTD *in vitro* in C57BL/6J mice. Recordings in temporal CA1 in presence of GBR (30nM) resulted in no change of LTP. However GBR application blocked LTD expression. Co-application of GBR and sulpiride conversely rescued significant LTD. To assess the behavioral consequences of acute and subchronic elevation of endogenous DA in the HP, we implanted bilateral canulae in the temporal CA1 subfield of naïve mice and tested their memory abilities in the MWM and recognition tasks following chronic injections of GBR. Daily infusion before training resulted in a deficit at the learning phase in the spatial MWM. However, treated mice remembered the platform location during the probe. Mice treated with GBR also exhibited a clear deficit in the novel object recognition test (NORT) and the object novel place preference test (ONPP) after 2 weeks of infusion, but not after acute injection. We are now attempting to rescue recognition memory in the GBR chronically infused animals with an antipsychotic treatment. Altogether, this ongoing study enforces the belief that a modulation of both DAT and D2R activities trigger homeostatic DA changes in the mouse HP function. It could also provide some explanations for reported discordant findings concerning the role of DA in learning and memory and adds new dimensions to this neurotransmitter's involvement in the pathophysiology of mental disorders.

Disclosures: J. Rocchetti: None. **C. Fasano:** None. **E. Guma:** None. **G. Dal Bo:** None. **E. Isingrini:** None. **S. El Mestikawy:** None. **T. Wong:** None. **B. Giros:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.15/D70

Topic: B.08. Synaptic Plasticity

Support: NIDA Grant R01 DA32701 (to MJB)

NIDA Grant R01 DA35821 (to CPF)

Title: Neurotensin induces presynaptic, calcineurin-dependent LTD of dopamine D2 autoreceptor-mediated neurotransmission in midbrain dopamine neurons

Authors: *E. PICCART[1], N. A. COURTNEY[2], C. P. FORD[2], M. J. BECKSTEAD[1]; [1]UTHSCSA, San Antonio, TX; [2]Dept. of Physiol. and Biophysics, Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstracts: Increased dopaminergic signaling is a hallmark of severe mesencephalic pathologies, including schizophrenia and psychostimulant abuse. Application of the modulatory peptide neurotensin to midbrain dopamine neurons transiently increases activity by decreasing dopamine D2 autoreceptor function. However, it is not known how neurotensin affects dendritic dopaminergic neurotransmission that strongly determines dopamine neuron activity. Here, we performed patch-clamp electrophysiology and fast-scan cyclic voltammetry in mouse brain slices to determine the effects of neurotensin on dopamine autoreceptor-mediated signaling. Neurotensin application produced two distinct effects: a transient depression of postsynaptic dopamine autoreceptor signaling and a persistent long-term depression (LTD-DA) of D2 autoreceptor-mediated inhibitory postsynaptic currents (IPSCs). This LTD-DA required activation of the type 2 neurotensin receptor and protein phosphatase calcineurin. NT application increased the paired pulse ratio and reduced somatodendritic dopamine release, suggesting that neurotensin-induced LTD-DA is expressed presynaptically. Surprisingly, we observed that stimulation-induced LTD-DA (which we reported previously) was also dependent on the type 2 neurotensin receptor and calcineurin. The fact that electrically-induced LTDDA is blocked by postsynaptic calcium chelation, in combination with present data, suggests that endogenous neurotensin may act as a retrograde messenger to decrease presynaptic dopamine release. The current research thus provides a mechanism through which increased neurotensin release can produce a long-lasting increase in membrane excitability of midbrain dopamine neurons.

Disclosures: E. Piccart: None. **N.A. Courtney:** None. **C.P. Ford:** None. **M.J. Beckstead:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.16/D71

Topic: B.08. Synaptic Plasticity

Support: Swedish Brain foundation

VR 2009–4477

VR 2010–3100

VR 2009–2289

Title: Nicotinic modulation of striatal synaptic transmission and plasticity in the dorsolateral striatum of rat

Authors: *A. LOTFI, L. ADERMARK;
Psychiatry and Neurochemistry, Univ. of Gothenburg, Gothenburg, Sweden

Abstracts: The ability to change behaviour likely depends on the selective strengthening and weakening of brain synapses, and this complexity and adaptability of neuronal communication is necessary for integrative and higher functions of the brain. Nicotine has repeatedly been shown to enhance cognitive performance in humans and laboratory animals and appears to decrease the threshold for induction of synaptic plasticity in the hippocampus. Thus, nicotine may either strengthen cell processes normally involved in learning or recruit additional processes. The aim for this study was to define the role of nicotine in modulating neurotransmission and endocannabinoid-mediated plasticity in the dorsolateral part of the striatum. *In vitro* electrophysiology was performed on acutely isolated brain slices containing the striatum and the overlaying cortex from juvenile rats (p23-28). Administration of nicotine (0.5-10 μM) depressed striatal neurotransmission in a dose-dependent manner that involved activation of nicotinic acetylcholine receptors (nAChRs), N-Methyl-D-aspartate receptors (NMDA), glycine receptors and gamma-aminobutyric acid (GABA) receptors, but not dopamine D2 receptors. Endocannabinoid-mediated long-term depression, induced by high frequency stimulation, was facilitated in slices pre-treated with nicotine, while short-term depression induced by the cannabinoid 1 receptor agonist WIN55,212-2 (1 μM) was not, suggesting that nicotine promotes endocannabinoid signalling at a level that is upstream from the presynaptic terminal. The data presented here indicates that nicotine enhances endocannabinoid-mediated plasticity by altering striatal microcircuitry and shifting the threshold for endocannabinoid production and release, which could be important for the cognitive enhancing properties of nicotine.

Disclosures: **A. Lotfi:** None. **L. Adermark:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.17/D72

Topic: B.08. Synaptic Plasticity

Support: U01AA16651

R01AA15167

Title: Replication of chronic intermittent ethanol-induced metaplasticity in C57Bl6/J DRD1a-TdTomato medium spiny neurons in the nucleus accumbens shell

Authors: *R. RENTERIA, III[1], T. R. BUSKE[2], R. MORRISETT[2];
[1]UT Austin - Inst. For Neurosci., Austin, TX; [2]Col. of Pharm., Univ. of Texas at Austin, Austin, TX

Abstracts: The purpose of this study was to determine the effects of *in vivo* ethanol exposure on the expression of plasticity in D1 and D2 dopamine receptor expressing medium spiny neurons (MSNs) in the nucleus accumbens (NAc) shell. The output of the nucleus accumbens can be divided into two distinct pathways: the direct and indirect pathways. Medium spiny neurons of the direct pathway express D1 dopamine receptors and project primarily to the VTA. In the indirect pathway, MSNs express D2 dopamine receptor and project to the ventral pallidum. To distinguish the two subtypes of MSNs in the nucleus accumbens we used DRD1a-TdTomato mice that have been back crossed onto C57BL6/J. The use of the transgenic mice allows us to identify MSNs that express D1 dopamine receptors (D1+) and those that do not (D1-). Our previous work in C57BL6/J has shown that the expression of LTD induced by low frequency stimulation is blocked by acute ethanol, while *in vivo* chronic intermittent ethanol (CIE) exposure with the same stimulation protocol results in long term potentiation (LTP). In this study we used whole cell patch clamp electrophysiology to measure excitatory post synaptic currents (EPSCs) in D1+ and D1- MSNs in the NAc shell. Plasticity was induced by pairing low frequency stimulation with post synaptic depolarization. In ethanol naïve mice, D1+ MSNs express LTD while D1- MSNs do not. Mice were treated with 16 hours of ethanol vapor for 4 consecutive days. In slices prepared from ethanol treated mice, D1+ MSNs express LTP. The expression of LTP in D1+ MSNs is accompanied by an increase in the frequency of spontaneous EPSCs as well as an increase in action potential firing in response to fixed current injections. D1- MSNs from ethanol treated mice express LTD and have a decrease in the frequency of spontaneous EPSCs as well as an increase in paired pulse ratio. The reversal in plasticity in D1+

MSNs and the expression of LTD in D1- MSNs may constitute an important neuroadaptation necessary for the expression of alcohol related behaviors.

Disclosures: R. Renteria: None. **T.R. Buske:** None. **R. Morrisett:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.18/E1

Topic: B.08. Synaptic Plasticity

Title: Endocannabinoid LTD at Nucleus Tractus Solitarius excitatory synapses depends on the nutritional status

Authors: A. KHLAIFIA, *F. J. TELL;
CRN2M, CNRS UMR 7286, Marseille, France

Abstracts: Presynaptic long-term depression (LTD) of synapse efficacy generally requires coordinated activity between presynaptic and post-synaptic neurons and a retrograde signal synthesized by the postsynaptic cell in an activity-dependentmanner. In this study, we examined LTD in the rat nucleus tractus solitarii (NTS), a brainstem nucleus that relays homeostatic information from the internal body to the brain. We found that coactivation of N-methyl-D-aspartate receptors (NMDARs) and type 1cannabinoid receptors (CB 1 Rs) induces LTD at the first central excitatory synapse between visceral fibers and NTS neurons.This LTD is presynaptically expressed. However, neither postsynaptic activation of NMDARs nor postsynaptic calcium influx are required for its induction. Direct activation of NMDARs triggers cannabinoid-dependent LTD. In addition, LTD is unaffected by blocking 2-arachidonyl-glycerol synthesis, but its induction threshold is lowered by preventing fatty acid degradation. Altogether, our data suggest that LTD in NTS neurons may be entirely expressed at the presynaptic level by local anandamide synthesis. Ongoing experiments indicates that this form of LTD depends on the nutritional status. Fasting impairs LTD induction while refeeding restores it. We are currently investigating the mechanisms involved in such regulation.

Disclosures: A. Khlaifia: None. **F.J. Tell:** None.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.19/E2

Topic: B.08. Synaptic Plasticity

Title: ATP consumption in molecular reactions of neuronal signaling

Authors: *N. RASUMOV, E. DE SCHUTTER;
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Abstracts: The human brain consumes 10^6 times less energy than the currently fastest supper computer [1], while maintaining a comparable performance in many demanding task [2]. These energetic efficiency has been suggested to result from primitive computations on a molecular level [3]. However, while the importance of ion channels on energy efficiency has been the primary focus of research [4], most computations occur at the molecular level prior to the amplification step and prior to the information transmission. We calculate the amount of energy consumed by such computations and compare their structural and functional properties. As a starting point, we chose the molecular reactions involved in long term depression and using our stochastic model [5] estimate the molecular energy consumption. To compare our feedback loop we investigate the energy consumption of millions of feedback loops in molecular signaling. For the first time we are able to go beyond the current size limit of 15 steps [6] and detect feedback loops with hundreds of molecular reactions. We find that the number of ATPs consumed is related with size of positive feedback loop. We conclude that the energy consumed by the long term depression is only marginally above the physical limit of storing information and above its silicon equivalent of Random Access Memory. Hence, this study provides the first systematic attempt to investigate the energy consumption of information-storing molecular computations and points towards energy efficient motifs for synthetic biology. Acknowledgements Both are funded by OIST GU, Japan. References 1.Niven JE, Laughlin SB: Energy limitation as a selective pressure on the evolution of sensory systems. Journal of Experimental Biology 2008, 211(11):1792-1804. 2.Ferrucci DA: Introduction to "This is Watson". Ibm Journal of Research and Development 2012, 56(3-4):15. 3.Mead C: Neuromorphic Electronic Systems. Proceedings of the Ieee 1990, 78(10):1629-1636. 4.Sengupta B, Stemmler M, Laughlin SB, Niven JE: Action Potential Energy Efficiency Varies Among Neuron Types in Vertebrates and Invertebrates. Plos Computational Biology 2010, 6(7). 5.Antunes G, De Schutter E: A Stochastic Signaling Network Mediates the Probabilistic Induction of Cerebellar Long-Term Depression. J Neurosci 2012,

32(27). 6.Ma'ayan A, Cecchi GA, Wagner J, Rao AR, Iyengar R, Stolovitzky G: Ordered cyclic motifs contribute to dynamic stability in biological and engineered networks. Proc Natl Acad Sci U S A 2008, 105(49):19235-19240.

Disclosures: **N. Rasumov:** A. Employment/Salary (full or part-time):; OIST GU. **E. De Schutter:** A. Employment/Salary (full or part-time):; OIST GU.

Poster

601. Long-Term Depression

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 601.20/E3

Topic: B.08. Synaptic Plasticity

Support: NIH Grant EY012347

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American Heart Postdoctoral Fellowship 11POST7020009

Brain & Behavior Foundation NARSAD Young Investigator Grant 20748

Title: Ca^{2+}/Calmodulin promotes postsynaptic release of PSD-95 by preventing palmitoylation

Authors: *L. MATT[1], Y. ZHANG[2], T. PATRIARCHI[1], D. K. PARK[1], D. CHOWDHURY[1], Z. A. MALIK[1], J. B. AMES[2], J. W. HELL[1];
[1]Sch. of Medicine, Dept. of Pharmacol., [2]Dept. of Chem., Univ. of California Davis, Davis, CA

Abstracts: Postsynaptic density protein-95 (PSD-95) is essential for anchoring AMPA and NMDA receptors to the glutamatergic postsynapse. It is rapidly relocated from dendritic spines following Ca^{2+} influx through NMDA receptors. Using pull-down and protein interaction studies we identified Ca^{2+}/Calmodulin (CaM) binding to residues 1-13 of the PSD-95 N-terminus. A structural model based on NMR data predicts that the first 16 residues of PSD-95 assume an alpha-helical conformation upon CaM binding and that CaM tightly interacts with Tyr12 of PSD-95 blocking access to the palmitoylation sites at Cys3 and Cys5. Confirming the model's prediction, rapid de-pamitoylation of PSD-95 following NMDA-treatment of acute forebrain slices is prevented by CaM inhibitors. Furthermore, mutating Tyr12 of PSD-95 to Glu (Y12E)

results in a CaM binding-deficient protein, which is no longer de-palmitoylated upon stimulation. Glutamate stimulation of cultured neurons leads to a rapid relocation of overexpressed wildtype PSD-95 from dendritic spines. This re-location is not observed for the CaM binding-deficient Y12E mutant. Interestingly, after stimulation, the concentration of Y12E PSD-95 in synaptic spines is even increased. In summary we find that the block of PSD-95 palmitoylation by CaM binding promotes the Ca^{2+}-induced dissociation of PSD-95 from the postsynaptic membrane. We believe that this increase in PSD-95 mobility contributes significantly to postsynaptic plasticity after NMDA receptor activation and Ca^{2+} influx by increasing structural flexibility.

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Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.01/E4

Topic: C.06. Developmental Disorders

Support: MH092696-01

AR100276

Title: Predictive and reactive precision grip force control in individuals with autism

Authors: *Z. WANG, G. MAGNON, R. GREENE, J. SWEENEY, M. MOSCONI;
Dept. of Psychiatry, UT Southwestern Med. Ctr., Dallas, TX

Abstracts: Sensorimotor abnormalities are present in the majority of individuals with autism spectrum disorders (ASD). The control processes and neurological substrates underlying these dysfunctions are not well understood. Here, we examined predictive and reactive visuomotor force production in 31 individuals with ASD and 21 healthy controls matched on age, sex and handedness. Participants pressed on opposing load cells with their thumb and index finger and received visual feedback in the form of a horizontal line that moved upwards on a monitor in front of them when they increased their force level. Participants were instructed to press on the load cells so that the horizontal line reached the level of a parallel, static target line. Each participant completed trials in which the target force level was set to 15%, 45% or 85% of their maximum force. Predictive control was examined by measuring the accuracy of initial (i.e.,

primary) responses completed prior to visual feedback being processed. Reactive control was examined by measuring the accuracy of participants' force level after making corrective adjustments to reach the target force level subsequent to the primary response. There were no differences in the accuracy of primary responses or the number of corrective adjustments made following the primary response for individuals with ASD and healthy controls. But, after completing their corrective adjustments, individuals with ASD overshot targets at 15% MVC compared to controls, and undershot targets at 85% MVC. Individuals with ASD also took longer to complete their corrective adjustments at 15% MVC. There were no differences in the peak rate of force increase during the primary response or during the total force increase phase. These results indicate that individuals with ASD are unable to precisely adjust their force output to task demands due to deficits making reactive adjustments to force levels based on visual feedback. Given that they applied the same number of corrective pulses as healthy controls, ASD participants appear to be able to perceive and utilize online visual feedback. However, they either fail to map their compensatory motor commands to the perceived performance error, or they are not able to execute the corrective motor commands as accurately and efficiently as healthy individuals. This reactive precision grip force control impairment in ASD is similar to the profile of grip force imprecision reported in multiple studies of patients with cerebellar disease. These findings thus provide new evidence for visuomotor alterations in ASD consistent with functional disturbances of the cerebellum.

Disclosures: Z. Wang: None. **G. Magnon:** None. **R. Greene:** None. **J. Sweeney:** None. **M. Mosconi:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.02/E5

Topic: C.06. Developmental Disorders

Support: NARSAD, Young Investigator Award (AWARD NO.: 18803)

Title: RBM8a regulates anxiety and social behaviors

Authors: *C. MCSWEENEY[1], A. ALACHKAR[2], P. D. HULLIHEN[1], D. ZOU[1], Y. ZHOU[1], F. DONG[1], D. DENG[1], Y. MAO[1];
[1]Penn State Univ., State College, PA; [2]Univ. of California Irvine, Irvine, CA

Abstracts: Nonsense-mediated mRNA decay (NMD) is an RNA surveillance mechanism that ensures the degradation of mRNAs carrying premature termination codons (PTCs). This mechanism relies on several factors, which form a tetramer known as the exon junction complex (EJC). Mutations in multiple EJC factors have been reported to cause X-linked mental retardation and autism (Tarpey et al., 2009; Laumonnier et al., 2010; Addington et al., 2010). This strongly indicates a potential role for NMD in the pathogenesis of autism. Various behavioral assays can be used to assess behaviors typically associated with neuropsychiatric disease. These tests include the open field test (anxiety), social interaction task (social preference), and contextual fear conditioning (learning and memory). Due to multiple papers associating core NMD factors with autism and mental retardation, we suspect that RBM8a (an EJC factor) may be involved in autism. In order to probe how RBM8a affects mouse behavior, WT mice were injected with RBM8a overexpression lentivirus or RBM8a shRNA lentivirus, targeted to the dentate (bilateral) and assessed on a variety of behavioral tasks. RBM8a overexpression and knockdown mice were found to exhibit an anxious phenotype when tested in the open field ($p<0.05$ for both groups). In the social interaction task, RBM8a overexpression mice were found to have impaired social interaction, while RBM8a knockdown mice exhibited enhanced social interaction ($p<0.05$ for both groups). In addition, RBM8a knockdown mice showed deficits in learning and memory in the contextual fear conditioning task ($p< 0.05$). The behaviors found to be altered in RBM8a overexpression and knockdown mice parallel those seen in autism (anxiety, sociability, learning and memory). Further dissection of the behavioral phenotypes associated with these mice, in addition to investigating the molecular mechanisms that cause these phenotypes may provide insights into causes of autism and mental retardation.

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Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.03/E6

Topic: C.06. Developmental Disorders

Title: Yoga as a co-therapeutic approach for autism spectrum disorders

Authors: *I. J. ROSEMBERG GARCIA[1], A. RUIZ-GARCÍA[2], J. VÁZQUEZ-RAMÍREZ[3], M. CORZO[4], P. ZÁRATE-GONZÁLEZ[5], H. SÁNCHEZ-CASTILLO[2];

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Abstracts: Is well know that children with Autism Spectrum Disorders (ASD) show alterations related with social interaction, affective regulation, language, stereotyped and anxious behaviors, between others. Most ASD therapies are based on behavioral treatment and those has shown to be effective specially in stereotyped, anxious behaviors and affective regulation. However it is important to considerer new alternative therapies, which can ameliorate symptoms observed in ASD. Moreover It has been shown that yoga has great benefits for mental and physical health, like cognition improvement in dementia patients, improve on anticipatory responses, upturn in adaptative skills, reduction of depressive symptoms and betterment in Attention Deficit Hiperactive Disorder. The aim of this study assess if yoga reduces symptoms related to ASD in children. Our sample includes eight children between 3 to 9 years old who practice yoga sessions ones a week per one hour in the National Rehabilitation Intitute of Mexico. Parents were asked to answer a check list related to changes in ADS symptoms in their sons and daughters . Parents refered changes after yoga sessions in following instructions, social interaction and language, these results highlights that yoga benefits ASD patients and shows that could be a complementary treatment in ASD symptoms. Nevertheless it is necessary to apply different evaluation methods that contributed with a deep analisys of the changes in ASD and yoga practice.

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Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.04/E7

Topic: C.06. Developmental Disorders

Support: Max Kade Foundation

ISNR Research Foundation

Title: Using HRV, skin conductance, and neurofeedback training thresholds to quantify progress in children diagnosed with ASD

Authors: *H. COURELLIS[1], A. COURELLI[1], E. FRIEDRICH[2], J. A. PINEDA[2];
[1]Bioengineering, [2]Cognitive Sci., UCSD, La Jolla, CA

Abstracts: Neurofeedback training (NFT) has been shown to elicit behavioral improvement in pediatric patients diagnosed with Autism Spectrum Disorder (ASD). A typical NFT schedule is comprised of multiple 45-min sessions that aim to suppress mu-brainwaves, 8-13 Hz rhythms specific to the sensorimotor cortex. As NFT training progresses, monitoring the outcomes of individual trainees is essential because it can contribute to the customization of the NFT protocol for each patient. A set of physiological measures that can quantify NFT progress includes Heart rate Variability (HRV), skin conductance, and the power of mu, beta, and theta bands present in EEG. HRV has been shown to predict mental effort in Brain-Computer Interface (BCI) activities, while tonic skin conductance is associated with attention tasks in a social context (e.g., during interaction with others). Pediatric patients diagnosed with ASD between 6 and 17 years of age participated in a series of NFT sessions. NFT was implemented through a BCI in which each participant interacted with the computer in a "video game" like scenario. Activity was rewarded if the "player" maintained their mu, theta, and beta brainwave power within specific ranges, which were progressively adjusted to increase the difficulty of the game and, consequently, increase the rigor of the training. The training was designed to downregulate beta and theta power and to upregulate mu power. Each session lasted for approximately one hour and EEG brain activity was recorded from one electrode over the right sensorimotor cortex (C4). During a period of five minutes before and five minutes after each NFT session, the participant's heart rate (electrodes placed at left wrist and neck) and skin conductance (electrodes placed at the pointer and ring finger of the left hand) were also recorded. The acquired data was analyzed and mu, theta, and beta training thresholds, the high frequency power of the HRV power spectrum, and the amplitude of tonic and phasic skin conductance were tabulated. In a number of cases, as the training progressed, the high frequency power of HRV showed a noticeable increase in pre- and the post- assessments, suggesting that the performance in social cognition of the participants (as implemented by the "video game") had improved. This observation was supported by the amplitude of the tonic skin conductance which was significantly elevated after the training. Finally, thresholds leading to successful upregulation for mu and downregulation for beta and theta brainwaves corroborated the level of progress that was achieved.

Disclosures: H. Courellis: None. **A. Courelli:** None. **E. Friedrich:** None. **J.A. Pineda:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

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Program#/Poster: 602.05/E8

Topic: C.06. Developmental Disorders

Support: Supported by the Innovative Medicines Initiative Joint Undertaking (n° 115300), resources composed of financial contribution from the EU 7th Framework Program (FP7/2007-2013), from the EFPIA companies in kind contribution and from Autism Speaks.

Title: The role of dietary polyunsaturated fatty acids in the pathogenesis and treatment of autism spectrum disorders

Authors: K. VAN ELST[1], J. E. MERKENS[1], H. BRUINING[1,2], B. BIRTOLI[3], C. TERREAUX[3], J. BUITELAAR[4], *G. M. RAMAKERS[5], M. J. H. KAS[1];
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Abstracts: The last decades have shown a spectacular rise in the autism spectrum disorders (ASD) prevalence that cannot solely be explained by known factors such as broader diagnostic criteria, diagnostic accretion or higher parental age. Further, environmental factors, including dietary composition, may also play a role in ASD pathogenesis. The rise in ASD seems to parallel changes in the diet. The replacement of cholesterol by omega-6 (n-6) polyunsaturated fatty acids (PUFAs) resulted in a drastically disturbed ratio of n-6/n-3. It has been shown that depletion of omega-3 (n-3) PUFAs, especially during early life, induces aberrant developmental changes in brain connectivity, synaptogenesis, behavior and cognition. We reasoned that the recent changes in fatty acid composition may be contributing to the etiology of neurodevelopmental conditions such as, among others, ASD. To investigate this, a longitudinal behavioral and neurodevelopmental study in 2 mouse inbred strains, C57BL/6J and BTBR T+ Itpr3tf/J, has been performed. The animals received a dietary supplementation or depletion of n-3 PUFAs in chow, thereby decreasing or increasing the n-6/n-3 PUFA ratios, respectively. Diets were given to the mothers prior to pregnancy and were continued throughout life. The effects of the different diets were measured in various ways: firstly, by assessing animal behaviors inside and outside the ASD behavioral domains of repetitive and social behavior by applying an extensive longitudinal behavioral test battery. Secondly, by taking blood samples at different developmental stages and parallel to the test battery, to determine the PUFA peripheral concentrations over time. The diets had the expected effect on PUFA blood concentrations in

mice, as blood sample PUFA ratios were in the same directions as in the diets. Preliminary findings indicated that n-3 PUFAs have a positive effect on behavioral outcome related to the autistic domain of repetitive behavior and on the cognitive domain during early life. Currently, changes in brain morphology and composition as a function of dietary exposure are under investigation. This study puts forward the interesting possibility of applying dietary intervention in the understanding and treatment of behavioral domains related to neurodevelopmental disorders, such as ASD.

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Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.06/E9

Topic: C.06. Developmental Disorders

Support: PRODEX (Belgium)

IAP (BELSPO, Belgium)

FNRS

ARC (Belgium)

ESA (European Union)

Fondation JED

ANR (France)

Title: Behavioral characterization of internal models and predictive mechanisms in autism

Authors: *C. EGO[1,2], L. BOHOMME[3], J.-J. ORBAN DE XIVRY[1,2], D. DA FONSECA[3], P. LEFÈVRE[1,2], G. S. MASSON[3], C. DERUELLE[3];
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Abstracts: Autism spectrum disorder (ASD) is a group of complex disorders of brain development characterized by difficulties in social interaction, verbal and nonverbal communication and repetitive behaviors. Previous studies reported that autism might also be characterized by a dysfunction in the ability to build prediction, a critical cognitive skill for both behavioral and social functions. Eye movements are useful to study prediction abilities. In the present study, we investigated the integrity of predictive mechanisms in autistic children during ocular tracking of moving objects that were temporarily blanked. Eye movements were recorded in 27 subjects aged from 11 to 21 years old. Among them, 18 suffer from Asperger's syndrome and 9 are aged-matched control subjects. Subjects were instructed to pursue a 0.5 deg red dot at the center of a moving bird stimulus. After an initial fixation and gap period, the target started to move towards the center of the screen at constant velocity. After 600ms, the target was blanked for 800ms and reappeared for 600ms. The target velocity (20 deg/s) and direction (right or left) were kept constant over the 20 trials of each of the 4 blocks to allow subjects to learn the target trajectory. During target blanking, pursuit eye velocity first decayed similarly for both groups. After a few trials, control subjects learned the timing of target reappearance and were able to increase eye velocity in anticipation of target reappearance. ASD subjects did not exhibit this predictive reacceleration, suggesting that they were not able to learn such timing. In all subjects, we observed some variability of the pursuit response during the blanking period. In control subjects, this variability was well compensated by the saccadic system, resulting in a negative correlation between pursuit and saccadic eye displacements during blanking. This negative correlation is a signature of internal models as it occurs in the absence of visual signals. Interestingly, this correlation tended to be weaker in ASD subjects suggesting that internal models might be altered in these subjects. In conclusion, our results suggest that ASD subjects have impaired predictive mechanisms and less reliable internal models.

Disclosures: C. Ego: None. **L. Bohomme:** None. **J. Orban de Xivry:** None. **D. Da Fonseca:** None. **P. Lefèvre:** None. **G.S. Masson:** None. **C. Deruelle:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.07/E10

Topic: C.06. Developmental Disorders

Support: UCL-NIMH Joint Doctoral Training Program in Neuroscience fellowship

NIMH IRP

Title: Social deficits in ASD are linked with greater task-driven neural synchrony under naturalistic conditions

Authors: *K. JASMIN[1,2], S. J. GOTTS[1], Y. XU[3], N. ABDULSABUR[3], S. LIU[3], J. E. INGEHOLM[1], I. W. EISENBERG[1], B. ORIONZI[1], A. R. BRAUN[3], A. MARTIN[1]; [1]NIMH/NIH, Bethesda, MD; [2]Inst. of Cognitive Neurosci., UCL, London, United Kingdom; [3]NIDCD/NIH, Bethesda, MD

Abstracts: A defining feature of autism spectrum disorders (ASD) is difficulty in social situations. However, previous functional brain imaging studies of ASD have relied on pre-recorded visual or auditory stimuli. These studies typically show attenuated brain responses to social stimuli relative to typically developed (TD) controls.. Similarly, rest-state studies typically show weaker correlations between brain regions in ASDs, especially in social areas. Whether the brains of ASD people show less correlation in a naturalistic social context has not been tested. We recruited 18 high-functioning males with ASD age 14-30 and 20 typically developed age and IQ-matched controls (TDs) for an ecologically valid social task: Subjects engaged in free, spontaneous conversations with an experimenter while they were scanned with functional magnetic resonance imaging (fMRI). Both parties could see and hear each other through MR-safe headphones and microphones. We used PCA to isolate and remove large BOLD fluctuations caused by speech-related movement. Cardiac pulse, respiration, head motion parameters, ventricles and localized white matter signals were also regressed from the data to markedly reduce noise while preserving task-related BOLD fluctuations. Across task runs, we correlated each voxel's time series with the mean of all gray-matter voxels in the brain. Correlation maps were then compared by group with an independent samples t-test. This analysis also controlled for degree of motion and percent time spent speaking (Speaking Time; ST), although neither measure differed significantly between groups. During conversation, activity across voxels in ASD brains was overall more highly correlated than for TDs. Increased synchrony was particularly prominent in the right hemisphere for ASDs, while the TDs' correlation maps were predominantly left-lateralized. Our result cannot be explained by differences in speaking time between groups, as ST was well-matched between groups (ASD=47%, TD=48%). Moreover, the ASD subjects who spoke the least showed higher levels of task-driven synchrony and more severe autistic symptoms. In contrast to studies showing links between decreased whole-brain correlation at rest and autism symptomatology, here we demonstrated a reversed pattern during live social interaction: the brains of people with ASDs showed increased task-driven neural synchrony compared to TDs. Moreover, greater synchrony was associated more severe autistic symptomatology. Our results suggest that ASD brains are less differentiated than TD brains, and therefore recruit a more bilateral, spatially distributed set of areas to perform real-world social tasks.

Disclosures: **K. Jasmin:** None. **S.J. Gotts:** None. **Y. Xu:** None. **N. Abdulsabur:** None. **S. Liu:** None. **J.E. Ingeholm:** None. **I.W. Eisenberg:** None. **B. Orionzi:** None. **A.R. Braun:** None. **A. Martin:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.08/E11

Topic: C.06. Developmental Disorders

Support: CNPq/CSF/PDEE

Title: Investigations in neuronal receptors expression in the animal model of autism based on the early blockade of GRPR: Potential role in the behavioral impairments observed in ASD

Authors: *J. PRESTI TORRES[1,2], M.-C. AUDET[3,2], J. JAMES[2], P. KENT[2], Z. MERALI[2]; [1]PUCRS, Porto Alegre, Brazil; [2]Inst. of Mental Hlth. Reseach, Univ. of Ottawa, Ottawa, ON, Canada; [3]Dept. of Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstracts: Autism spectrum disorder (ASD) is a neurodevelopmental disorder defined by impairments in social interaction and social communication, as well as restricted interests and activities. In addition to environmental factors that have been linked to ASD, dysfunction of serotonin processes during early brain development has been suggested to play a role in its etiology. Hyperserotonemia during neurodevelopment was reported to modulate a number of molecular events associated with social interaction impairments and anxiety. Preclinical and human genetic studies have suggested that gastrin-releasing peptide receptor (GRPR) may be associated with this disorder. In this vein, our group has previously shown that pharmacological blockade of the GRPR during the neonatal period in rats produced behavioral features reminiscent of ASD at different stages of development. Importantly, administration of the atypical antipsychotic clozapine reversed the social interaction deficits elicited by neonatal blockade of GRPR, suggesting that social disturbances elicited in this model could be mediated by dopamine and/or serotonin dysfunction.. Here we evaluated the effects of neonatal blockade of the GRPR on social interactions and on the mRNA expression of serotonin receptors in the medial prefrontal cortex (mPFC) of rats at the juvenile stage. Male Wistar rats received intraperitoneal injections of the GRPR antagonist, RC-3095, or of a saline vehicle on postnatal days (PND) 1-10. One set of rats was tested for social interaction on PND30. For the determination of mRNA expression of serotonin receptors in mPFC, a second set of rats

submitted to the same treatment was sacrificed and brain tissue was collected on PND32 Pharmacological blockade of the GRPR during neurodevelopment significantly decreased patterns of social interaction, such as following, sniffing, and play fighting behaviours. As well, this treatment increased the prefrontal expression of serotonin receptors, 5-HT2C, but failed to alter that of other serotonin receptors. This data supports the idea that the social interaction deficits elicited by early blockade of the GRPR may be related to alterations in the serotonin system. Further investigations are needed to characterize alterations in the expression of additional brain receptors that could be related to ASD symptomatology.

Disclosures: J. Presti Torres: None. **M. Audet:** None. **J. James:** None. **P. kent:** None. **Z. Merali:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.09/E12

Topic: C.06. Developmental Disorders

Title: Intrinsic motivation and theory of mind in adolescents with autism

Authors: *M. FIRESTEIN[1], P. F. GERHARDT[2], H. HOCH[3];
[1]Psychiatry, Columbia Univ., New York, NY; [2]JPG Autism Consulting, New York, NY;
[3]Psychology, Barnard Col., New York, NY

Abstracts: Numerous studies have suggested that theory of mind (ToM), a cognitive process by which an individual is able to recognize that others have their own internal mental states that are different than his or her own, is delayed or impaired in individuals with autism. The ability to understand that these internal mental states direct behavior is a crucial skill needed for forming social relationships with others. Thus, it is possible that the social behavior deficits associated with autism may be partly the result of an inability to employ ToM. The purpose of this study was to investigate how intrinsic motivation affects the ability of adolescents with autism to employ ToM during a false-belief task. Specifically, we were interested in manipulating the content of the task, so that the task itself, as opposed to a consequential reward, would be motivating enough to increase the adolescent's attention and desire to engage in the task. Participants (n=4, mean age=14 years) were tested on three conditions of the Sally-Anne Task. Performance on the "standard condition" of the Sally-Anne Task was recorded and compared to performance on two novel, modified versions of the task. These modified conditions were

identical to the standard condition, except that they incorporated personalized and intrinsically motivating objects and people in order to examine how performance might vary depending on the content of the task. In our modified "familiar person condition" neutral dolls were replaced with personalized hand-made dolls of each participant's close friends. In our "intrinsically motivating object condition" we replaced the neutral hidden object (a marble) with each participant's preferred object (e.g. M&Ms). None of the participants passed the false-belief question in the standard condition. Of the four participants, two passed the false-belief question in the familiar person condition and three passed the false-belief question in the intrinsically motivating object condition. We have demonstrated that by including personalized, intrinsically motivating stimuli into the Sally-Anne Task, three adolescents with autism were able to pass this false-belief task and thus demonstrate ToM. The implications of these findings not only provide novel insight into ToM abilities in individuals with autism, but also suggest potential educational techniques that may be advantageous for this clinical population.

Disclosures: **M. Firestein:** None. **H. Hoch:** None. **P.F. Gerhardt:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.10/E13

Topic: C.06. Developmental Disorders

Title: What eye movements reveal about the reliability of the Autism-Spectrum Quotient

Authors: ***J. L. STEVENSON**[1], K. R. HART[2], K. A. WILLIAMS[3], L. S. MULLER[3];
[1]Psychology, [2]Mathematics and Computer Sci., [3]Neurosci., Ursinus Col., Collegeville, PA

Abstracts: The Autism-Spectrum Quotient (AQ) is a 50-item self-report questionnaire that assesses the number of autistic characteristics in adults. Participants respond to statements about autistic characteristics (e.g., I prefer to do things the same way over and over again) using a 4-point Likert scale (i.e., definitely disagree, slightly disagree, slightly agree, or definitely agree). While the AQ was designed to be a screening questionnaire for individuals on the autism spectrum, a growing body of research uses the AQ to characterize neurotypical individuals as either having a high degree of autistic traits or a low degree of autistic traits. The current study investigated the reliability of the AQ in a neurotypical sample. Thirty-six undergraduate students (M = 19.00 years, SD = 0.79; 23 females, 13 males) completed two computerized versions of the AQ. The first version was completed online (M = 111.44, SD = 19.35) and the second was

completed in the laboratory (M = 108.75, SD = 17.80) while participants' eye-movements were monitored using a Tobii T60 eye-tracker. Analyses focused on the five subscales of the AQ: attention switching, communication, attention to detail, imagination, and social skill. Regardless of subscale, male participants displayed a significantly greater change in AQ subscale scores than female participants ($F(1, 34) = 5.61$, $p = .024$; male: 95% CI: 0.48 - 2.29; female: 95% CI: -0.62 - 0.74). A 2 x 5 mixed-design ANOVA was conducted to investigate the effects of gender and subscale on the time spent fixating on the selected option (corrected for the time spent fixating on all possible options). There was a significant interaction between gender and subscale ($F(4, 136) = 3.65$, $p = .007$). Males tended to spend more time than females fixating on their selected response option for the attention to detail subscale ($p = .06$), whereas females tended to spend more time than males fixating on their selected response option for the social skill subscale ($p = .06$). Additional analyses examining the relation between eye movement patterns and consistency of AQ scores will be presented. These results have the potential to shed insight on the reliability of AQ subscale scores for neurotypical males and females. Furthermore, these results could inform research on whether AQ scores in the neurotypical population reflect state or trait characteristics.

Disclosures: **J.L. Stevenson:** None. **K.R. Hart:** None. **K.A. Williams:** None. **L.S. Muller:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.11/E14

Topic: C.06. Developmental Disorders

Support: The Danish Ministry of Science, Innovation, and Education (MINDLab)

The A.P. Moller Foundation for the Advancement of Medical Science

Title: Autistic behavior in adolescent Fmr1 knockout mice - With parallels to the valproate rat model of autism

Authors: E. SORENSEN[1], T. BANKE[2], F. BERTELSEN[1,3], J. SCHEEL-KRUGER[1], A. MOLLER[1,3], *K. DRASBEK[1];
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Abstracts: The prevalence of the currently incurable developmental disabilities, known as autism spectrum disorders (ASDs), has been increasing during the past years. This steep rise in the prevalence of ASDs is foreseen to continue in the next decade thereby necessitating a better understanding of the background of ASDs, hopefully leading to the development of effective treatment strategies. Animal models present valuable tools in uncovering the etiology of ASDs as well as platforms for testing novel intervention strategies. The aim of this study is to evaluate and compare a genetic and a pharmacologic animal model of ASDs. The Fmr1 knockout (KO) mouse model is a monogenic animal model that mimics the fragile X syndrome (FXS). This model exhibits developmental abnormalities affecting cognitive functions including learning disabilities, anxiety, and hyperactivity, which are all symptoms related to autism. About one third of human FXS cases exhibit ASDs. In parallel, we will study the valproate (VPA) rat model of autism that is induced by VPA exposure during embryogenesis. As presented previously by our group, daily injections of clinically relevant doses of VPA in pregnant rats cause several autistic features in the offspring, including a 60 % reduction in play behavior during adolescence. In the human clinic, alterations in play behavior are one of the first signs seen in autistic children. Using a C57BL/6 mouse background, autistic behavior in adolescent (5-9 weeks) Fmr1 KO mice is studied using a battery of behavioral tests. Firstly, anxiety level was assessed using the marble burying test. It showed that Fmr1 KO mice had a lowered anxiety level as they buried less marbles than wildtype mice. Currently, we are establishing tests for social interaction and preference for social novelty (3-chamber test) and recognition memory (novel object recognition test). It is generally accepted that autism has a largely genetic etiology, but environmental factors also have a significant effect, which is utilized in the VPA rat model of autism. Most behavioral tests are originally developed in rats and the relatively high anxiety level seen in the C57BL/6 mouse can potentially interfere with the mice's performance when testing for sociability and memory functions. Therefore, anxiety must be extensively studied and conditions should be optimized to reduce stress and anxiety during testing. Until now, most behavioral research in the Fmr1 KO mouse has focused on adult mice. In humans, autistic symptoms appear very early in life. Therefore, to be valid as an animal model of autism, the Fmr1 KO mouse should have autistic behavior in adolescence.

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Poster

602. Autism Behavioral Analysis I

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.12/E15

Topic: C.06. Developmental Disorders

Support: College of Arts and Sciences, Quinnipiac University

Faculty for Undergraduate Neuroscience

Title: Perinatal exposure to benzyl butyl phthalate (bbp) induces alterations in neuronal development/maturation proteins, estrogen responses, and fear conditioning in rodents

Authors: D. DEBARTOLO[1], S. JAYATILAKA[2], R. VONTELL[4], R. RAMOS[5], M. ROSE[2], N. SIU[5], R. MERVIS[6], *A. J. BETZ[3];
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Abstracts: Benzyl Butyl Phthalate (BBP) is an industrial plasticizer that has an unknown action in the central nervous system. Phthalates have recently been associated with behavioral actions that are linked to their endocrine disrupting properties. The purpose of this study was to investigate the behavioral, anatomical and molecular effects of indirect perinatal BBP exposure in offspring of BBP treated pregnant dams. We administered BBP (10.0 μg/ml) in sweetened food pellets to pregnant dams until pups were weaned at post natal day 23. In Experiment 1, offspring were sacrificed at PND23 and we found a significant decrease in anogenital length and a decrease in body weights in both male and female exposed offspring, suggesting BBP exposure s an leads to reproductive abnormalities. Further analysis showed no migrational or lamination deficits in the hippocampus or cortex of exposed male offspring, but nickel staining with MAP2 of the hippocampus demonstrated unusual blunting of axons. In Experiment 2, offspring were sacrificed at PND65 and we found an increase in serum levels of 17β-Estradiol in male offspring suggesting BBP can invoke changes in the endocrine system. BBP exposed offspring have a decrease freezing response in tests of fear conditioning and no gross motor changes. Lastly, we found altered protein levels associated with normal neuronal development, synaptic plasticity and dendritic morphology in the amygdala, dorsal and ventral hippocampus. MeCP2 and Ube3a were found to be dysregulated in exposed offspring are also targets of investigation in Autism Spectrum Disorders. Our studies suggest that indirect perinatal BBP exposure to offspring disrupts normal learning and social behavior. These effects could be related to alterations in brain development. These findings indicate a compelling need for evaluation of acceptable levels of exposure to phthalates present in the environment.

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Poster

602. Autism Behavioral Analysis I

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Topic: C.06. Developmental Disorders

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NSC Grant 101-2627-B-002-002

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National Taiwan University 10R81918-03

National Taiwan University 101R892103

National Taiwan University 102R892103

Title: Altered amygdala-cortical gray-matter structural covariance in males with autism spectrum disorder

Authors: *Y. C. CHEN[1], ,. Y. LIN[2], J. O. S. GOH[3], S. S. F. GAU[2];
[1]Dept. of Psychiatry, Natl. Taiwan university Hosp. & Col. of Med., Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Psychiatry, Natl. Taiwan Univ. Hosp. and Col. of Med., Taipei, Taiwan; [3]Grad. Inst. of Brain and Mind Sciences, Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstracts: Inter-regional covariation of brain structural volumetry has been suggested to reflect systemic neurodevelopmental architectural processes. In autism spectrum disorder (ASD), several pathophysiological brain structural abnormalities are involved across various regions. In particular, aberrant morphometry of the amygdala underpins social-emotional impairment in ASD. How brain systems structurally covary with the amygdala in ASD remains unclear. In this study, we compared how amygdala morphometry relates to various structural indices across cortical regions between ASD and typically developing (TD) individuals. We hypothesized that to the extent that aberrant amygdala structure captures systemic neurodevelopmental disorders in ASD, positive associations should be observed across various cortical areas. By contrast, in TD, amygdala structure should be less associated with the morphometry of other cortical regions. Structural MRI (3T) images of 117 males with ASD (mean age±SD, 14.6±4.4 years) and 108 TD males (mean age±SD, 15.0±5.9 years), with age ranging from 7 to 30 years. Volume-based

morphometry analysis was implemented using Freesurfer ver. 5.2.0, which parcellated gray matter into various brain regions including cortical areas and amygdala for each participant. Multiple regression models were then used to assess the differences in covariance between the amygdala and cortical volumes between ASD and TD males, controlling for age, intelligence and intracranial volume. All imaging results were corrected for multiple comparisons by Monte Carlo simulation ($p<0.05$). In the TD group, the volume of the left amygdala positively correlated only with left entorhinal gyrus volume. In the ASD group, right amygdala volume positively correlated with bilateral rostral middle frontal, the left superior frontal, left superior parietal, and right lateral occipital areas. In direct group comparisons, co-varying relationships between the left superior parietal gyrus-left amygdala pair, alongside the left rostral middle frontal gyrus-right amygdala and the superior parietal lobule-right amygdala pairs, was significantly greater in ASD than TD males. In general, amygdala-cortical structural correlations were more widespread and positive in ASD, while the relationships were reduced or even negative in the TD group. Our findings point to a systemic pattern of neuropathological gray matter development across various brain regions in ASD that is distinct from more heterogeneous inter-regional morphometry in TD individuals.

Disclosures: **Y.C. Chen:** None. **„Y. Lin:** None. **J.O.S. Goh:** None. **S.S.F. Gau:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.14/F2

Topic: C.06. Developmental Disorders

Support: NIH Grant R01MH099660

Title: Over-expression of Tbx1 or COMT in the mouse hippocampus partially recapitulates behavioral phenotypes of 22q11.2 duplication

Authors: ***S. BOKU**[1], T. IZUMI[1], T. TAKAHASHI[1], G. KANG[1], A. HISHIMOTO[1], A. NISHI[1], S. KATO[2], K. KOBAYASHI[2], K. TANIGAKI[3], T. HIRAMOTO[1], N. HIROI[1]; [1]Psychiatry and Behavioral Sci., Albert Einstein Col. of Med., Bronx, NY; [2]Fukushima Med. Univ., Fukushima, Japan; [3]Shiga Med. Res. Inst., Moriyama, Japan

Abstracts: Duplication of human chromosome 22q11.2 is associated with high rates of autism spectrum disorders (ASDs) and intellectual disability (ID). Because the duplicated region

contains more than 30 genes, specific 22q11.2 genes that contribute to these neuropsychiatric disorders are not understood in humans. We tested the hypothesis that over-expression of two 22q11.2 genes, the transcription factor Tbx1 and catechol-O-methyl-transferase (COMT), in the hippocampus contributes to behavioral phenotypes relevant to these disorders in mice. Because our own analysis showed that Tbx1 is enriched in postnatal and adult neural progenitor cells and Comt mRNA is present in postnatal neural progenitor cells, we designed a lentiviral vector that carried Tbx1 or COMT, together with enhanced green fluorescent protein (EGFP), under murine stem cell virus (MSCV) promoter. These vectors were infused into the hippocampus of C57BL/6J mice and mice were tested 10 days later for social interaction, anxiety-related behavior in the elevated plus maze, working memory in spontaneous alternation and motor activity and thigmotaxis in an inescapable open field. The vectors preferentially infected postnatal neural progenitor cells in the hippocampal dentate gyrus, as evidenced by colocalization of nestin and EGFP of the vector. Tbx1 over-expression increased social interaction, reduced anxiety-related behavior and impaired working memory at 2 months of age; it had no effect on thigmotaxis or motor activity. Over-expression of COMT impaired working memory and elevated basal levels of anxiety-related behaviors and thigmotaxis at 2 months, but not 1 month of age; it had little effect on other behaviors. Our data suggest that 1) Tbx1 over-expression in postnatal neural progenitor cells in the hippocampus contributes to working memory deficits but paradoxically opposes social interaction deficits associated with 22q11.2 duplication and 2) COMT over-expression selectively impairs working memory and elevates anxiety-related along developmental trajectory. Our data suggest that a dose elevation of these two 22q11.2 genes induces distinct behavioral phenotypes related to ASDs and ID during postnatal development.

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Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

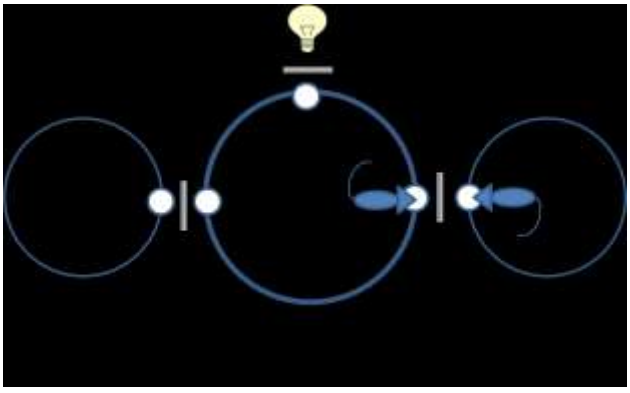
Program#/Poster: 602.15/F3

Topic: C.06. Developmental Disorders

Title: Sensory and social reinforcement monitor for identification of an autism-like phenotype in rats

Authors: *J. B. RICHARDS[1], J. PERON[2], R. WANG[3], D. R. LLOYD[4], R.-Y. SHEN[4], S. HAJ-DAHMANE[4];
[1]Res. Inst. On Addictions, BUFFALO, NY; [2]Ctr. for Assistive Technol., [3]Res. Institue on Addictions, [4]Res. Inst. on Addictions, State Univ. of New York at Buffalo, Buffalo, NY

Abstracts: Stereotyped behavior and impaired social interaction are core diagnostic features of autistic spectrum disorder (ASD). It has been hypothesized that sensory reinforcement underlies the occurrence of repetitive stereotyped behaviors that are characteristic of ASD. In rats an established measure of sensory reinforcement is responding to produce a light onset. Rats display a large variety of social approach behaviors. Here we described a novel operant methodology designed to compare the relative frequency of responding for sensory and social stimuli. Rats emit separate (but topographically equivalent) responses to gain access to a stimulus rat (social reinforcer) or light onset (sensory reinforcer). We hypothesize that this methodology can measure an ASD-like phenotype in rats. An ASD-like phenotype would be indicated by high levels of responding for light onset and low levels of responding for access to a conspecific. The figure illustrates the apparatus that we used to measure choice between a social reinforcer (a rat), a sensory reinforcer (light onset) and a control (empty compartment). The three holes in the center compartment are blocked by metal flaps that can be locked down by electromagnets to control access to the social and sensory stimuli. Turning off the electromagnet releases the flap and allows access. Infrared sensors monitor snout pokes. During testing access to sensory and social reinforcers is contingent upon snout poking according to concurrent Variable Interval 2 min schedules of reinforcement. Six rats were tested with light alone, stimulus rat alone, or with concurrent light and stimulus rat. Dependent measures were the number pokes and the duration of contact with response contingent stimuli while the blocking flap was released. Rats responded at higher rates into the holes associated with sensory and social stimuli than control holes. These results demonstrate that the methodology asses the effects of social and sensory stimuli presented both alone and simultaneously in rats.



Disclosures: J.B. Richards: None. **J. Peron:** None. **R. Wang:** None. **D.R. LLoyd:** None. **R. Shen:** None. **S. Haj-Dahmane:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.16/F4

Topic: C.06. Developmental Disorders

Title: Hyper-fear memory formation is associated with hyper-plasticity in the amygdala in the VPA rat model of autism and hypo-fear memory formation is associated with hypo-plasticity in the amygdala in the MAM model of schizophrenia

Authors: *D. LA MENDOLA, V. DELATTRE, H. MARKRAM, K. MARKRAM;
EPFL, Lausanne, Switzerland

Abstracts: Autism and schizophrenia are two neurodevelopmental disorders associated with changes in cognition and emotion as well as impaired social abilities. Animal models of both disorders can be induced during gestation by injection of valproic acid (VPA) or methylazoxymethanol (MAM), respectively. Offspring exposed to either these drugs exhibit substantial neuroanatomical, behavioral and electrophysiological symptoms of autism and schizophrenia. In this study we compared rat models of autism (VPA) and schizophrenia (MAM) on a social interaction task and in a fear conditioning paradigm. We observed that both VPA- and MAM-treated offspring exhibited reduced social interactions, but that fear conditioned memories were increased in the autism model, whereas they were decreased in the schizophrenia model. Fear memory formation is mediated by the amygdala, and we hypothesized that the amygdala is differentially affected in the two rat models of autism and schizophrenia. We then compared long-term potentiation (LTP) in the amygdala in both rat models, VPA and MAM, using the patch-clamp technique paired with extracellular stimulation in acute brain slices *in vitro*. We report significantly increased LTP in the VPA rat model of autism and significantly decreased LTP in the MAM rat model of schizophrenia. In the VPA rat model of autism, the observed amygdaloid hyper-plasticity agrees with the hyper-fear memory formation, whereas the amygaloid hypo-plasticity agrees with the hypo-fear memory formation in the MAM model of schizophrenia. The speculate that autism and schizophrenia may lie on opposite extremes of one neurophysiological spectrum, that in both cases leads to similar apparent symptoms, such as social withdrawal, but might be caused by opposite neurophysiological activity patterns in the amygdala.

Disclosures: D. La Mendola: None. **V. Delattre:** None. **H. Markram:** None. **K. Markram:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.17/F5

Topic: C.06. Developmental Disorders

Support: FNP Grant HOMING PLUS/2012-6/6

PSPB 210/2010

Title: Transfer of remote emotional information in a mouse model of autism

Authors: *K. Z. MEYZA, T. NIKOLAJEW, K. KONDRAKIEWICZ, K. ZIEGART, A. PUSCIAN, E. KNAPSKA;
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Abstracts: Autism spectrum disorder (ASD) is a behaviorally defined neurodevelopmental disorder diagnosed by two major clusters of symptoms: 1. deficits in social communication and social interaction, and 2. restricted, repetitive patterns of behavior (DSM-V 299.0). The first set is responsible for the lack of empathy observed in autistic patients. Even though empathy is considered a human-specific trait, growing evidence suggests that it is possible to develop animal models of this phenomenon. Here we used a behavioral paradigm developed initially for rats (Knapska et al. 2006, 2009) to test empathy-like behavior in highly social c57BL/6J (B6) mice and a well-defined mouse model of ASD, the BTBR T+tf/J (BTBR) mice. The animals were housed in same-strain pairs for 4 weeks prior to the onset of the experiment and habituated (for at least 10 days) to being handled, transported and to a brief separation (10 min each day). On the test day, one of the mice (Demonstrator) was removed from the cage, moved to the conditioning chamber and either left undisturbed or subjected to a fear conditioning training. The other mouse (Observer) was left undisturbed in the home cage. After the animals were reunited in their home cage, their behavior was recorded for 10 minutes. The behavior of the Observers exposed to stressed Demonstrators was strikingly different in B6 and BTBR mice. B6 mice increased the number and duration of contacts, the number of nose-to-nose and nose-to-anogenital sniffs and the number and duration of digging episodes as compared with same-strain Observers exposed to non-stressed Demonstrators. BTBR mice on the other hand reduced the duration of contacts and nose-to-anogenital sniffing and dug less in response to a stressed Demonstrator as compared to a non-stressed one. In order to look for neuronal correlates of these striking behavioral differences we evaluated the expression of c-Fos protein in the medial prefrontal cortex, amygdala and the ventral hippocampus of these mice. While prelimbic and infralimbic medial prefrontal cortex and

the basolateral and central (lateral part) nuclei of the amygdala proved to be involved in empathic behavior, medial and cortical amygdala and the ventral hippocampus did not.

Disclosures: **K.Z. Meyza:** None. **T. Nikolajew:** None. **K. Kondrakiewicz:** None. **K. Ziegart:** None. **A. Puscian:** None. **E. Knapska:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.18/F6

Topic: C.06. Developmental Disorders

Support: NIH grant NS065957

NSF grant IOS0843585

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Title: Effects of a ketogenic diet in BTBR mice using a novel test-retest protocol in the 3-chamber sociability test

Authors: *D. N. RUSKIN, J. H. ALTSCHULER, H. S. REUMAN, S. A. MASINO; Trinity Col., HARTFORD, CT

Abstracts: Sociability can be impaired or abnormal in psychiatric/neurological conditions such as autism, schizophrenia, neurofibromatosis type I and brain injury. Treatments that could aid social functioning would be of great benefit. Mouse models exist for many of these conditions, and there are several ways to assess sociability. For instance, play behavior and ultrasonic vocalization can be studied in juveniles, and a common test for sociability in adults is the 3-chamber test. The relevant apparatus has one center and two side chambers. After a side preference assessment phase, a stranger mouse (novel to the test mouse) is contained in a small enclosure in one side chamber, the opposite side chamber contains an empty enclosure, and the test mouse is allowed to wander among the three chambers. Normal mice prefer to be social and spend more time in the side with the mouse. In contrast, on average, mice with impaired sociability, such as the BTBR mouse model of autism, are asocial and do not have a preference. However, a group of BTBR mice may contain individuals that are social and others that are

antisocial (preference for the empty chamber). To understand the reliability of sociability over time we performed a retest implementation of the 3-chamber test. Mice (either the social control strain CD-1 or the autism model strain BTBR) underwent a 3-chamber test at five weeks of age. Mice were retested at eight weeks of age, with different stranger mice in the small enclosure (to maintain social novelty). In addition, between tests some BTBR mice were fed a Ketogenic diet, a metabolic therapy we have shown enhances sociability in this test in this mouse strain (Ruskin et al 2013 PLOS ONE). We found that CD-1 mice were stably social over the two tests, and BTBR mice on their normal chow diet were stably asocial (no preference as a group) over the two tests. Notably, the Ketogenic diet-fed BTBR mice, confirmed to be asocial as a group in the first test, were significantly social in the second test, confirming the benefits of the ketogenic diet. In addition, if analysis was restricted to the BTBR mice that preferred to spend time in the empty chamber, the ketogenic diet also significantly enhanced sociability in this population of identified antisocial animals. This test-retest implementation might have several useful applications, including reducing animal use, identifying social and antisocial subpopulations before further experimentation, and assessing reversibility of experimental treatments. It may have particular utility in environmental models of animal autism, in which environmental insults early in development may produce inconsistent effects on adult sociability.

Disclosures: D.N. Ruskin: None. **J.H. Altschuler:** None. **H.S. Reuman:** None. **S.A. Masino:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.19/F7

Topic: C.06. Developmental Disorders

Support: DoD AR120065

Title: Effect of GABAAR subtype-selective positive allosteric modulators in the BTBR mouse model of autism

Authors: *R. F. YOSHIMURA, M. B. TRAN, D. J. HOGENKAMP, R. J. EGUSQUIZA, T. K. GEE, K. W. GEE;
Pharmacol., Univ. of California, Irvine, Irvine, CA

Abstracts: Autism spectrum disorder (ASD) is characterized by core behavioral symptoms including deficits in social interaction, communication and repetitive/stereotypical behavior. ASD has significant comorbidities with epilepsy, cognitive impairment and anxiety which suggest the potential involvement of the γ-aminobutyric acid-A receptor (GABAAR). In support of this, studies on ASD patients have reported a downregulation in the enzymatic synthesis of GABA and a selective reduction in GABAAR expression. Amelioration of this deficit in inhibitory neurotransmission could be a promising pharmacological target for the treatment of ASD. The BTBR mouse strain is characterized by a behavioral phenotype with core deficits similar to that of ASD patients. BTBR mice show decreased sociability in the three-chamber social approach paradigm and increased repetitive behavior in the self-grooming paradigm, both of which are reversed using the mGluR5 negative allosteric modulator, GRN-529. We have tested the beta-subunit subtype-selective GABAAR positive allosteric modulator, 2-261, in both the social approach and self-grooming behavioral paradigms and compared it to GRN-529. The data from these studies provide encouragement that novel treatments for ASD can be developed from GABAAR subtype-selective positive allosteric modulators.

Disclosures: R.F. Yoshimura: None. **M.B. Tran:** None. **D.J. Hogenkamp:** None. **R.J. Egusquiza:** None. **T.K. Gee:** None. **K.W. Gee:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

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Program#/Poster: 602.20/F8

Topic: C.06. Developmental Disorders

Support: NIMH Grant 084961

Title: Moment-to-moment task adaptation and maintenance of task set in ASD

Authors: *R. LUDLUM[1], X. YOU[1], K. DUDLEY[2], Y. GRANADER[2], L. KENWORTHY[2], W. D. GAILLARD[2], C. J. VAIDYA[1,2];
[1]Psychology Dept., Georgetown Univ., Georgetown Univ, DC; [2]Res. Inst., Children's Natl. Med. Ctr., Washington, DC

Abstracts: Cognitive control encompasses a range of abilities that allow for flexible allocation of mental resources and direction of behavior depending on one’s current goals. Previous research has shown that cognitive control impairments play a large role in the pathology of

Autism Spectrum Disorders (ASD), which are characterized by social and communication deficits, as well as general mental inflexibility. The current study aimed to further elucidate the role of cognitive control in late childhood and ASD by using a social and non-social conflict adaptation task. Thirty-seven children with ASD and thirty-two typically developing (TD) ages 7 to 15 completed the tasks. Participants were asked to respond to a target word (LEFT or RIGHT) in the presence of task-irrelevant stimuli (an arrow or face with averted gaze) that were congruent or incongruent with the target word. Trials on both tasks were embedded in blocks that varied by percent of incongruent trials, placing demands on either moment-to-moment control (25% incongruent trials) or task-set maintenance (75% incongruent trials). Participants' parents also filled out questionnaires regarding the child's executive and social behaviors, including the Behavior Rating Inventory of Executive Function (BRIEF) and Social Responsiveness Scale (SRS). Accuracy was calculated using the percent of Incongruent trials correctly answered. Accuracy was analyzed separately for the two block types using Group x Task repeated measures ANOVAs. As predicted, ASD participants were more error prone than their TD counterparts on both tasks during the Moment-to-Moment Adjustment blocks, $F(1,67) = 4.81$, p .05. Furthermore, performance on Moment-to-Moment Adjustment blocks for the Arrows task negatively correlated with parent ratings on the BRIEF, $r(59) = -.38$, $p < .05$, and its subscales for Inhibit, $r(59) = -.36$, $p < .05$, Shift, $r(59) = -.25$, $p = .05$, and Emotional Control, $r(59) = -.31$, $p < .01$, as well as the SRS subscales for Social Awareness, $r(62) = -.27$, $p < .05$, and Autistic Mannerisms, $r(62) = -.25$, $p < .05$, suggesting that worse cognitive control skills in daily life were related to greater difficulty making fast adjustments to conflicting non-social stimuli. Performance on Task-Set Maintenance blocks for the Faces task negatively correlated with the BRIEF subscales for Emotional Control, $r(59) = -.34$, $p < .05$, and Monitor, $r(59) = -.26$ $p < .05$, and SRS subscales for Social Awareness, $r(62) = -.25$, $p < .05$, Social Communication, $r(62) = -.29$, $p < .05$, and Autistic Mannerisms, $r(62) = -.25$, $p < .05$, suggesting that worse social skills were related to greater difficulty maintaining a task set with social stimuli.

Disclosures: **R. Ludlum:** None. **X. You:** None. **K. Dudley:** None. **Y. Granader:** None. **L. Kenworthy:** None. **W.D. Gaillard:** None. **C.J. Vaidya:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.21/F9

Topic: C.06. Developmental Disorders

Support: Conacyt Scholarship No. 287846 to CNCC

Title: Impact of virtual stimulation on motor skills of autistic children

Authors: *C. N. CRESPO CORTÉS[1], P. SAFT[2], G. A. CORIA-AVILA[2], L. I. GARCIA[2], M. E. HERNANDEZ[2], J. MANZO[2];
[1]Univ. Veracruzana, Veracruz, Mexico; [2]Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico

Abstracts: Previous studies showed the benefits of virtual stimulation for motor deficits. In autism, some children have motor problems, including low muscle tone. Thus, there is a huge effort to face this challenge. Present work stimulated the movement of 10 autistic children between 4 and 12 years old with a similar diagnosis of autism, from the Child Rehabilitation Center of Veracruz, Mexico. We used the Wii-Nintendo video game to stimulate the subjects with the "Wii sports" for 10 weeks, twice a week, 20 minutes each session. The sports used on the screen were two pairs, two with a fixed target (boxing and bowling), and two with a target in motion (tennis and baseball). At first the facilitator played the games to catch the attention and stimulate the subjects to imitate the movements and to play by themselves. Data collection was made by direct observation and organized in eleven categories according to movements observed in subjects per session. Results indicated that attention was poor at the first four tests. Starting at the fifth test a long attention was observed in all subjects until the end of the study. The fifth session represented also the time when subjects took the remote without any assistance. At session 1 only 30% of subjects could mimic de movement, however at session 7, 90% of the kids were capable to mimic the specific movement for a sport. At session 1 only 10% pressed the A button of the remote, but at session 6 all the individuals press button A to start the game. And this learning remained until the end. Pressing button B was a more complex concept, because the button is not visible, however at the 9th session, 70% of subjects used it. At the beginning no subject could make a movement toward the target, however starting at the 6th session 70% of the kids made a movement toward a target. Subjects became more skilled in sports with a fixed target. Data showed that autistic children are sensitive to virtual stimulation and responds to it following a two-week stimulation, and the acquisition of the skill remains for a long term. Thus, virtual stimulation not only enhanced the motor skill of the subjects but also the process of learning and memory of what is the goal of a challenge. Our hypothesis is that fixed target were more stimulating, and preferred by the subjects, because they can direct a movement to a target, but following a target is a more complex task to achieve. However, we suggest that they will be able to follow targets with further stimulation.

Disclosures: C.N. Crespo Cortés: None. **P. Saft:** None. **G.A. Coria-Avila:** None. **L.I. Garcia:** None. **M.E. Hernandez:** None. **J. Manzo:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.22/F10

Topic: C.06. Developmental Disorders

Support: NIH AA019482

Title: Animals reared in impoverished conditions are slower to habituate to the reinforcing effects of sensory reinforcers

Authors: *R. WANG, J. B. RICHARDS, D. R. LLOYD, S. HAJ-DAHMANE, R.-Y. SHEN; Res. Inst. on Addictions, SUNY at Buffalo, Buffalo, NY

Abstracts: Background Restricted, repetitive and stereotyped patterns of behavior are one of three core diagnostic features of autistic disorder. It has been hypothesized that stereotyped behavior is maintained by sensory reinforcement (Often referred to as automatic reinforcement). Stereotyped behaviors such as “rocking” are displayed to some degree by most individuals. Autistic individuals have more frequent and persistent stereotyped behaviors. We hypothesize that increased frequency and persistence of stereotypy in autism disorder is due to a failure of normally occurring habituation of reinforcer effectiveness. We (Lloyd et al., 2012) have previously reported that habituation modulates responding for a sensory reinforcer (light onset). This research examined how environmental factors can modulate habituation in responding for sensory reinforcers (light onset). Rats were reared in impoverished (IC), enriched (EC), and social (SC) conditions after weaning. Dishabituation challenges were used to rule out motor and/or sensory fatigue explanations of within-session declines in responding. Method EC (n=10), IC (n=16), and SC (n=10) rats were placed into dark test chambers for 1-hour/day. Entries into 2 snout poke holes were counted. During test sessions 1-10 snout poking had no programmed effect. Beginning with test session 11, light onset (5 s) was made contingent upon responding into one of the 2 snout poke holes (active). Dishabituation challenges were performed by presentation of a loud warbling tone and by altering the location of the response-contingent light onset. Results EC rats habituated much more rapidly than SC and IC rats, and responding was higher in SC and IC rats during all phases of the study. Tests of dishabituation produced robust dishabituation in SC and IC rats, and produced smaller inconsistent effects in EC rats. Conclusion The results indicate that the reinforcing effects of sensory stimuli habituate more rapidly in EC than in IC and SC rats, resulting in less stereotypy. The dishabituation challenges

indicate that these differences are not due to adaptation or sensory fatigue. Therefore, enriched environment could be effective in reducing stereotypy in individuals with autistic disorder.

Disclosures: **R. Wang:** None. **J.B. Richards:** None. **D.R. Lloyd:** None. **S. Haj-Dahmane:** None. **R. Shen:** None.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.23/F11

Topic: C.06. Developmental Disorders

Support: NeuroDevNet

CIHR scholarship 290993

FRSQ scholarship 27512

Title: Behavioral motor deficits in juvenile mice induced by unilateral cerebellar haemorrhage and inflammation

Authors: ***S. TREMBLAY**, D. GOLDOWITZ;
CMMT, Vancouver, BC, Canada

Abstracts: Background: Extreme preterm infants are exposed to multiple stressors during their postnatal development including perinatal cerebellar haemorrhage (CBH) and postnatal infection, two major risk factors for neurodevelopmental impairments. In order to understand how cerebellar development is altered by those insults, we developed an animal model using a cerebellar haemorrhagic insult combined with an early or late inflammatory event in mice pups. Methods: Unilateral intraparenchymal CBH was induced by using local injection of bacterial collagenase (0.15U) at postnatal day 1 (P1) combined with intraperitoneal lipopolysaccharide (LPS) injection (300µg/kg) concomitantly (early inflammatory state, EIS) or at P5 (late inflammatory state, LIS). Mice were behaviourally tested on a modified mouse neurological exam every day until P15 and cerebellar tissues were collected at P2 and P15. At P15, tests were performed to further assess locomotor activity, motor coordination and muscular strength. Results: Unilateral cerebellar haemorrhages induced at P1 delays forelimb grasp acquisition in neonatal mouse pups by 24-48 hours and increases locomotor activity in P15 mice during open-field recording (1357±287 vs 2583±562sec, p=0.048). Early inflammatory state induction also

leads to comparable delay in neonatal forelimb grasp acquisition followed by a significant decrease in grip strength (0.036±0.002 vs 0.027±0.003kg, p=0.037) and muscular strength measured by the inverted screen task in juvenile mice (18.4±2.1 vs 8.6±1.9sec, p=0.003). However, late inflammatory state did not induce any significant neonatal or juvenile behavioural impairment. Combined model of cerebellar haemorrhage with either an early or late inflammatory state did not worsen juvenile muscular strength or motor function. Preliminary histological data looking at 24 hours after the haemorrhagic event combined or not with inflammation shows an increase in total microglial cells in the single and double insult models in both hemispheres along with a greater increase of microglial cells in the ipsilateral white matter area in relation with cerebellar haemorrhage. Conclusions: Early insults in the developing neonatal brain alters juvenile motor milestones in a mouse model of cerebellar haemorrhage combined with early systemic inflammatory stress. This new model will allow us to measure short and long-term impact of combined early insults on cerebellar development, their impact on neurobehavior and test neuroprotective strategies to improve extremely preterm infant neurodevelopmental outcomes.

Disclosures: **S. Tremblay:** None. **D. Goldowitz:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.24/F12

Topic: C.06. Developmental Disorders

Support: NIH GRANT 5K02MH070031-11

Title: The role of mTOR signaling in parvalbumin interneuron function

Authors: *A. KOEPPEN-BABCOCK;
Psychiatry, Children's Hosp. of Philadelphia, Philadelphia, PA

Abstracts: Mutations in phosphatase and tensin homolog on chromosome ten (Pten), a tumor suppressor gene that inhibits the mammalian target of rapamycin (MTOR) pathway, have been linked with a subset of autistic patients that exhibit spontaneous seizures and macrocephaly. Recent studies using Nse-Cre conditional Pten knockouts found that haplo-insufficient mice had significant decreases in cytochrome c oxidase activity and increased mtDNA mutations. Because of this increase in mitochondrial stress, GABAergic parvalbumin interneurons, which are fast

spiking and non-accommodating, should be particularly vulnerable to these effects due to their high metabolic needs. To test this idea, PTEN-loxP mice have been crossed to Lhx6-Cre, in which Cre is mainly expressed in PV and SST- interneuron subgroups of the cerebral cortex and striatum. Both knockout and haplo-insufficient mice have larger parvalbumin interneurons in the cortex, hippocampus, and striatum at P21. No apoptosis was observed at P15 or P21. Experiments to measure the electrophysiological activity of parvalbumin interneurons, to quantify the accumulation of mitochondrial mutations, and to examine social behavior of the haplo-insufficient mice are ongoing.

Disclosures: A. Koeppen-Babcock: A. Employment/Salary (full or part-time):; Children's Hospital of Philadelphia.

Poster

602. Autism Behavioral Analysis I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 602.25/G1

Topic: C.06. Developmental Disorders

Support: NIMH 1R01 MH081023

Title: Diagnostic prediction in autism using random forest and classification trees of functional connectivities

Authors: *C. P. CHEN[1], A. JAHEDI[2], R. MUELLER[3];
[1]Computat. Sci. Res. Ctr., [2]Mathematics and Statistics, [3]San Diego State Univ., San Diego, CA

Abstracts: Although autism spectrum disorder (ASD) is considered a neurological disorder, there are no established brain biomarkers. Supervised machine learning approaches to diagnostic classification are ideally suited to reveal potentially complex patterns of biomarkers. We used Random Forest, Classification and Regression Trees on resting state functional connectivity MRI data. While the Random Forest procedure identifies the variables (connections) most informative for diagnostic prediction, interpretation of the interaction between these informative variables requires Classification and Regression Trees (CART). Methods We used resting state fMRI data from 252 low-motion participants (126 ASD, 126 TD) from the Autism Brain Imaging Data Exchange (ABIDE), matched on age, nonverbal IQ, and head motion. FMRI data were motion corrected, aligned to high-resolution anatomical, standardized to the MNI152 template, and blurred to a 6mm global full-width-at-half-maximum. Six rigid-body motion parameters, time

series from white matter and ventricles, and derivatives were modeled as nuisance regressors. Time points with motion >.25mm (and their neighbors) were censored. We chose 220 regions of interest (ROIs) from Power et al. (2011), using 10mm spheres to extract an average signal from each. For each ROI pair, a feature was defined as functional connectivity (time series correlation; 24090 total features). We used the top features selected from binary classification and modeled their interaction. Random forest is a multivariate machine learning method that uses an ensemble of classification trees and aggregates their results, capitalizing on linear and non-linear interactions of features. CART is a decision tree technique for dataset segmentation that provides rules applied to new datasets for prediction. Results RF achieved a peak 91% accuracy with 100 informative features. To reduce complexity, we selected 20 most informative features (83% accuracy) for the CART model. Prominent among these were somatosensory/motor and salience network ROIs, accompanied by ventral attention, default mode, and subcortical ROIs. Conclusions While RF machine learning achieved high diagnostic classification accuracy with features involving >100 ROIs, CART - albeit at a lower accuracy - allowed for improved interpretation of findings with respect to most informative regions and their complex interactions. The pivotal role of ROIs in somatosensory, motor, and salience networks adds novel evidence to the growing literature on these systems in ASD.

Disclosures: **C.P. Chen:** None. **A. Jahedi:** None. **R. Mueller:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.26/G2

Topic: C.06. Developmental Disorders

Support: NIH Grant R01- MH094604

Title: Differences in neuronal activation and gene expression in the fragile X mouse

Authors: ***T. D. ROGERS**, C. G. FORSBERG, J. VEENSTRA-VANDERWEELE; Vanderbilt Univ., Nashville, TN

Abstracts: Abnormal social behavior is a core symptom of autism spectrum disorders (ASD) and fragile X syndrome (FXS). Interestingly, previous studies have demonstrated an association of amygdala activation with both social approach and social avoidance behavior. Further, both functional and structural neuroimaging studies have implicated the amygdala and prefrontal

cortex (PFC) in ASD. It is currently unclear whether molecular changes occurring in the amygdala and PFC could mediate altered social behavior observed in ASD and FXS. We used the Fmr1 knockout (KO) mouse, which displays altered social behavior, to further investigate this relationship. To determine the impact of a loss of Fmr1 expression on neuronal activation patterns, we used immediate early gene activation (cFos) immunohistochemistry and compared neuronal activation patterns in different brain regions across conditions and genotypes following exposure to a sociability task. The presentation of a novel mouse, as compared to the presentation of a novel item, elicited increased levels of activation in the lateral amygdala (LA) and medial amygdala (MA) and decreased levels of activation in the PFC in wildtype animals ($p < 0.05$). Fmr1 KO mice displayed hyperactivation in LA and MA and hypoactivation in PFC ($p < 0.05$) across both behavioral conditions as compared to littermate controls ($p < 0.01$). Additionally, levels of activation in the LA and MA were negatively correlated with those of the PFC ($p < 0.001$ and $p < 0.005$, respectively), while LA and MA activation levels were positively correlated ($p < 0.005$). In order to identify molecular changes that might mediate altered patterns of neuronal activation and abnormal social behavior, we used RNA sequencing to examine differential gene expression in LA, MA, and PFC of mice from each condition and genotype. An analysis of the interaction between condition and genotype indicated that genes that might affect neurotransmission, such as Th, Gad1, and Oxtr, were differentially expressed in the amygdala ($p < 0.05$). This analysis also indicated that multiple autism candidate genes, including Reln, Sema5a, and Met, were significantly differentially expressed ($p < 0.05$). Real-time PCR is currently underway to confirm the differential expression of genes of interest. The current findings indicate that the amygdala and PFC display differences in neuronal activation and gene expression following exposure to social stimuli as compared to non-social stimuli. Differences in gene expression in these two brain areas in response to social challenge may suggest a mechanism for changes in the neuronal activation patterns and altered behavioral responses in Fmr1 KO mice.

Disclosures: **T.D. Rogers:** None. **C.G. Forsberg:** None. **J. Veenstra-VanderWeele:** None.

Poster

602. Autism Behavioral Analysis I

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Program#/Poster: 602.27/G3

Topic: C.06. Developmental Disorders

Support: Histopathology Limited, Hungary

Allegheny College Class of '39 Senior Research Fund

Title: Choroid plexus target-based therapy in rats with chronic hydrocephalus: Use of an aquaporin-1 antibody treatment

Authors: H. L. PHILLIPS[1], S. SURASH[2], M. G. LUCIANO[3], J. CROSS[1], *J. R. HOLLERMAN[1];
[1]Psychology/Neuroscience, Allegheny Col., MEADVILLE, PA; [2]Nuffield Hlth., Newcastle upon Tyne, United Kingdom; [3]Pediatrics and Neurocognitive, Cleveland Clinic, Main Campus, Cleveland, OH

Abstracts: Hydrocephalus is characterized by an excessive accumulation of cerebrospinal fluid (CSF) in the ventricular system, which causes a swelling of the lateral, 3rd and 4th ventricles, also referred to as ventriculomegaly. Current treatments for the disorder include procedures such as implantation of a shunt system or a 3rd ventriculostomy. These options are invasive and tend to fail within three to five years, resulting in multiple surgeries in a patient's life. Aquaporin-1 (AQP1), a water channel showing high expression on the apical surface of the choroid plexus has shown to play an important role in CSF formation. Previous research has predicted that an AQP1 antibody could serve as a potential adjunct therapy in treating patients with hydrocephalus. The aim of the present study was to analyze whether an AQP1 antibody treatment in the right lateral ventricle of a hydrocephalic rat model could improve the common physiological and behavioral deficits characterized by the disorder. Anatomical analysis and immunohistochemistry (IHC) was used to assess uptake of the antibody as well as effects on ventricle size. IHC was also used to assess possible uptake of antibody in another AQP1 expressing organ, the kidney. Behavioral changes were measured with the Morris water maze and the object preference test. Results showed that animals treated with an AQP1 antibody in the right lateral ventricle expressed positive staining, indicating antibody uptake, as well as significantly smaller right and left ventricles nearest the pump implant compared to saline treatment animals. There was no positive staining of the antibody in the kidneys. Behavioral improvements in memory and cognition tasks across the 14-day treatment were observed. This study indicates a viable relationship between AQP1 antibody therapy and improvement in behavioral and physiological symptoms of hydrocephalus.

Disclosures: H.L. Phillips: None. **S. Surash:** None. **M.G. Luciano:** None. **J. Cross:** None. **J.R. Hollerman:** None.

Poster

603. Autism Genetic Models

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Topic: C.06. Developmental Disorders

Support: UCDavisMINDInstitute

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Title: Evaluation of ganaxolone in B6 mice and the BTBR mouse model of autism

Authors: *T. M. KAZDOBA[1,5], D. ZOLKOWSKA[2], M. A. ROGAWSKI[2], R. J. HAGERMAN[3], J. N. CRAWLEY[4];
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Abstracts: Excitatory and inhibitory neurotransmission abnormalities, particularly reduced GABAergic inhibition and fewer inhibitory interneurons, are hypothesized to contribute to the symptoms of autism spectrum disorder. BTBR T+ Itpr3tf/J (BTBR) is an inbred strain of mice that models idiopathic autism. BTBR exhibit deficits in social approach, reciprocal social interactions and associated ultrasonic vocalizations, high repetitive self-grooming, and cognitive deficits. Recently, BTBR mice were reported to show reduced spontaneous GABAergic neurotransmission, and low doses of benzodiazepines rescued sociability deficits in BTBR (Han et al., 2014), suggesting that positive allosteric modulators (PAMs) of $GABA_A$ receptors may improve certain aspects of the autism phenotype. Ganaxolone, a synthetic neurosteroid that acts as a PAM at synaptic and extrasynaptic $GABA_A$ receptors, displays anticonvulsant properties in rodent models of seizure and epilepsy, and has an anxiolytic-like profile in the elevated plus maze (Reddy and Rogawski, 2012). Here we evaluated ganaxolone in BTBR mice and in the control social strain C57BL/6J (B6) to determine whether this PAM of $GABA_A$ receptors affects mouse behaviors relevant to diagnostic and associated symptoms of autism. Acute ganaxolone treatment was assessed for its effects on sociability in the three-chambered social approach task, and for its impact on male-female reciprocal social interactions and concomitant vocalizations, spontaneous repetitive self-grooming, and marble burying and digging. In addition, we assessed anxiety-related behaviors in the elevated plus maze and light↔dark exploration test, and exploratory locomotor activity in a novel open field. Evaluation of novel treatments in the BTBR mouse model, particularly those targeting GABA neurotransmission, may lead to the preclinical identification of compounds that show clinical promise for ameliorating the core symptoms of autism.

Disclosures: **T.M. Kazdoba:** None. **J.N. Crawley:** None. **D. Zolkowska:** None. **M.A. Rogawski:** None. **R.J. Hagerman:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.02/G5

Topic: C.06. Developmental Disorders

Support: NIH Grant MH065702

Title: Social deficits with the Neurofibromatosis type 1 mutation, a role for the extracellular signal-regulated kinase (ERK) pathway

Authors: *D. H. ARENDT[1,2], C. BERNABE[3], L. FEDERICI[3,4], S. FITZ[2], W. D. CLAPP[5,6], A. I. MOLOSH[2,4], P. L. JOHNSON[3,7], A. SHEKHAR[2,7,4,8];
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Abstracts: Neurofibromatosis type 1 (NF1) is a genetic disorder that affects approximately 1 in 3500 individuals and often presents with social and cognitive disabilities. In the past, we have shown that Nf1 knockout mice exhibit deficits in longterm social memory coupled with increased phosphorylation of ERK in Nf1 neuronal cultures. Despite the social deficits, the Nf1 deletion does not impair the animal's ability to distinguish a novel and familiar object. When the P21-activated kinase 1 (Pak1) gene, which also regulates the ERK pathway, was co-deleted with Nf1 (Nf1/Pak1), social deficits seen with a single Nf1 deletion were rescued. Since phosphorylation of ERK is a common feature of both these regulatory genes (Nf1 and Pak1) we further investigated this pathway as it related to social memory. For this work we focused on the amygdala as a range of preclinical and fMRI data have implicated this area in social recognition and memory. Furthermore, our previous work has shown that projection neurons within the basolateral amygdala (BLA) have enhanced miniature inhibitory post-synaptic currents and decreased long-term potentiation in Nf1 knockout mice. Therefore, we hypothesized that acute inhibition of Pak1 with an inhibitor (IPA3) in the BLA would rescue the deficits seen in the Nf1 knockout mice similar to the Nf1/Pak1 co-deletion as we have previously demonstrated. Social memory was tested by utilizing a standard three chamber test for mice. While Nf1 knockout animals did display increased short term preference to the novel mouse with Pir3.5, they did not show a long term social preference. However, when IPA3 (but not Pir3.5, its inactive enantiomer) was administered, long term social memory was restored in NF1 knockouts as

measured by an elevation in contact time with the novel partner. Finally, pharmacologically enhanced levels of pERK within the BLA of WT animals were attenuated with the application of IPA3, further confirming its ability to block ERK phosphorylation. This study adds to a growing body of evidence that ERK activation plays a Key role in social memory. Elucidating this exact mechanism may be critical in treating disorders that present with social deficits.

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Poster

603. Autism Genetic Models

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.03/G6

Topic: C.06. Developmental Disorders

Support: NIHAA022448

Title: Purinergic P2X4 knockout mice exhibit altered expression of dopaminergic proteins. Implications of P2X4 receptors in multiple neurological disorders

Authors: ***S. KHOJA**[1], L. ASATRYAN[2], M. W. JAKOWEC[3], D. L. DAVIES[2];
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Abstracts: Purinergic ionotropic P2X receptors (P2XRs) are homo- and heterotrimeric cation permeable channels activated by extracellular adenosine-5'-triphosphate (ATP). Of the seven subunits of P2XRs, P2X4 receptors (P2X4Rs) are the most abundant in the central nervous system. P2X4Rs have been implicated in regulation of various functions including neuroendocrine functions and hippocampal synaptic plasticity. P2X4Rs have been reported to interact with other major neurotransmitter systems including the GABAergic, glutamatergic and dopaminergic systems. Presently, there is a paucity of information regarding the the role of P2X4Rs in pathophysiology of neuropsychiatric disorders. Using a series of behavioral tests, we reported that P2X4R KO mice exhibit deficits in sensorimotor gating and socio-communication compared to wildtype (WT) mice. Such behavioral deficits are relevant to various neurological disorders including autism spectrum disorders, schizophrenia, and bipolar disorder. In addition, male P2X4R KO mice exhibit higher intake of and preference for ethanol compared to WT mice suggesting that P2X4Rs may contribute to regulation of ethanol intake and play a role in

pathophysiology of alcohol addiction. Furthermore, we reported significant changes in expression of multiple subunits of glutamate ionotropic receptors including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-Methyl-D-aspartate receptors (NMDARs) and of $GABA_A$ receptors in P2X4R KO mice. The current study tests the hypothesis that dysregulated dopamine function may contribute to behavioral deficits in P2X4R KO mice. We measured expression of tyrosine hydroxylase (TH), dopamine transporter (DAT), dopamine D1 and D2 receptors (D1Rs and D2Rs) and dopamine and cAMP regulated phosphoprotein of 32kDa (DARPP-32) in dorsal and ventral striatum, midbrain and prefrontal cortex of P2X4R KO mice using western blotting. P2X4R KO mice exhibited reduced expression of TH in dorsal, ventral striatum and midbrain compared to WT mice. In addition, there was significant increase in expression of DAT in ventral striatum of P2X4R KO mice compared to WT mice. Further, we found significant increase in expression of D2Rs in midbrain in P2X4R KO mice. Taken together, these findings suggest potential association between P2X4Rs and dopamine neurotransmission. Perturbation of this cross talk may be one of the underlying mechanisms for the behavioral deficits in P2X4R KO mice. Overall, these findings suggest that P2X4Rs may have a role in pathophysiology of severe neuropsychiatric disorders through regulation of dopamine function.

Disclosures: S. Khoja: None. **L. Asatryan:** None. **M.W. Jakowec:** None. **D.L. Davies:** None.

Poster

603. Autism Genetic Models

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Program#/Poster: 603.04/G7

Topic: C.06. Developmental Disorders

Support: Simons Foundation Autism Research Initiative

Title: Behavioral consequences of disrupted MET signaling

Authors: *B. L. THOMPSON[1], W. RODRIGUEZ[2], P. LEVITT[2];
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Abstracts: Our laboratory discovered that the gene encoding the receptor tyrosine kinase, *MET*, contributes to autism risk. These studies utilized genetically modified mouse lines to dissect the roles of *Met* in the neurobiological underpinnings of neurodevelopmental disorders to test our

hypothesis that disruption of Met signaling during development has functional consequences on the maturation of cortical circuits and specific behaviors that are altered in autism. We generated two conditional mouse lines in which *Met* is deleted from select populations: 1) $Met^{fx/fx}/Emx1^{cre}$ (deleted from all cells arising from the dorsal pallium) and 2) $Met^{fx/fx}/Nestin^{cre}$ (deleted from all neural cells). A battery of behavioral tests was performed to assess cognitive, emotional, and social disturbances that are observed in multiple neurodevelopmental disorders, including autism spectrum disorders, and that, are in part sub-served by circuits that express Met. Multiple cohorts of mice were tested in early adulthood on rotarod, activity chamber, elevated plus maze, spontaneous alternation in the t-maze, olfactory dishabituation, social novelty preference, marble burying, contextual fear conditioning, and direct social interaction with simultaneous USV recordings. We found that the null $Met^{fx/+}/Emx1^{cre}$ mice display significant hypoactivity in the activity chamber and in the t-maze despite normal performance on the rotarod. Additionally, these animals show a deficit in spontaneous alternation. These mice show normal anxiety, olfactory dishabituation, social novelty preference, contextual fear conditioning, and marble burying. In comparison, the null $Met^{fx/fx}/Nestin^{cre}$ mice display deficits in contextual fear conditioning, and a weak deficit in sociability in the social novelty preference task. These mice show normal performance on rotarod and activity chamber, anxiety, spontaneous alternation, olfactory dishabituation, and marble burying. These data suggest a complex contribution of Met in the development of social, emotional, and cognitive behavior. The impact of disrupting developmental Met expression is dependent upon the circuit-specific deletion pattern. The null $Met^{fx/fx}/Nestin^{cre}$ mice (*Met* deleted from every cortical cell) show behavioral phenotypes consistent with autism. In contrast, the null $Met^{fx/+}/Emx1^{cre}$ mice (*Met* deleted from cells arising from the dorsal pallium) show a different behavioral repertoire, with decreased exploratory behavior and memory. Future studies will determine the impact of environmental interactions with the deletion of Met from the $Met^{fx/fx}/Nestin^{cre}$ mice on further social, emotional, and cognitive behaviors.

Disclosures: B.L. Thompson: None. **P. Levitt:** None. **W. Rodriguez:** None.

Poster

603. Autism Genetic Models

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Program#/Poster: 603.05/G8

Topic: C.06. Developmental Disorders

Support: Pennsylvania Department of Health (SAP # 4100042728)

NIMH 1P50MH096891 (EB)

NINDS T32NS007413 (SF)

Title: Haploinsufficiency of the autism-linked gene Protocadherin 10 causes male-specific behavioral deficits

Authors: H. SCHOCH[1], *S. L. FERRI[1], A. S. KREIBICH[2], H. C. DOW[2], S. HIRANO[3], R. T. SCHULZ[4], E. S. BRODKIN[2], E. ABEL[1];
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Abstracts: Boys are five times as likely to be affected by autism spectrum disorders (ASD) as girls, but the genetic and hormonal contributions of this sex difference are not clear. The gene PCDH10 has been linked to ASD in human studies. Its product, the calcium-dependent adhesion molecule Pcdh10, plays an important role in pruning of excitatory synapses in the brain. Pcdh10 is expressed in several brain areas associated with cognition and social behavior. Mice heterozygous for a deletion of Pcdh10 (Pcdh10+/- mice) display reduced sociability in the Social Approach Test and impairment in fear conditioning tasks. Remarkably, these impairments were present exclusively in males. Pcdh10+/-mice showed no deficits in locomotor activity in the rotarod test, no deficits in olfactory acuity or discrimination to social or non-social odorants in the olfactory habituation-dishabituation task, and no alternation in nonsocial anxiety-related behaviors in the elevated zero maze or open field test. Here, we explore the role of gonadal hormones in mediating sex-specific behavioral deficits in Pcdh10+/- mice.

Disclosures: **H. Schoch:** None. **S.L. Ferri:** None. **A.S. Kreibich:** None. **H.C. Dow:** None. **S. Hirano:** None. **R.T. Schulz:** None. **E.S. Brodkin:** None. **E. Abel:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.06/G9

Topic: C.06. Developmental Disorders

Support: NS085709

Title: A touchscreen version of delayed non-match to position (DNMTP) in inbred mice

Authors: *P. T. LEACH[1], J. N. CRAWLEY[2];
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Abstracts: Mouse models of neurodevelopmental disorders with intellectual impairments have been extensively characterized, but more challenging cognitive phenotyping analyses are needed, especially on complex working memory tasks. Touchscreen operant chambers may be useful for the further characterization of mouse models of neurodevelopmental disorders, particularly those characterized by more subtle cognitive deficits. Here we demonstrate effective methods for conducting delayed non-matching to position in touchscreen chambers. To confirm high baselines that will enable detection of deficits in mutant mouse models, the task was first validated in two inbred strains of mice, C57BL/6J and FVB/AntJ, which display good cognitive abilities in other tasks, and are frequently used as the genetic background for targeted mutations. Briefly, mice were trained to press the touchscreen in a random spatial position, indicated by an illuminated square. When a nose poke occurs in the food magazine at the rear of the chamber, the illuminated square appears in the initially presented position and in a novel position. Successful non-matches to position, i.e., pressing on the novel position, are rewarded with 20 µl strawberry Ensure milkshake. Unsuccessful choices are followed by a timeout and a correction trial. After reaching the criterion of 80% correct responses for 2 days, delays of 1, 3, and 10 seconds are introduced. FVB mastered the non-match rule in an average of ~25 days, and demonstrated delay-dependent performance in this touchscreen task.

Disclosures: P.T. Leach: None. **J.N. Crawley:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.07/G10

Topic: C.06. Developmental Disorders

Support: P50 HD055751

Title: Shank3+/- mice exhibit probabilistic learning deficits, but not altered repetitive behaviors

Authors: A. SYED[1], D. A. AMODEO[3], J. A. SWEENEY[4], *M. E. RAGOZZINO[2];
[1]Psychology, [2]Univ. Illinois Chicago, CHICAGO, IL; [3]Psychology, Univ. of Illinois at Chicago, Chicago, IL; [4]Psychiatry, Univ. of Texas Southwestern, Dallas, TX

Abstracts: The Shank3 gene codes for a post-synaptic scaffolding protein found at glutamatergic synapses. Alterations in the Shank3 gene have been repeatedly linked to autism spectrum disorder (ASD) including 22q13 deletion syndrome in which autistic behaviors are exhibited. One of the core features observed in ASD is an "insistence on sameness" or behavioral inflexibility. While Shank3 null mutant mice exhibit reversal learning deficits, it is unclear whether Shank3 heterozygote mice also display behavioral inflexibility. In a recent study, we found that individuals with ASD acquire a two-choice spatial discrimination with probabilistic reinforcement as quickly as controls, but were impaired during reversal learning. The present experiment examined whether Shank3+/- mice exhibit learning and/or reversal learning deficits with probabilistic reinforcement. In addition, mice were tested for repetitive grooming and marble burying behavior to determine whether Shank3+/- may display other repetitive behaviors. Shank3+/- and B6 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. For grooming behavior, mice were individually placed in a clear plastic cage and the cumulative time spent grooming all body regions across 10 min was recorded. For marble burying, 20 glass marbles were placed on top of 3 cm of clean woodchip bedding in a clear plastic cage. Mice were allowed to explore the container and marbles for 30 min. Marbles were considered buried if $\geq 2/3$ of the surface area was covered in woodchip bedding. In the spatial discrimination test, Shank3+/- mice exhibited an initial learning deficit, as well as a reversal learning deficit suggesting that there is a basic learning impairment when feedback is provided in a probabilistic manner. Shank3+/- mice more frequently shifted back to the incorrect choice when mice were not reinforced for a correct response. In contrast, Shank3+/- mice did not exhibit increased grooming or marble burying behavior. Because Shank3 mutations associated with ASD and 22q13 deletion syndrome are heterozygous, studying the Shank3+/- mouse can be useful for understanding the pathophysiology related to certain ASD features.

Disclosures: **A. Syed:** None. **D.A. Amodeo:** None. **M.E. Ragozzino:** None. **J.A. Sweeney:** None.

Poster

603. Autism Genetic Models

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Program#/Poster: 603.08/G11

Topic: C.06. Developmental Disorders

Support: NIH Grant 1F05MH097457-01

Title: Mutant DISC1 produces smaller Purkinje cells and impairs recognition memory and social behavior in adult mice

Authors: *A. V. SHEVELKIN[1,2], B. N. ABAZYAN[2], G. L. RUDOW[3], J. C. TRONCOSO[3], C. A. ROSS[2], M. V. PLETNIKOV[2,4];
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Abstracts: Disrupted-In-Schizophrenia-1(DISC1) and its variants have been associated with neurodevelopmental disorders, including schizophrenia and autism spectrum disorders (ASD). Purkinje cells (PC) express DISC1. We generated a mouse model of inducible and selective expression of mutant DISC1 in PC. Here, we sought to analyze the brain and behavioral alterations in this transgenic mouse model. We evaluated volume of the cerebellum and PC in mice at postnatal (P) day 21 and assessed behavioral phenotype in male and female mice of 3-7 months of age using a series of tests relevant to schizophrenia and ASD, including novelty-induced activity, elevated plus maze, Y maze, object and place recognition, fear conditioning and rotarod. We found a significant decrease in Purkinje cells size. Neither total number of PC nor volume of the cerebellum were significantly altered in mutant DISC1 mice. No cellular markers of inflammation were observed in mutant mice. Neurobehavioral phenotyping showed abnormal social interaction, hyperactivity in open field and deficient novel object recognition in male mice. We observed no group differences in elevated plus maze, spontaneous alteration or spatial recognition in Y maze. Electrophysiological analysis of PC activity is in progress. Our findings indicate that mutant DISC1 affects PC morphology and produces cognitive and social abnormalities in adult mice. This may have the potential to advance our knowledge of the role for DISC1 in maturation and function of the cerebellum related to neurodevelopmental disorders.

Disclosures: A.V. Shevelkin: None. **B.N. Abazyan:** None. **G.L. Rudow:** None. **J.C. Troncoso:** None. **C.A. Ross:** None. **M.V. Pletnikov:** None.

Poster

603. Autism Genetic Models

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Topic: C.06. Developmental Disorders

Support: Autism Science Foundation

R01HD069560

Autism Speaks

The Hartwell Foundation

BRAINS for Autism Foundation

Title: Autism-associated insertion mutation in shank3 results in impaired behavior and synaptic transmission

Authors: *M. KOUSER[1], H. E. SPEED[1], J. M. REIMERS[1], Z. XUAN[1], C. F. OCHOA[1], C. M. POWELL[1,2];
[1]Neurol. and Neurotherapeutics, [2]Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstracts: Deletion and other loss-of-function mutations of SHANK3 have been strongly implicated in autism. SHANK3 deletions and mutations have also been linked with Phelan-McDermid Syndrome associated with autism and intellectual disability. At least 3 different Shank3 mutant mice have been published to date, each deleting one or more exons. We have created a novel genetic animal model of an autism-associated SHANK3 insertion mutation in exon 21 with high molecular construct validity. We hypothesize that this autism-associated Shank3 genetic model will result in reduced Shank3 levels and will lead to behavioral abnormalities and functional changes in excitatory synaptic transmission. This autism-associated exon 21 point mutation results in loss of predominant higher molecular weight isoforms of Shank3 protein similar to exon 21 deletion mutation. We find alterations in multiple behaviors including motor coordination and response to novelty, among others, testing littermate, sex-matched progeny of heterozygous by heterozygous matings. We also report abnormalities in hippocampal synaptic transmission and plasticity. In summary, our novel, Shank3 point mutant mouse model of autism provides high construct validity for autism and provides valuable insights into the role of Shank3 in synaptic function and behavior.

Disclosures: M. Kouser: None. **H.E. Speed:** None. **J.M. Reimers:** None. **Z. Xuan:** None. **C.F. Ochoa:** None. **C.M. Powell:** None.

Poster

603. Autism Genetic Models

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Topic: C.06. Developmental Disorders

Support: Simons Foundation Autism Research Initiative (SFARI)

Title: Male-specific deficits in reinforcement learning, motivation, striatal volume, and white-matter integrity in the 16p11.2del/+ mouse model of autism

Authors: N. M. GRISSOM[1], S. MCKEE[1], J. LIDSKY-EVERSON[1], H. SCHOCH[2], R. HAVEKES[2], M. KUMAR[3], S. PICKUP[3], V. KUMAR[4], H. POPTANI[3], T. NICKL-JOCKSCHAT[4], T. M. REYES[1], *T. G. ABEL[2];
[1]Pharmacol., [2]Dept. of Biol., [3]Radiology, Univ. of Pennsylvania, PHILADELPHIA, PA;
[4]Psychiatry, Univ. Hosp. Aachen (Universitätsklinikum Aachen), Aachen, Germany

Abstracts: One prominent theory conceptualizing autism spectrum disorders (ASD) focuses on deficits in "social motivation", positing that developmental deficits in reward mechanisms directly impede social learning, resulting in the array of social skill deficits that characterize ASD. Understanding the value of rewards and learning to associate actions with their outcomes is supported by striatal circuitry, including nucleus accumbens and dorsal striatum. We have tested motivation and reward-based learning in 16p11.2del/+ mice, which model one of the most common copy number variations associated with ASD. A mouse model of 16p11.2 hemideletion on a mixed 129/b6 background (the chr7qF3 deficient mouse) has previously been generated (Horev et al, PNAS 2011). Adult male and female 16p11.2del/+ mice were tested for operant learning and motivation using mouse operant 9-hole chambers with a sweetened liquid reinforcer. Compared to wildtype mice, 16p11.2del/+ male, but not female, mice have early impairments in the ability to associate a response with a reward as measured by Fixed Ratio responding. Once fully trained on the fixed ratio task, animals were probed with a Progressive Ratio schedule, which assesses motivation to continue responding as task demands become more challenging. 16p11.2del/+ males demonstrated significantly reduced progressive ratio responding, indicating deficits in motivation to seek reward, while female 16p11.2del/+ were unaffected. Importantly, sucrose preference when given freely in home cage was unaltered in 16p11.2del/+ mice, indicating that mechanisms of reward preference are unaffected. These animals do not display deficits in hearing seen in a model of 16p11.2 hemideletion on a pure b6 background, which might confound changes in striatal function. A separate cohort of animals were perfused and the brains were imaged for structural MRI analysis to assess striatal volume, and Diffusion Tensor Imaging (DTI) to assess white matter integrity. Male del/+ animals had significantly reduced dorsal striatum volume compared to wildtype males, while no differences were observed in females. DTI revealed that the corpus callosum and external capsule were

reduced in integrity, while the fornix was increased in integrity, in male del/+, suggesting diminished corticostriatal connectivity and enhanced hippocampal connectivity. These data indicate that fundamental mechanisms of goal-directed behavior, likely mediated by dorsal striatum, are altered in 16p11.2del/+ male mice while sparing females. These findings provide strong support for the idea that sex differences in reward and motivation are involved in the pathophysiology of ASD.

Disclosures: **N.M. Grissom:** None. **T.G. Abel:** None. **S. McKee:** None. **J. Lidsky-Everson:** None. **H. Schoch:** None. **R. Havekes:** None. **M. Kumar:** None. **S. Pickup:** None. **V. Kumar:** None. **H. Poptani:** None. **T. Nickl-Jockschat:** None. **T.M. Reyes:** None.

Poster

603. Autism Genetic Models

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.11/H2

Topic: C.06. Developmental Disorders

Support: MIND Institute, University of California Davis

Title: GABA-B receptor agonist r-baclofen reverses social deficits and reduces repetitive behavior in two mouse models of autism

Authors: *J. L. SILVERMAN, M. C. PRIDE, J. E. HAYES, K. R. PUHGER, J. N. CRAWLEY;
UC Davis Sch. of Med., Sacramento, CA

Abstracts: Autism spectrum disorder (ASD) is diagnosed by two core behavioral criteria, unusual reciprocal social interactions and communication, and repetitive behaviors with restricted interests. Excitatory/inhibitory imbalance is a prominent hypothesis for the etiology of autism. Reductions in excitatory glutamatergic neurotransmission with antagonists and negative modulators, and increases in inhibitory neurotransmission with GABAergic agonists and positive modulators, have reversed abnormalities in mouse models of ASD and Fragile X syndrome on synaptic plasticity, spine morphology, seizures and behavioral phenotypes (Kreuger and Bear, 2011; Silverman et al., 2013; Vorstman et al., 2014). Initial clinical trials with STX209 (Arbaclofen) yielded variable but promising results on the ABC-Social Avoidance, Vineland II-Socialization, Social Responsiveness and ABC-irritability scales (Berry-Kravis et al., 2012; Erickson et al., 2014). We tested the hypothesis that activation of the GABAB receptor with the

commercially available r-baclofen enantiomer, but not the less potent s-baclofen enantiomer, would rescue ASD-relevant phenotypes in the BTBR and C58/J inbred strain mouse models of ASD. BTBR display well-replicated deficits in sociability and long bouts of repetitive self-grooming. C58/J exhibit high levels of stereotyped vertical jumping. Two independent cohorts of BTBR showed improved social approach following acute r-baclofen treatment. Both BTBR and C58 showed reductions in repetitive behaviors after r-baclofen. These data support further investigations of the GABAB agonist target, in other preclinical models and in further clinical studies, to evaluate the benefits of enhancing inhibitory synaptic transmission to treat the core diagnostic symptoms of autism.

Disclosures: J.L. Silverman: None. **M.C. Pride:** None. **J.E. Hayes:** None. **K.R. Puhger:** None. **J.N. Crawley:** None.

Poster

603. Autism Genetic Models

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Topic: C.06. Developmental Disorders

Support: the Japan Foundation for Neuroscience and Mental Health

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the Mother and Child Health Foundation

the NOVARTIS Foundation for the Promotion of Science

Title: Axonal localization of Ca2+-dependent activator protein for secretion 2 is critical for subcellular locality of brain-derived neurotrophic factor and neurotrophin-3 release affecting proper development of postnatal mouse cerebellum

Authors: *T. SADAKATA;
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Abstracts: Ca2+-dependent activator protein for secretion 2 (CAPS2) is a protein that is essential for enhanced release of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) from cerebellar granule cells. We previously identified dex3, a rare alternative splice variant of CAPS2, which is overrepresented in patients with autism and is missing an exon 3 critical for axonal localization. We recently reported that a mouse model CAPS2αex3/αex3 expressing dex3 showed autistic-like behavioral phenotypes including impaired social interaction and cognition, and increased anxiety in an unfamiliar environment. Here, we verified impairment in axonal, but not somato-dendritic, localization of dex3 protein in cerebellar granule cells and demonstrated cellular and physiological phenotypes in postnatal cerebellum of CAPS2αex3/αex3 mice. Interestingly, both BDNF and NT-3 were markedly reduced in axons of cerebellar granule cells, resulting in a significant decrease in their release. As a result, dex3 mice showed developmental deficits in dendritic arborization of Purkinje cells, vermian lobulation and fissurization, and granule cell precursor proliferation. Paired-pulse facilitation at parallel fiber-Purkinje cell synapses was also impaired. Together, our results indicate that CAPS2 plays an important role in subcellular locality (axonal vs. somato-dendritic) of enhanced BDNF and NT-3 release, which is indispensable for proper development of postnatal cerebellum.

Disclosures: T. Sadakata: None.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.13/H4

Topic: C.06. Developmental Disorders

Support: Italian Ministry of Health Grant (GR3), Young Researcher 2008

Telethon – Italy (GGP09134)

Compagnia di San Paolo grant

Title: Behavioral and neuro-anatomical characterization of synapsin 1, 2 and 3 mutant lines

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Abstracts: Autism spectrum disorders (ASD) are heterogeneous neurodevelopmental disorders characterized by deficits in social communication and restricted interests and repetitive behaviors. Abnormalities in language development, mental retardation and epilepsy are often observed in ASD children and, conversely, several forms of epilepsy also display ASD. Given the high comorbidity between ASD and epilepsy, the possibility of a common genetic basis for both diseases has been proposed. Synapsins (Syns) are a family of synaptic vesicle phosphoproteins encoded by the SynI, SynII and SynIII genes. The Syn gene family is a good candidate for the synaptic epilepsy/ASD pathway, as Syns regulate synaptic transmission and plasticity with distinct roles in excitatory and inhibitory neurons and SynI and II mutations have been recently associated with ASD in humans. Mice lacking SynI or SynII genes experience epileptic seizures starting at 2-3 months of age and display social and mild cognitive impairments and repetitive behaviours also before the onset of seizures. Aim of our study was to analyze whether deletion of SynI, SynII or SynIII gene in mice causes motor and social communication deficits during infancy and adulthood. During the first two postnatal weeks, we recorded spontaneous locomotor activity and ultrasonic vocalizations in pups in response to social separation, while, at adulthood, we evaluated social behaviors and ultrasonic vocalizations emitted by male mice in interaction with wild-type females in estrous. Moreover, we used Magnetic Resonance Imaging (MRI) to map in the three mutant lines regional gray matter volume and white matter fractional anisotropy (FA), two imaging readouts that have been extensively used in ASD patient populations. Our results showed different phenotypes in the three Syn mutant lines. Deficits in spontaneous behaviors and ultrasonic vocalizations measured during the first two postnatal weeks of age and social investigation and vocal patterns at adulthood were detected in SynI and SynII mutant mice. By contrast, SynIII mice did not show deficits at either infancy or adulthood. MRI did not reveal volumetric or FA alterations in SynII and III mutants, whereas significant increases in FA in callosal and frontal areas were observed in SynI-/-. Overall, motor and social communication deficits observed in SynI-/- and Syn II-/- mice support the view that these genes are involved in the expression of autistic-like behavioral traits and identify these mutant lines as useful experimental models of ASD and epilepsy.

Disclosures: **M.L. Scattoni:** None. **M. Morini:** None. **M. Pagani:** None. **M. Blesa:** None. **A. Gozzi:** None. **F. Benfenati:** None. **C. Michetti:** None. **A. Caruso:** None. **M. Sabbioni:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.14/H5

Topic: C.06. Developmental Disorders

Support: NPO Rett Syndrome Supporting Organization

Brain Mapping by Integrated Neurotechnologies for Disease Studies

Title: Generation and analysis of neurodevelopmental disorder model marmoset

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Abstracts: In the human brain, there are two major functional domains. One has been conserved in all mammals through evolution and governs fundamental functions such as reward, emotion and memory; the other is unique to primates, and is acquired through the enlargement of the cerebral cortex governing special functions such as tool use, language, and self-consciousness. Thus, to properly understand these brain functions, we need appropriate animal models for studying each function. Animal models that are used to analyze brain functions are different in each case. In the former, a reductive approach is adopted based on gene manipulation using models such as genetically-modified fish and rodents, while in the latter, the main approach is psychological and involves complex behavior analysis using non-human primates such as macaque monkeys. Many researchers believed that the complementary nature of genetic engineering technologies in rodent and fish models and cognitive neuroscience techniques in primate research would lead to progress in this research field. However, due to lack of appropriate animal models that can be analyzed in both aspects of the brain's functions, contact points between these two approaches have been limited. The development of genetically engineered non-human primates has attracted attention for its potential to connect the two research fields. Recently, we succeeded in creating the world's first transgenic primate using marmosets. This technological breakthrough provides a potential paradigm shift by enabling researchers to analyze both the brain functional domains using various model marmosets. Currently, we are developing a technique for creating knockout marmosets using zinc finger nuclease (ZFN) technology. By combining this technique with the development of cognitive information for marmoset brain analysis, innovative MRI imaging technology and marmoset genetic analysis tools, we aim to create and analyze marmoset models suitable for research on autism spectrum disorders (ASD), to understand the pathogenesis, and to contribute to new therapeutic strategies to treat ASD.

Disclosures: **N. Kishi:** None. **K. Sato:** None. **M. Okuno:** None. **H.J. Okano:** None. **E. Sasaki:** None. **H. Okano:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

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Program#/Poster: 603.15/H6

Topic: C.06. Developmental Disorders

Support: NRF Grant 2013R1A1A4A01012426

Title: Is erbb4 a susceptibility gene for autism spectrum disorders endophenotype? an association study in korean population

Authors: *R.-S. WOO[1], S. KIM[2];
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Abstracts: Background: Autism spectrum disorder (ASD) and schizophrenia are major complex psychiatric disorders, and there are several reports concerning the influence of candidate genes and polymorphisms common to both conditions on pathogenesis and behavioral phenotype. **Methods:** To assess whether the *ERBB4* gene, which is a strong candidate gene for schizophrenia, could also be implicated in the vulnerability to ASD, we conducted a family based association study between three common single nucleotide polymorphisms (SNPs) in the *ERBB4* gene (rs7598440, rs839523, rs707284) and 250 Korean ASD multiplex families using the family-based association test (FBAT) program (n = 959). **Results:** It did not reveal any genetic association between SNPs/haplotype in *ERBB* and ASD affection. However, when ASD phenotypes were analyzed using clinical assessment tools, three polymorphisms were associated with scores obtained using the Autism Spectrum Quotient-Children's Version (AQ-C) (dominant model; rs7598440 $p = 0.003$, rs839523 $p = 0.001$, rs707284 $p = 0.002$). Furthermore, the rs7598440(C)-rs839523(A)-rs707284(A) haplotype revealed an association with AQ-C scores (FDR $p = 0.003$ under the dominant model). The rs7598440(C)-rs839523(A)-rs707284(A) "risk" haplotype dominant group demonstrated higher scores for the AQ-C (52.53 ± 27.21 [n = 181]) than the rs7598440(T)-rs839523(G)-rs707284(G) haplotype recessive group (43.20 ± 26.13 [n = 66]) ($p = 0.017$). **Conclusions:** This study suggests a possible association between *ERBB4* variants and the Korean ASD endophenotype.

Disclosures: **R. Woo:** None. **S. Kim:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

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Program#/Poster: 603.16/H7

Topic: C.06. Developmental Disorders

Support: National Institute of Health Grant P01 DA 12408

The Lundbeck Foundation

Danish Medical Research Council

Title: Early-onset parkinsonism and psychiatric disorder in a patient with missense mutation in the C-terminal tail of DAT

Authors: *F. H. HENRIKSEN[1], T. SKJØRRINGE[2], N. V. ARENDS[1], S. YASMEEN[2], K. ERREGER[3], H. J. G. MATTHIES[3], A. GALLI[3], L. E. HJERMIND[4], L. B. MØLLER[2], U. GETHER[1];
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Abstracts: Dopamine disturbances are critical in the pathophysiology of Parkinson's disease and have also been implicated in a spectrum of psychiatric disorders including ADHD, schizophrenia, autism, and addiction. The sodium-coupled dopamine transporter (DAT) controls dopamine homeostasis, but its contribution to disease remains poorly understood. We have recently described an adult patient with compromising missense mutations in the DAT gene, who suffered from early onset parkinsonism and ADHD. Here, we present an unrelated patient that carries a missense mutation in the C-terminal PDZ binding domain of DAT and who suffers from early-onset parkinsonism and a complex psychiatric disorder. We have previously reported that the PDZ binding domain of DAT is critical for striatal targeting of DAT *in vivo*, but not for surface expression in heterologous cells. Consistently the missense mutation carried by the patient generates only modest changes in DAT function, when expressed in heterologous cells, as supported by 20-30% reduction in dopamine uptake capacity (~75% of WT DAT), and a corresponding reduction in surface expression and amphetamine induced efflux. Interestingly, a SPECT scan of the patient shows clear bilateral reduction of DAT ligand binding. The

identification of an independent patient with a missense mutation in the DAT gene and concurrent early-onset parkinsonism and neuropsychiatric disorder supports that abnormal DAT function may constitute a risk factor for psychiatric disorders and possibly also for early-onset parkinsonism.

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Poster

603. Autism Genetic Models

Location: Halls A-C

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Program#/Poster: 603.17/H8

Topic: C.06. Developmental Disorders

Support: The Simons Foundation

The National Institute of Mental Health Intramural Research Program and the University of California Davis MIND Institute

National Institutes of Health Pioneer Award

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National Science Foundation

Title: 16p11.2 deletion syndrome mice display ultrasonic vocalization deficits during social interactions

Authors: *M. YANG[1], E. J. MAHRT[2], F. C. LEWIS[1], G. M. FOLEY[1], T. PORTMANN[3], R. E. DOLMETSCH[3,4], C. V. PORTFORS[2], J. N. CRAWLEY[1];
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Abstracts: Deletions and duplications at the chromosome 16p11.2 region are variably associated with speech delay, autism spectrum disorder, schizophrenia and cognitive impairments. Social communication deficits are a primary diagnostic symptom of autism. Here we investigated

ultrasonic vocalizations in young adult male 16p11.2 deletion mice during a novel three-phase male-female social interaction test to evaluate responses to the presence and absence of a social partner. Strikingly fewer vocalizations were discovered in most 16p11.2 heterozygous deletion males (+/-) during Phase 1, the first exposure to an unfamiliar estrus female, as compared to wildtype littermates (+/+), replicated in two independent cohorts of subject mice. During Phase 2, when the female was removed, +/+ mice emitted calls, but fewer than during Phase 1, while +/- mice called minimally. Sensory and motor abnormalities were detected in +/-, including higher nociceptive thresholds and a complete absence of acoustic startle responses, along with stereotyped jumping and backflipping in a small percentage of individuals. Hearing loss in all +/- was confirmed by lack of auditory brainstem responses to low 8-32 kHz and high 50-100 kHz frequencies. However, these sensory and motor phenotypes could not directly explain the low vocalization rates in +/-, since both genotypes reduced their calling when the female was removed, and +/- subsequently displayed normal abilities to emit vocalizations during their Phase 3 re-exposure to the female. Together these findings support the concept that vocalizations represent a response to social cues, and that 16p11.2 deletion mice are deficient in their initial USV responses to social cues.

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Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.18/H9

Topic: C.06. Developmental Disorders

Support: NIH R01 MH039085

Tsai Family Fund

Title: In search of biomarkers and treatment for a subpopulation of autism spectrum disorder (ASD) with high serotonin (sASD)

Authors: K. CHEN[1,2], Y. CHENG[3,1], C.-S. CHEN[4,1], C.-Y. YANG[5,1,6], *J. C. SHIH[7,1]; [1]USC-Taiwan Ctr. for Translational Res., Los Angeles, CA; [2]Dept. of Pharmacol. and Pharmaceut. Sci., USC, Los Angeles, CA; [3]Inst. of Neuroscience, Natl. Yang-Ming Univ. and Dept. of Rehabilitation, Natl. Yang-Ming Univ. Hosp., Taipei, Taiwan; [4]Grad. Inst. of Systems

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Abstracts: MAOA KO mice display high serotonin (5-HT) levels, particularly during early developmental stages. We have shown that MAOA KO mice exhibit behavioral hallmarks of autism spectrum disorder (ASD), such as sociocommunication impairments, stereotypical responses, and behavioral inflexibility; They also displayed neuropathological alterations, including reduced thickness of the corpus callosum, increased dendritic arborization of pyramidal neurons in the prefrontal cortex and disrupted microarchitecture of the cerebellum, similar to ASD (Bortolato, 2012). Thus, MAOA KO mice are useful animal model of sASD. More importantly we found that reducing 5-HT at P1 to P7, rescued the autistic like behaviors seen in MAO A KO mice. This result suggests the early treatment of sASD may cure the sASD before the symptoms appeared (Bortolato, 2013). In order to identify biomarkers of sASD and comorbid anxiety, we probed MAO A KO mouse serum with human proteome microarray to analyze the serum autoantibody. We found that Up-regulated autoantibodies include SLCA18 (glycine), LSP1(lymphocyte specific protein) TWF2 (neurite development), PTS (SNP in autism, catalyzes synthesis of BH4 , the co-factor for 5-HT and DA synthesis); Down-regulated autoantibodies include 5-HTR2c (SR1), GPR119,TGM1, CAMKA1, ADH6, DTK2B, EPS8, Sox5, Ogfr and ICT1. Recently, we identified two families in Taiwan with sASD. Their plasma serotonin levels were significantly higher (36.51 ± 1.84 ng/mL) than controls (0.14 ± 1.52 ng/mL). Interestingly, the subjects with sASD showed reduced emotional mismatch negativity when we measured the electrophysiological index of anxiety with the presentation of emotionally (neutrally, angrily, fearfully) spoken meaningless syllables dada in a passive auditory oddball paradigm (Fan & Cheng, 2014). The molecular basis of this autistic family includes the altered genes and autoantibodies are currently studied, will be discussed and compared with the data found in MAO A KO mice. This new information will shed lights on the potential treatment and diagnosis of this subpopulation of ASD (sASD).

Disclosures: K. Chen: None. **Y. Cheng:** None. **C. Chen:** None. **C. Yang:** None. **J.C. Shih:** None.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.19/H10

Topic: C.06. Developmental Disorders

Support: Connecticut Technology Talent Bridge Program

Title: Genetic abnormalities in autistic disorder

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Abstracts: Background: Autism is a highly heritable complex neurodevelopmental disorder characterized by distinct impairments of cognitive function, social interactions and speech development. Mutations in different set of genes may be involved in different autistic individuals. By identifying genetic markers inherited with autism in family studies, the actual mutations that increase the risk for autism may be identified. **Method:** Lymphoblast cell lines from a family of 5 members with autistic fraternal twin brothers were cultured and processed for chromosomal studies and fluorescence in-situ hybridization (FISH) studies to investigate if the autistic twins had any genetic abnormalities and if the unaffected parents and other siblings were carriers of the same genetic abnormality. Lymphoblast cell lines were maintained in culture in PB-Max media. After 72hrs of culture, 50µl of Colcemid was added to arrest the cells in metaphase stage. Then cultures were harvested and fixed following Genesys Diagnostic's harvesting protocol. Cells were dropped and stained using GTG banding technique. Cytogenetic analysis was done by using Ikaros (Metasystems) software system. In order to further investigate the abnormality, FISH procedure was carried out by following the Genesys laboratory protocol. Chromosome enumeration DNA probe Cep-9 and probes for Prader-Willi (PW) and Angelman (AS) syndromes, located on chromosome 15 (bands 15q11-13) (Cytocell) were applied and cells were scored by fluorescent microscopy. Isis software system was used to capture the Images. **Results:** With karyotyping, it was observed that the mother, father, a normal sister were found to be chromosomally normal. The autism affected twins had very low level of mosaicism of trisomy 9, in 2/50 and 3/50 metaphases, respectively. The scoring of aneuploidy by interphase FISH with the chromosome enumeration DNA probe Cep 9 confirmed low level of mosaicism (3.4%) for an extra signal for CEP9, consistent with trisomy 9. However, FISH for the loci at 15q11-13 for AS and PW syndromes showed normal diploid signal patterns for AS and PW in the affected twins. **Conclusions:** Low level of chromosomal mosaicism in twins with autism suggests that low-level mosaicism can have clinical manifestation. Most cytogenetic studies in autism are performed by array CGH techniques, which are less efficient for mosaicism studies.
Funding Provided by Connecticut Tech Talent

Disclosures: **D. Ahuja:** A. Employment/Salary (full or part-time):; part-time. **C. Smiley:** A. Employment/Salary (full or part-time):; Full time. **P. Nuni:** A. Employment/Salary (full or part-time):; full time.

Poster

603. Autism Genetic Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.20/H11

Topic: C.06. Developmental Disorders

Support: Pennsylvania Department of Health (SAP # 4100042728)

1P50MH096891

T32 MH017168

Title: Abnormal dendritic spines in amygdala of mice haploinsufficient for Protocadherin 10, an autism-associated gene

Authors: *H. SCHOCH[1], A. KREIBICH[2], H. DOW[2], S. HIRANO[4], R. T. SCHULTZ[5], T. ABEL[3], E. BRODKIN[2], D. FELDMEYER[6,7];
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Abstracts: Behavioral and cognitive impairments observed in autism spectrum disorder (ASD) are thought to arise from abnormal neuronal connectivity in the developing brain, but the molecular basis of these deficits is largely unknown. Protocadherin 10 (Pcdh10) is a cadherin superfamily neural cell adhesion molecule that has been associated withASD in human genetic studies. Mouse Pcdh10 is expressed highly in olfactory, limbic, and striatal regions, and plays an important role in activity-dependent synaptic pruning. Male mice lacking a single allele of Pcdh10 display reduced social approach behavior and impaired amygdala-dependent conditioned fear. To determine whether abnormal neural connectivity may underlie the behavioral deficits we observed, we measured dendritic length and spine density in Golgi stained lateral and basolateral amygdala neurons from Pcdh10+/- males. We found that Pcdh10+/- neurons have increased dendritic spine density, specifically, increased thin elongated filopodia-type spines. These data suggest that abnormal regulation of synapse formation in the amygdala may underlie the behavioral deficits observed in Pcdh10+/- mice.

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Poster

603. Autism Genetic Models

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Program#/Poster: 603.21/H12

Topic: C.06. Developmental Disorders

Support: EVMS Research Enhancement Grant

Title: Rapamycin, an inhibitor of mTORC1 signaling activity, improved measures of sociability in the BTBR T+ Itpr3tf/J mouse model of autism spectrum disorder

Authors: *J. A. BURKET[1], A. D. BENSON[1], A. H. TANG[2], S. I. DEUTSCH[1];
[1]Psychiatry and Behavioral Sci., [2]Microbiology and Mol. Cell Biol., Eastern Virginia Med. Sch., Norfolk, VA

Abstracts: mTOR signaling overactivity is a common pathological point of convergence for several syndromic forms of autism spectrum disorders (ASDs), such as Tuberous Sclerosis Complex and fragile X syndrome, stimulating interest in inhibiting mTORC1 activity as a therapeutic strategy for syndromic and nonsyndromic forms of ASDs. Administration of mTORC1 inhibitors (e.g. rapamycin) in syndromic mouse models of ASDs improved behavior, cognition, and neuropathology. However, since only a minority of ASDs are due to the effects of single genes (~10%), there is a need to explore inhibition of mTOR activity in mouse models that may be more relevant to the majority of nonsyndromic presentations, such as the genetically-inbred BTBR T+Itpr3tf/J (BTBR) mouse model of ASDs. BTBR mice have social impairment and exhibit increased stereotypic behavior. Thus, we wondered if inhibiting mTORC1 with rapamycin would improve the sociability of this strain. We reported previously that D-cycloserine, a partial glycineB site agonist of the NMDA receptor, had prosocial effects in the BTBR mouse. NMDA receptor activation is an important regulator of mTORC1 signaling activity. The current study investigated the ability of rapamycin to improve sociability and stereotypic behavior in BTBR mice. Using a standard paradigm to assess mouse social behavior, rapamycin improved several measures of sociability in the BTBR mouse. Whereas vehicle-treated Swiss Webster control mice showed a preference for exploring/sniffing the enclosed stimulus mouse ($p<0.01$), the vehicle-treated BTBR mice did not show this preference. However, rapamycin-treated BTBR mice spent more time engaged in exploring/sniffing the enclosed stimulus mouse ($p<0.01$). During free interaction between test and stimulus mice, vehicle-treated BTBR and Swiss Webster mice did not differ from each other in terms of discrete episodes of social approach and anogenital sniffing. However, treatment of the BTBR strain with rapamycin significantly increased their discrete episodes of social approach ($p<0.05$) and anogenital sniffing

($p<0.001$), relative to the vehicle-treated BTBR mice. Rapamycin treatment did not affect episodes of social approach and anogenital sniffing made by the Swiss Webster mice. In summary, treatment with a centrally-effective dose of rapamycin improved the sociability of the BTBR mouse strain on several reliably-obtained measures (i.e., exploring/sniffing of the enclosed stimulus mouse and measures of social approach and anogenital sniffing during free interaction of test and stimulus mice). These data support therapeutic targeting of upregulated mTORC1 signaling activity to improve sociability.

Disclosures: J.A. Burket: None. **A.D. Benson:** None. **A.H. Tang:** None. **S.I. Deutsch:** None.

Poster

603. Autism Genetic Models

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Program#/Poster: 603.22/I1

Topic: C.06. Developmental Disorders

Support: R01HD069560

Autism Speaks

The Hartwell Foundation

BRAINS for Autism Foundation

Title: Multiple Shank3 mutant mouse models of autism result in decreased synaptic homer1b/c in striatum

Authors: *C. F. OCHOA[1], J. M. REIMERS[1], Z. XUAN[1], T. C. JARAMILLO[1], M. KOUSER[1], H. E. SPEED[1], C. M. DEWEY[3], S. LUI[1], C. M. POWELL[1,2];
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Abstracts: Autism Spectrum Disorders (ASDs) are characterized by dysfunction in two major behavioral domains including impaired social communication/interactions and restrictive and repetitive behaviors (DSM-5, May 2013). Mutations and deletions in the SHANK3 gene have been independently implicated in both idiopathic autism spectrum disorders (ASD) and 22q13 Deletion Syndrome or Phelan-McDermid Syndrome (PMS). Approximately 75-80 % of patients with PMS meet diagnostic criteria for autism, with the rest exhibiting symptoms of ASD. In an

effort to better understand autism and PMS, we and others have created multiple autism-relevant genetic models in mice based on mutations in the Shank3 gene. These include Shank3 exon 4-9 deletion, exon 13 stop, exon 21 deletion, and exon 21 point mutation mouse models. Because each of these models has some level of constructive validity for autism and PMS, we are interested in identifying convergent molecular abnormalities among these models as potential treatment targets for this form of ASD. Because Shank3 is the major Shank isoform expressed in striatum and because each mouse model has behavioral abnormalities that may be modulated by dorsal striatum, our initial studies have focused on this brain region. By making synaptosomes from the dorsal striatum of these multiple Shank3 mutant models, we have identified alterations in synaptic levels of multiple postsynaptic density proteins in Shank3 mutants. The most striking and consistent alteration is a decrease in Homer1b/c levels in the dorsal striatum of all Shank3 mutants. This finding suggests that Shank3 is crucial for maintaining Homer1b/c levels at the synapse. Furthermore, these data suggest that mGluR/Homer interactions may be altered in these Shank3 mutants. This may have important consequences for synaptic function, a focus of our future studies.

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Poster

603. Autism Genetic Models

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 603.23/I2

Topic: C.06. Developmental Disorders

Title: The role of CHD8, an autism spectrum disorder risk gene, in brain development

Authors: *O. DURAK[1], Y. KAESER-WOO[2], A. MORTELL[2], A. NOTT[2], L.-H. TSAI[1]; [1]Brain and Cognitive Sci., Picower Institute, MIT, Cambridge, MA; [2]Picower Inst. for Learning and Memory, Cambridge, MA

Abstracts: Autism spectrum disorder (ASD) is a complex developmental disability which is typically characterized by social deficits, communication difficulties, stereotyped behaviors and cognitive delays. It is well established that ASD has a strong genetic origin as supported by a number of genome-wide association and exome sequencing studies. Several studies identified de novo mutations in chromodomain helicase DNA-binding protein 8 (CHD8) in patients with ASD

and provide strong evidence in favor of CHD8 as a genuine autism risk factor. CHD8 is a member of the chromodomain helicase binding (CHD) family of proteins which are thought to regulate gene expression. CHD8 initially was isolated as a regulator of β-catenin-dependent transcription, and previous studies suggest that CHD8 directly interacts with β-catenin to inhibit its transcriptional activity. β-catenin-dependent transcriptional activity is implicated in the regulation of neural progenitor proliferation, dendritogenesis, axon establishment and guidance, and synaptogenesis. However, it remains unknown what role CHD8 plays in ASD etiology and neuronal development. In the current study, using the *in utero* electroporation technique we have shown that CHD8 knockdown affects neural progenitor proliferation in developing cortex, and dendritic arborization of pyramidal neurons in postnatal cortex. Consistent with the change in proliferation, Wnt-signaling activity was significantly altered following CHD8 knockdown. Additionally, we have identified a previously unknown role of CHD8 in regulation of β-catenin mRNA levels, which potentially could explain why proliferation and dendritogenesis is altered. Finally, *in utero* knockown of CHD8 in layer 2/3 pyramidal neurons results in deficits in social interaction behavior in adult mice. Currently, we are using transgenic mouse models and human induced pluripotent stem cells to further understand how CHD8 mutations could contribute to underlying causes of phenotypes associated with ASD.

Disclosures: O. Durak: None. **Y. Kaeser-Woo:** None. **A. Mortell:** None. **A. Nott:** None. **L. Tsai:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.01/I3

Topic: C.06. Developmental Disorders

Support: Ministry of Education, Science, Technology, Sports and Culture of Japan 23590124

Title: Role of A2BP1, a candidate gene for ASD, in establishing the architecture of the developing cerebral cortex

Authors: *K.-I. NAGATA, N. HAMADA, H. ITO, I. IWAMOTO, R. MORISHITA, H. TABATA;
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Abstracts: Ataxin-2-binding protein 1 (A2BP1 also known as Fox1 or Rbfox1) is an RNA-binding protein necessary for proper pre-mRNA splicing events in a variety of genes crucial for neuronal functions. Critical functions of A2BP1 in brain development have been approved by human mutations in A2BP1 gene that cause neurodevelopmental disorders including autism spectrum disorder (ASD). To elucidate the pathophysiological relevance of A2BP1, we here performed cell biological analyses of the neuron-specific isoforms (A016 and A030) during cerebral development *in vitro* and *ex vivo*. Knockdown of A2BP1 isoforms by the *in utero* electroporation method caused abnormal neuronal positioning during corticogenesis, which were most likely to be attributed to impaired cell migration. Knockdown of the A2BP1 isoforms induced distinct morphological defects in migrating cortical neurons, while the cell cycle of neuronal progenitor cells were not affected by silencing of the 2 isoforms. Aberrant morphology was confirmed in *in vitro* analyses; A2BP1-silencing in primary cultured hippocampal neurons resulted in the reduction of primary axon length and total length of dendrites. In addition, when A2BP1-A016 and -A030 were silenced in cortical neurons in one hemisphere, axonal growth to the contralateral hemisphere was severely and moderately suppressed, respectively. Taken together, impaired cortical neuron migration and disturbed interhemispheric axon development may induce structural and functional defects of the cerebral cortex, and consequently contribute to emergence of the clinical symptoms of neurodevelopmental disorders such as ASD.

Disclosures: K. Nagata: None. **N. Hamada:** None. **H. Ito:** None. **I. Iwamoto:** None. **R. Morishita:** None. **H. Tabata:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Program#/Poster: 604.02/I4

Topic: C.06. Developmental Disorders

Title: KRAS mediated enhancement of ERK signalling during brain development results in synaptic plasticity deficits and learning disabilities

Authors: *R. BRAMBILLA[1], A. PAPALE[1], N. SOLARI[1], R. D'ISA[1], M. CAMBIAGHI[1], M. CURSI[1], L. LETIZIA[1], M. CEROVIC[2], N. HARDINGHAM[2], E. MENNA[3], M. MATTEOLI[3]; [1]San Raffaele Scientific Inst., Milano, Italy; [2]Cardiff Univ., Cardiff, United Kingdom; [3]Univ. of Milan, Milan, Italy

Abstracts: Ras-ERK signalling activation in the adult mammalian brain is a necessary step to establish both long-term plasticity and long-term memories, as conclusively demonstrated by a number of electrophysiological and behavioural studies. Dysregulation of this cascade gives rise to a class of genetic syndromes that collectively goes under the name of "Rasopathies". Interestingly, patients affected by these pathologies may manifest a certain degree of mental retardation and some learning disabilities. In order to determine the exact role of Ras-ERK signalling in the postnatal forebrain we generated new mouse models in which a potent constitutive active form of KRAS, the G12V mutation, can be conditionally induced without overexpression, leading to a selective abnormal ERK activation in specific neuronal types. We found that a sustained activation of KRAS in excitatory cells of the forebrain has no effect on behaviour suggesting that Ras signalling in this subset of neurons does not significantly alter synaptic functions. On the contrary, a wider expression of the KRAS mutant leads to an increased activity of GABAergic interneurons, already occurring in the early phases of postnatal development. Importantly, this enhancement of ERK activity in interneurons leads to a permanent increase in the inhibitory tone throughout the brain, resulting in a reduced synaptic plasticity in the hippocampus and in a severe form of intellectual disability.

Disclosures: R. Brambilla: None. **A. Papale:** None. **N. Solari:** None. **R. d'Isa:** None. **M. Cambiaghi:** None. **M. Cursi:** None. **L. Letizia:** None. **M. Cerovic:** None. **N. Hardingham:** None. **E. Menna:** None. **M. Matteoli:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Program#/Poster: 604.03/I5

Topic: C.06. Developmental Disorders

Title: The molecular basis of altered emotional learning in an environmentally induced animal model of Autism

Authors: *A. BANERJEE, J. A. LUONG, A. MORALES, A. HO, B. SAULS, J. E. PLOSKI; Univ. of Texas At Dallas, Richardson, TX

Abstracts: Autism Spectrum Disorders (ASD) are complex neurodevelopmental disorders characterized by core symptoms including repetitive behavior, impaired social interactions and deficits in social communication. Apart from these core symptoms, a significant number of ASD individuals display higher levels of anxiety and some studies indicate that a subset of ASD

individuals are impaired in their ability to be fear conditioned. Therefore we sought to further examine emotional learning in an environmentally induced animal model of ASD, where pregnant rats are exposed to the known teratogen, valproic acid (VPA) on day 12.5 of gestation. Specifically we exposed dams to either one of two different doses of VPA (500 and 600 mg/kg) or vehicle on day 12.5 of gestation. Resultant progeny at 60 days of age were auditory fear conditioned to a 5 kHz 75 dB tone. Animals exposed to 500 mg/kg VPA displayed normal acquisition of fear conditioning, but exhibited reduced extinction of fear memory. However we observed that rats exposed to 600 mg/kg of VPA exhibited a significant reduction in acquisition of fear conditioning. To examine the molecular basis of VPA induced impairment in fear learning in animals exposed to VPA (600 mg/kg), we performed whole genome gene expression analysis using DNA microarrays to examine differences in gene expression within the amygdala from rats exposed to VPA and vehicle. Homer1a was one of the genes which exhibited a significant upregulation within the amygdala. Homer1a is an activity induced immediate early gene (IEG) which is formed from a longer homer1 gene by alternative transcription termination. It acts as a dominant negative regulator and uncouples long forms of homer-ligand interaction resulting in molecular reorganization of the synapse. We chose to focus on Homer1a due to its critical role in the normal functioning of the glutamatergic system, its involvement in the process of fear learning and its previous implication in ASD. To validate the microarray data and to additionally examine how homer1a mRNA levels varied within the basolateral nucleus of the amygdala (BLA) in animals exposed to either 500mg/kg or 600 mg/kg we performed quantitative PCR (qRT-PCR) using homer1a specific primers. We observed a dose dependent increase in homer1a mRNA levels in VPA exposed animals indicating that the precise levels of homer1a within the BLA may be critical for proper fear learning. We have generated adeno-associated (AAV) viruses designed to express homer1a which we have bilaterally infused into the rat BLA to examine how over expression of homer1a can influence fear learning.

Disclosures: **A. Banerjee:** None. **J.A. Luong:** None. **A. Morales:** None. **A. Ho:** None. **B. Sauls:** None. **J.E. Ploski:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Topic: C.06. Developmental Disorders

Support: Columbia University CTSA grant (NCRR/NIH)

Einhorn Family Charitable Trust ULI RR024156

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Title: Oxytocin counteracts inflammatory effects of bacterial endotoxin in gut cells

Authors: *H. TAMIR[1,6], B. Y. KLEIN[2], D. L. HIRSCHBERG[3], R. J. LUDWIG[2], M. M. MYERS[2,4,5], S. GLICKSTEIN[7], M. G. WELCH[1,5,2,4];
[1]Pathology & Cell Biol., [2]Developmental Neurosci., [3]Ctr. for Infection and Immunol., [4]Pediatrics, [5]Psychiatry, Columbia Univ. Col. of Physicians and Surgeons, New York, NY; [6]Mol. Imaging and Neuropathology, New York State Psychiatric Inst., New York, NY; [7]EB Sci., Oakland, CA

Abstracts: It is increasingly likely that the gut is impacted at a cellular level in a subset of individuals with autism spectrum disorders (ASD). This is supported by metanalyses showing that children with ASD experience significantly more nonspecific gastrointestinal (GI) symptoms and abnormal bacterial endotoxin lipopolysaccharide (LPS) responses, when compared to non-affected peers. We have previously reported that OT and its receptor (OTR) modulate the signaling of multiple molecules in the cellular stress pathway, including PI3K, Akt and importantly, the mammalian target of rapamycin complex1 (mTORC1) in gut cell culture; Akt and mTORC1, which modulate cellular stress signaling, and OT, known to modulate affiliative behaviors, have been associated with ASD etiology through genetic screens and functional analysis. Gut inflammation may be modulated by OT/OTR in a subset of individuals with ASD and gut symptoms: (1) OT/OTR reduces inflammation and restores normative function in an experimental model of colitis in rats and mice. (2) Mice lacking the OTR show altered gastrointestinal structure, motility, inflammation, macromolecular permeability, and mucosal maintenance. All of these findings suggest that understanding the pathophysiology of OT/OTR in the gut could lead to a better understanding of the pathophysiology of ASD phenotypes. In the current study, we stimulated the Caco2BB enterocyte gut cell line with bacterial 100- 400 ng/ml LPS for 90 min to induce an inflammatory response and treated the cells with 7.8 - 62.5 nM OT to test whether OT could modulate the response. LPS alone activated Akt, mTORC1 substrate S6K1 and induced the NF-KB inflammatory transcriptional program. Treatment with OT after LPS inhibited the activation of Akt and S6K1, and attenuated increases in pIKB. Conversely, LPS alone inhibited the activation of IRE1, a sensor of endoplasmic reticulum stress. OT treatment after LPS counteracted this inhibition. Further, LPS alone inhibited IRE1a substrate X-box binding protein 1, thus arresting transduction of the unfolded protein response. OT treatment after LPS counteracted the same inhibition and reactivated the response. We also demonstrated the OTR dependence of this response using the OTR-specific antagonist OTA. Results show that OT can reverse multiple LPS-induced inflammatory signals and suggest that OT may protect enterocytes from stress. These findings add to our understanding of abnormal intracellular stress

mechanisms in the gut during inflammation, and may lead to new treatments, particularly for a subset of autistic children with GI symptoms.

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Poster

604. Autism Synaptic and Cellular Mechanisms

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Topic: C.06. Developmental Disorders

Support: Autism Speaks pilot grant

Title: A novel *in vivo* assay of Pten autism alleles reveals necessary roles for Pten in cortical GABAergic interneuron development

Authors: *D. VOGT, K. K. CHO, A. T. LEE, V. S. SOHAL, J. L. RUBENSTEIN;
Dept. of Psychiatry, Univ. of California San Francisco, San Francisco, CA

Abstracts: Pten is a strong autism candidate gene and is involved in signaling events that are a hub for many other autism candidate genes. Pten inhibits signaling downstream of PI3K and its loss of function is associated with neuronal overgrowth, including soma, axon and dendrite hypertrophy. While Pten's role in cancer and glutamatergic neurons has been studied, its role in GABAergic neuron development has not been extensively explored. Cortical GABAergic interneurons are highly diverse and have been implicated in neuropsychiatric disorders, including autism. Here, we examine the role of Pten in cortical GABAergic interneuron development and show its necessity for multiple events in cortical interneuron development. Moreover, we have developed an *in vivo* assay to test the functionality of human variants reported in autism populations. We transduced medial ganglionic eminence (MGE) cells with Pten alleles associated with autism, then transplanted these cells to assay the effects of these alleles in critical steps in cortical interneuron development. Specifically, we assessed the role of Pten in MGE-derived cortical interneuron development, including the evaluation of somatostatin and parvalbumin subgroups. We found that Pten is necessary for cortical GABAergic interneuron development and introduce novel assays to quickly assess autism alleles *in vivo*.

Disclosures: **D. Vogt:** None. **K.K. Cho:** None. **A.T. Lee:** None. **V.S. Sohal:** None. **J.L. Rubenstein:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: The National Research Foundation of Korea: 2005-0093836

The Korean Health Technology R&D Project: A092042

The Asan Institute for Life Sciences: 2012-193

Title: Thimerosal induces autistic behaviors in mice: Possible involvement of metalloprotease activation, zinc dyshomeostasis, and BDNF upregulation

Authors: ***M. YOO**[1], J. LIM[1], T.-Y. KIM[1], H.-R. BYUN[1], B.-R. SEO[1], J. A. CHOI[1], J.-Y. KOH[1,2];
[1]Neural Injury Res. Ctr., Asan Life Sci. Res. Inst., Seoul, Korea, Republic of; [2]Dept. of Neurol., Asan Med. Ctr., Seoul, Korea, Republic of

Abstracts: Whereas a number of genetic factors for autism spectrum disorder (ASD) have been identified, still the majority of cases remain idiopathic. Of diverse candidate environmental factors, childhood vaccination, especially with those containing thimerosal (TM), was proposed as a risk factor for idiopathic ASD. Although the role of TM has long been controversial, a recent epidemiological study seems to support the possibility (Geier et al., Transl Neurodegeneration, 2013). Since some ASD cases have been associated with increased BDNF levels, in the present study, we examined the possible link between TM and BDNF levels *in vitro* and *in vivo*. In addition, we tested whether TM treatment induces typical autistic behaviors in mouse. To mimic human vaccination schedules, mice were inoculated intramuscularly at postnatal day (p) 7, p9, p11, and p15 with 14.2, 10.8, 9.2, or 5.6 μg/kg of TM respectively. Control mice of the same age received corresponding volumes of saline. To assess autistic behaviors, the three-chamber sociability/social novelty test and open field interaction test were performed when mice were 4 weeks old. Compared with control mice, TM administered mice showed autistic behaviors such as preference for exploring an empty chamber to an unfamiliar

mouse (stranger1), and preference for the stranger 1 to a novel stranger (stranger 2). Postmortem brain examinations revealed that BDNF levels were substantially increased in neurons and microglia of cortex and hippocampus. In addition, intracellular free zinc levels and MMP activity were found increased in the same cells. Considering organic mercurials such as APMA are often potent MMP activators, we then examined whether TM is also a direct MMP activator, using cortical cell culture. Exposure of cortical cultures to 10μM TM markedly induced MMP-2, 9 activation, assessed by *in situ* zymography. At the same time, intracellular zinc levels as well as BDNF levels were significantly increased. All these changes were completely blocked by GM6001, a pan-MMP inhibitor, indicating that TM effects were indeed mediated by MMP activation. Present results demonstrate that TM, an organic mercurial compound, activates MMP, which then increases free zinc levels and BDNF levels both *in vitro* and *in vivo*. Such molecular changes may contribute to autistic behaviors that TM-treated mice exhibit. MMPs may prove a potential drug target that can prevent early changes associated with idiopathic ASD.

Disclosures: **M. Yoo:** None. **J. Lim:** None. **T. Kim:** None. **H. Byun:** None. **B. Seo:** None. **J.A. Choi:** None. **J. Koh:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Topic: C.06. Developmental Disorders

Support: KAKENHI (to T.S.)

NIMH (to M.N.)

NIMH (to A.S.)

Title: Comprehensive analysis of gene expression associated with the prefrontal circuit maturation

Authors: *S. UEDA[1], M. NIWA[2], A. SAWA[2], T. SAKURAI[1];
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Abstracts: The prefrontal cortex (PFC) is a pivotal brain region for cognitive functions, including working memory, planning, reasoning, and decision-making. Recent studies have

identified abnormalities (e.g. deficient myelination, aberrant neurotransmission, excitatory/inhibitory imbalance) in the PFC of developmental psychiatric disorders, namely autism and schizophrenia, supporting an idea that dysregulation of the circuitry development in PFC may be involved in these disorders. Knowledge on how these processes proceeds during normal brain development is crucial to understand involvement of developmental dysregulation in pathogenesis of these disorders. To this end, we performed comprehensive quantitative polymerase chain reaction (qPCR) analysis using mouse brains. Expression of oligodendrocyte/myelin related genes (e.g. *Cldn11*, *Plp1*) and fast-spiking interneuron marker gene, *Pvalb*, were dramatically increased between postnatal days 7 (P7) and P21, and peaked at P21 and P35, respectively. On the other hand, there were little changes in genes for neurotransmitter receptors (dopamine, AMPA, and NMDA receptors), postsynaptic molecules, and cell adhesion molecules. These patterns are unique to PFC compared to any other brain regions. In addition, we characterized responsiveness to NMDA antagonist by measuring extracellular glutamate level in the mouse PFC using *in vivo* microdialysis after a MK-801 administration. While basal extracellular glutamate level reached nearly equal adult level, MK-801 sensitivity was still high at P42, and then it was stabilized to adult level at P56. These results indicate that timing of prefrontal circuit maturation may be around P42 or later. These basic data on normal development should help us to have better ideas on the events in normal and pathological situations taking place in the PFC.

Disclosures: **S. Ueda:** None. **M. Niwa:** None. **A. Sawa:** None. **T. Sakurai:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Support: NIH Grant MH099504

NIH Grant MH100172

Title: The chromosome 20p12.1 autism genome-wide significant association signal implicates the long non-coding RNAs RPS10P2AS and MACROD2-AS1

Authors: *D. B. CAMPBELL, N. GREPO;
Psychiatry, USC, Los Angeles, CA

Abstracts: We recently reported that the long non-coding RNA *MSNP1AS* (moesin pseudogene 1 anti-sense) is a functional element revealed by an autism genome-wide association study (GWAS) signal on chromosome 5p14.1. *MSNP1AS* expression was increased in postmortem temporal cortex of individuals with autism and increased *MSNP1AS* expression was correlated with the autism-associated genotype. These data indicated that the non-coding RNA *MSNP1AS*, rather than the flanking protein-coding genes *CDH9* and *CDH10*, was the functional element implicated by the chromosome 5p14.1 autism GWAS signal. Shortly after the first autism GWAS report was published, another autism GWAS report indicated genome-wide significant association of the chromosome 20p12.1 marker rs4141463. Although rs4141463 lies within an intron of the protein-coding *MACROD2* (MACRO domain containing 2) gene, expression of *MACROD2* is neither altered in postmortem temporal cortex of individuals with autism nor correlated with rs4141463 genotype. Our bioinformatics approaches revealed two non-coding RNA transcripts near the autism susceptibility signal: *RPS10P2AS* (ribosomal protein S10 pseudogene 2 anti-sense) and *MACROD2-AS1* (MACROD2 anti-sense 1). In a panel of 15 human tissues, qPCR revealed that both *RPS10P2AS* and *MACROD2-AS1* were expressed at higher levels than the protein-coding *MACROD2* in fetal temporal cortex and adult peripheral blood. In postmortem temporal cortex, expression of *RPS10P2AS* was increased 7-fold in individuals with autism (P=0.02) and increased 8-fold in individuals with the autism-associated rs4141463 genotype (P=0.01). Similarly, *MACROD2-AS1* was increased 6-fold in individuals with autism (P=0.03) and increased 9-fold in individuals with the autism-associated rs4141463 genotype (P=0.02). Ongoing experiments will determine the impact of over-expression of the long non-coding RNAs *RPS10P2AS* and *MACROD2-AS1* on neuronal gene expression and morphology. These data indicate that multiple genome-wide significant associations with autism implicate long non-coding RNAs. Because long non-coding RNAs are more abundant in human brain than protein-coding RNAs, this class of molecules is likely to contribute to autism risk.
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Disclosures: **D.B. Campbell:** None. **N. Grepo:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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The Lily Avraham Gildor Chair for the Investigations of Growth Factors

AMN Foundation

Title: Activity-dependent neuroprotective protein (ADNP) interacts with the eukaryotic translation initiation factor 4e (eIF4E): an autism upstream regulator

Authors: *I. GOZES, A. MALISHKEVICH, N. AMRAM, G. HACOHEN-KLEIMAN; Sackler Sch. Med/Tel Aviv Univ., Tel Aviv, Israel

Abstracts: Activity-dependent neuroprotective protein (ADNP) discovered in our laboratory, is essential for brain formation(1, 2). De novo mutations in ADNP in humans lead to autism(3), and partial deficiency in ADNP in mice is associated with microtubule (MT) deficiencies and brain dysfunction, which is translated into social and cognitive deficits. The deficits are exacerbated with aging and are coupled to tau hyperphosphorylation and neuronal cell death(4). A recent publication showed that knockout of the eukaryotic translation initiation factor 4E (eIF4E) - binding protein 2 (4E-BP2)-an eIF4E repressor or eIF4E overexpression led to increased translation of neuroligins, which are postsynaptic proteins that are causally linked to autism spectrum disorders (ASDs)(5). Here, bioinformatics identified binding sites on ADNP for eIF4E and co-immunoprecipitation revealed a direct association between ADNP and eIF4E. Further expression studies showed increased eIF4E expression in the ADNP-deficient male mice. These studies position ADNP as an upstream candidate regulator of autism, controlling the expression of translation initiation factor (s) influencing key output behaviors. We thank Prof. O. Elroy-Stein for the antibodies. 1.A. Pinhasov et al., Activity-dependent neuroprotective protein: a novel gene essential for brain formation. Brain Res Dev Brain Res 144, 83 (Aug 12, 2003). 2.S. Mandel, G. Rechavi, I. Gozes, Activity-dependent neuroprotective protein (ADNP) differentially interacts with chromatin to regulate genes essential for embryogenesis. Dev Biol 303, 814 (Mar 15, 2007). 3.C. Helsmoortel et al., A SWI/SNF-related autism syndrome caused by de novo mutations in ADNP. Nat Genet 46, 380 (Apr, 2014). 4.I. Vulih-Shultzman et al., Activity-dependent neuroprotective protein snippet NAP reduces tau hyperphosphorylation and enhances learning in a novel transgenic mouse model. J Pharmacol Exp Ther 323, 438 (Nov, 2007). 5.C. G. Gkogkas et al., Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature 493, 371 (Jan 17, 2013).

Disclosures: I. Gozes: None. **A. Malishkevich:** None. **N. Amram:** None. **G. Hacohen-Kleiman:** None.

Poster

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Topic: C.06. Developmental Disorders

Support: Aarhus University

Aarhus University Hospital

Title: Reduced striatal 5-HT2A receptor but not serotonin transporter levels in the subchronic valproate model

Authors: *M. M. SKOVBORG[1], F. BERTELSEN[1,2], D. FOLLONI[1], P. WEIKOP[3], J. SCHEEL-KRÜGER[1], A. MØLLER[1,2], A. M. LANDAU[1,2];
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Abstracts: Introduction: Alterations in the serotonin (5-HT) system are often detected in patients with Autism Spectrum Disorders (ASD). ASD is characterised by impaired social interactions, impaired verbal and non-verbal communication, and by restricted, repetitive or stereotyped behaviour. In order to classify as ASD, the symptoms must be apparent before 3 years of age. Because prenatal exposure to Valproate (VPA) is associated with ASD, we have developed a novel animal model of autism in which pregnant rats were exposed to subchronic doses of VPA resulting in increased neuronal cell number, behavioural deficits and decreased striatal 5-HT levels in the VPA exposed rats compared to controls. In light of the latter finding, the aim of the current study is to further investigate the serotonin system with focus on the 5-HT_{2A} receptor and the serotonin transporter (SERT). **Methods:** Pregnant Wistar rats were treated with VPA (20 or 100 mg/kg) or saline from day 12 until the end of pregnancy. Brains from the male offspring (n=7/group) were removed and fresh frozen at postnatal day 50 and then sliced into 20 μm thick sections. We performed *in vitro* autoradiography of striatal $5HT_{2A}$ receptors using [^{3}H]Ketanserin as the radioligand and mianserin to assess non-specific binding. We assessed striatal SERT levels using [^{3}H]DASB as the tracer and citalopram to detect non-specific binding. Statistics were done on the specific binding values using a one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test. **Results:** The 5-HT_{2A} receptor binding was significantly decreased in dorsolateral and ventrolateral striatum in rats prenatally exposed to VPA compared to saline controls ($p<0.05$ and $p<0.01$ respectively). The post hoc test revealed that the decrease in dorsolateral striatum was only significant for the rats exposed to 20 mg/kg/day whereas the decrease in ventrolateral striatum was significant in both VPA-groups. However, VPA did not induce changes in striatal [^{3}H]DASB binding. **Conclusions:** The lower 5-HT_{2A} receptor binding combined with reduced levels of 5-HT in striatum indicate a down-regulation of the serotonin system in the VPA-exposed rats consistent with imaging studies in human in which 5-HT_{2A} receptor levels are altered. The lack of difference in SERT-binding is in

contrast to human imaging studies which detect reduced SERT availability, however methodological and species differences may account for the differences in data. The changes at the receptor and not the transporter level in our study may suggest changes in serotonin metabolism and release coupled to 5-HT_{2A} receptor regulations.

Disclosures: **M.M. Skovborg:** None. **F. Bertelsen:** None. **D. Folloni:** None. **P. Weikop:** None. **J. Scheel-Krüger:** None. **A. Møller:** None. **A.M. Landau:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Program#/Poster: 604.11/J1

Topic: C.06. Developmental Disorders

Support: Alberta Children's Hospital Research Institute

Title: Modulation of aberrant ERK signaling as a potential therapeutic strategy in the BTBR mouse model of autism

Authors: ***N. CHENG**[1], Y. AHN[1], R. MYCHASIUK[1], R. TOBIAS[1], D. RUSKIN[2], S. MASINO[2], J. M. RHO[1];
[1]Univ. of Calgary, Calgary, AB, Canada; [2]Trinity Col., Hartford, CT

Abstracts: Autism spectrum disorder (ASD) is a common life-long neurodevelopmental disorder defined by three core symptoms: impaired social interactions, communication deficits, and stereotyped repetitive behaviors. Unfortunately, there are no effective treatments that directly address these core deficits and the underlying mechanisms remain poorly understood. Notably, the most common genetic linkages to ASD include ones that involve the extracellular-signal-regulated kinase (ERK) pathway. This pathway is highly conserved in all eukaryotes and critically involved in neurodevelopmental processes such as cellular proliferation, differentiation, and apoptosis. It involves a cascade of kinase activation where phosphorylated MEK (pMEK) in turn phosphorylates and activates ERK (pERK). pERK subsequently activates downstream targets including cAMP response element binding protein (CREB), a transcription factor involved in a variety of cellular processes. Here, we hypothesize that there is aberrant ERK signaling in ASD, and interventions aimed at normalizing this pathway could alleviate ASD symptoms. We used the BTBR mouse model because it robustly exhibits all three core behavioral features of ASD. We found that protein expression levels of three key components of

the ERK pathway were slightly but significantly increased in P35 BTBR neocortex compared with control B6 mice (pMEK: 104% increase, $p<0.04$; pERK: 43% increase, $p<0.05$; and pCREB: 26% increase, $p<0.05$). Based on these findings, we asked if pharmacological inhibition of ERK signaling would mitigate ASD-like behaviors in BTBR mice. U0126 (a highly selective and potent MEK inhibitor) was injected i.p. daily (12.5 mg/kg/day) in BTBR and B6 mice from P24 to P35; control groups were sham-injected with vehicle. At P35, we assessed all groups of mice with two behavioral tests: inchworming (a novel motor stereotypy recently identified in BTBR mice by our lab) and the established 3-chamber test of sociability. U0126 treatment significantly reduced inchworming behavior in BTBR mice ($p<0.0002$), and led to more social interactions in the 3-chamber assay ($p=0.11$). Our preliminary results suggest that ERK signaling is aberrantly up-regulated in BTBR mice and its inhibition may have therapeutic potential in reversing some of the core symptomatology of ASD.

Disclosures: N. Cheng: None. **Y. Ahn:** None. **R. Mychasiuk:** None. **R. Tobias:** None. **J.M. Rho:** None. **D. Ruskin:** None. **S. Masino:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.12/J2

Topic: C.06. Developmental Disorders

Title: sncRNA expression differs more with age and by region in typical brain development than in ASD

Authors: B. STAMOVA[1], B. P. ANDER[1], N. BARGER[2], F. R. SHARP[1], *C. SCHUMANN[2]; [1]Neurol., [2]Psychiatry and Behavioral Sci., UC Davis MIND Inst., Sacramento, CA

Abstracts: Small non-coding RNAs (sncRNA), including microRNA (miRNA) and snoRNA, play a fundamental role in brain development throughout lifespan. Yet, little is known about their function in typical or atypical human brain development. To provide a baseline for contextualizing aberrant patterns of development, we first assessed differential expression of sncRNAs in typically-developing (TD) postmortem human brains in two temporal cortical regions - superior temporal sulcus (STS) and adjacent primary auditory cortex (PAC). STS is association cortex involved in social perception and commonly implicated in neurodevelopmental and psychiatric disorders; PAC processes basic auditory sensory information. We then similarly assessed age-matched brains from individuals with autistic

spectrum disorder (ASD) for comparison. Using Affymetrix miRNA 3.0 arrays, we explored how sncRNA expression patterns vary within TD and within ASD brains: 1) between spatially proximate, but functionally distinct, regions and 2) within these regions from childhood to adulthood. We found distinct regional and age-related changes of differentially expressed sncRNAs in TD in STS and PAC, however these differences were attenuated in ASD. Spatially, between STS and PAC, 22 sncRNAs were differentially expressed in TD cases, versus only 11 in ASD. In STS, 18 mature miRNAs changed with age in TD, while only 4 in ASD. In PAC, 27 mature miRNAs changed with age in TD, while only 2 in ASD. Age-related miRNAs in TD brains, based on similarity of expression profiles, group into defined positive and negative co-expression clusters, suggesting co-regulation of downstream mRNA targets. In contrast, ASD brains display significant deviations from this coordinated miRNA expression, thus potentially dysregulating a large number of downstream mRNA targets. In TD, putative targets of differentially expressed miRNAs are enriched in processes such as neurotransmitter signaling pathways, mTOR signaling, and in transcriptional regulation. ASD brains share common pathways with TD, and have enrichment of pathways not observed in TD, such as PTEN, PI3K, Erb, CNTF, neurotrophin, dopamine and cholecystokinin signaling. Several differentially expressed miRNAs found in our regional and age-related analyses have been previously associated with ASD dysregulation, providing additional support for their relevance to aberrant brain development across distinct brain regions. Our TD findings provide a necessary baseline for identifying sncRNA deviations in several disorders, including ASD, where we found evident attenuation of spatial and age-related differentially expressed miRNAs within temporal cortex.

Disclosures: B. Stamova: None. **B.P. Ander:** None. **N. Barger:** None. **F.R. Sharp:** None. **C. Schumann:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.13/J3

Topic: C.06. Developmental Disorders

Support: NIH Grant MH094681

Autism Science Foundation

Title: Interneuronal subpopulations in prefrontal cortex (BA 46) in autism

Authors: *V. MARTINEZ-CERDENO, E. HASHEMI, J. ARIZA;
Pathology, UC Davis, Sacramento, CA

Abstracts: An alteration in the number of interneuron types in a specific region of the cortex would likely alter the pattern of connections between cortical areas and could produce disturbances in cognitive functioning similar to those seen in autism. An alteration in the balance of excitatory neurons and inhibitory neurons is already known to be related with diseases such as squizophrenia and epilepsy. Here, we investigate the contribution of distinct cortical interneuron populations within the area BA46 of human prefrontal cortex, to the pathogenesis of autism. Parvalbumin (PV), calbindin (CB), and calretinin (CR) are markers of separate populations of interneurons defined by their morphology, their laminar distribution, and their developmental pattern. Even there is a small amount of colocalization, CR+, CB+, and PV+ neurons represent morphologically non-overlapping populations of neurons (Hof, 99). We obtained 10 autism cases and 10 age-matched controls (from the Autism Tissue Program), and based on gross cortical neuroanatomy in each case we isolated a block containing area BA 46. We cut 14 µm sections, stained one with Nissl, and based on cytoarchitecture selected a 2 mm wide bin extending through the thickness of the cortical grey matter in BA 46. On the adjacent section we performed triple immunostaining using different color combinations, and counterstained with Nissl. Using Stereo-investigator, we quantified the number of single PV+, CB+, CR+, double and triple stained cells within supragranular and infragranular layers in the area of interest, and compared the number of cells using paired t-tests.

Disclosures: V. Martinez-Cerdeno: None. **E. Hashemi:** None. **J. Ariza:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Program#/Poster: 604.14/J4

Topic: C.06. Developmental Disorders

Support: NIH Grant R01 MH097236

NIH Grant T32 MH073124

Title: Developmental trajectories of neuron numbers across amygdaloid nuclei in Autism Spectrum Disorder

Authors: *N. BARGER[1], M. V. VARGAS[2], C. M. SCHUMANN[3];
[1]Univ. of California, Davis - MIND Inst., Sacramento, CA; [3]Psychiatry and Behavioral Sci.,
[2]MIND Institute, Univ. of California, Davis, Sacramento, CA

Abstracts: Concordant with the core features of Autism Spectrum Disorder (ASD), we have previously found that neuron numbers in the amygdala, a brain region implicated in social and emotional behavior, are reduced in ASD adults. Given that a large subset of individuals with ASD exhibit atypical amygdala growth trajectories in MRI analyses, we sought to address whether neuronal developmental trajectories in the amygdala were additionally altered in children and across the lifespan. The amygdala is structurally and connectively heterogeneous and not all of its nuclei evidence reduced neuron numbers. Therefore, we assessed developmental patterns in four individual amygdaloid nuclei, the lateral, basal, accessory basal, and central nuclei, to understand how the process of cellular development may vary across nuclei to produce significant variation in the adult phenotype. We used the optical fractionator technique to estimate neuron number in Nissl-stained sections from postmortem samples of autistic and control cases 2-44 years of age. Neuron numbers in each diagnostic group were regressed against subjects' numeric age for each nucleus. In controls, age and neuron number evidenced no significant relationship in any nucleus analyzed ($p > 0.05$). In our preliminary analyses of ASD, lateral nucleus neuron numbers shared a significant, negative relationship with age ($p = 0.02$), while the neuron numbers in the basal nucleus showed a trend towards a negative linear relationship with age ($p = 0.11$). Regressions for the accessory basal and central nuclei were not significant. We are collecting data on additional cases in each diagnostic group to further test these findings. We have previously found significant reductions in neuron numbers in the lateral nucleus of the amygdala in adults with ASD. These preliminary findings suggest that autistic individuals are not born with a deficit of lateral nucleus neurons, but may experience increased rates of neuron loss through development compared with typically developing individuals. Additionally, our data indicate that neurons may be lost in the basal nucleus through development in ASD, but perhaps not at rates that yield significant average changes across groups in smaller samples of autistic adults. Increasing our sample population can provide even greater insight into developmental processes in the basal nucleus. Our findings highlight the importance of assessing autistic neuropathology across age groups to identify both potential mechanisms underlying significant variation in adult brains as well as aberrant developmental trends that may not be readily apparent in the adult phenotype due to changes across lifespan.

Disclosures: **N. Barger:** None. **M.V. Vargas:** None. **C.M. Schumann:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

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Program#/Poster: 604.15/J5

Topic: C.06. Developmental Disorders

Support: NSC102-2320-B-010-009 Taiwan

NSC102-2321-B-010-025 Taiwan

Title: NMDA receptor dysfunction contributes to impaired long-term depression in a valproate-induced rat autism model

Authors: *H.-F. WU[1,2], H.-J. YEN[2], P.-S. CHEN[3], H.-C. LIN[2];
[1]Physiol., [2]Physiol. and Brain research center, Natl. Yang-Ming Univ., Taipei, Taiwan;
[3]Psychiatry and Addiction Res. Ctr., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstracts: Autism-like phenotypes in male valproate (VPA)-exposed offspring has been linked to high glutamatergic neurotransmission in the thalamic-amygdala pathway. Amygdala, in the brain area, is related to socio-emotional behavior. Additionally, the activation of NMDA receptor (NMDAR) is the major cellular components of long term potentiation (LTP) and long term depression (LTD). However, the regulation of synaptic NMDAR activity within amygdala in autism disorders remains poorly understood. In this study, we characterized the LTD of amygdala in the VPA exposed offspring. By using the behavioral tracking technique to observe the social interaction, anxiety and depression-like responses *in vivo* and electrophysiology technique of extracellular recording of the amygdala brain slices *in vitro*. We found that the VPA-exposed offspring showed the less social interaction whereas higher emotional-related behaviors. Furthermore, the input-output relationship was not changed but the NMDA receptor-dependent long term depression was impaired in cortico-amygdala circuit in the VPA exposed offspring. The results suggested that the NMDA receptor regulates the synaptic activity in the amygdala which involved in VPA- exposed autism mode.

Disclosures: **H. Wu:** None. **H. Yen:** None. **P. Chen:** None. **H. Lin:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.16/J6

Topic: C.06. Developmental Disorders

Support: IMI EU Grant

Title: EU-AIMS - a boost for pre-clinical and clinical autism Research

Authors: *W. SPOOREN;
Hoffmann-La Roche, Basel CH 4070, Switzerland

Abstracts: Autism spectrum disorder (ASD) is a severe neurodevelopmental condition affecting 1% of the population. Recent identified genetic risk factors have given insight into the neurobiology. To move this field into evidence based medicines, fourteen leading academic centers across Europe have partnered with Autism Speaks, Autism Europe and six industry partners, i.e. Eli Lilly, J&J, Pfizer, Servier, Vifor and Roche. This consortium named EU-AIMS, was set-up in the context of the Innovative Medicines Initiative (EU) in 2012 and is led by Roche together with Kings College (UK) and has a budget of over 50 million $. The goal is to create a fully integrated pre-clinical and clinical research effort to advance this field significantly. We now report our first results: (1) EU-AIMS validated protocols to generate neurons from patient keratinocytes through reprogramming IPS cells. (2) Using a reversible KO model, EU-AIMS demonstrated that mice lacking NLGN3 share a convergent molecular signature with fragile-X syndrome, which could be rescued in adulthood. (3) By using MRI we have characterized endophenotypes of four animal models carrying mutations from ASD patients. 4) Two unprecedented naturalistic observational studies will be started in 2014 across Europe characterizing patients ageing from 6 months - 30 years: a) The Infant-at-Risk study prospectively studies 300 infants at high genetic risk and 100 low-risk infants from over 36 months. The aim is to identify factors that predict ASD. b) The Longitudinal European Autism Project (LEAP) is the largest autism project to date and includes 400 participants with ASD and 250 control participants from 6-30 years. Each participant will be seen at two time-points and all participants are comprehensively clinically characterized. Finally, EU-AIMS is setting-up an unprecedented European wide clinical network and has recruited more than 70 centers in 37 countries to share information, align and run clinical studies in a way that is unique in the world. NATURE REVIEWS-DRUG DISCOVERY VOLUME 11 | 2012 | 815 Web-site: http://www.eu-aims.eu/

Disclosures: **W. Spooren:** A. Employment/Salary (full or part-time):; F. Hoffman-La Roche.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.17/J7

Topic: C.06. Developmental Disorders

Title: Synaptic characterization of Nlgn3 KO Rats

Authors: *R. NAIR[1], E. SYLWESTRAK[1], P. SCHEIFFELE[2], A. GHOSH[1];
[1]F.Hoffmann La Roche, Basel, Switzerland; [2]Biozentrum, Univ. of Basel, Basel, Switzerland

Abstracts: Autism spectrum disorders (ASDs) are neurodevelopmental disorders, diagnosed early in development during the time of circuit formation. ASDs are characterized by impairments in social interactions and communication, and can be accompanied by repetitive behaviors. Genetic factors contribute strongly to the etiology of autism. A large number of genes encoding synaptic proteins have been implicated in ASDs suggesting that synaptic dysfunctions contribute to the pathogenesis of ASDs. In particular, one class of monogenic heritable form of autism is associated with mutations in neuroligin (NLGN) genes that encode postsynaptic cell adhesion molecules, which are essential for normal synapse function. Mutations in neuroligin3 (NL3) consisting of a single amino acid substitution from the Arg451 to Cys451 (R451C) as well as complete loss of NL3 have been implicated in autism. In this study, we have made use of a knockout rat line of NL3, which has been generated using zinc finger nuclease (ZFNs) technology. The rat is a preferred model system for studying many neurological disorders such as autism, mainly for its social behavior, size and, physiology. The major goal of this study is to understand the fundamental defects in autism models and hopefully reverse the changes using pharmacological tool. In the layer 2/3 of the somatosensory cortex, we found an increase in inhibitory synaptic transmission. However in the CA1 region of the hippocampus, no obvious changes were detected.

Disclosures: **R. Nair:** A. Employment/Salary (full or part-time):; Hoffmann La Roche, Biozentrum. **E. Sylwestrak:** None. **P. Scheiffele:** None. **A. Ghosh:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Program#/Poster: 604.18/J8

Topic: C.06. Developmental Disorders

Support: Autism Research Training (ART) Program Studentship

Canadian Institutes of Health Research (CIHR) Fellowship Program

Alberta Children's Hospital Research Institute (ACHRI)

Canadian Institutes of Health Research

Title: Decreased PI3K-Akt-mTOR signaling pathways in human autism and in rats exposed to valproic acid

Authors: *M. FAHNESTOCK[1], C. NICOLINI[2], Y. AHN[3], B. MICHALSKI[2], J. M. RHO[3]; [1]Dept Psychiatry & Behavioural Neurosciences, [2]Psychiatry & Behavioural Neurosciences, McMaster Univ., Hamilton, ON, Canada; [3]Depts. of Paediatrics & Clin. Neurosciences, Alberta Children's Hosp. Res. Inst., Univ. of Calgary, Calgary, AB, Canada

Abstracts: The molecular mechanisms underlying autistic behavior remain to be elucidated. Maternal exposure to the anticonvulsant valproic acid (VPA) during pregnancy induces autism-like phenotypes in both humans and rodents. Here, using both human and rat tissue, we provide two molecular pathways that contribute to autistic behaviour by adversely affecting spines: the Akt/mTOR pathway by disrupting spine protein translation and the TrkB/PI3K/Akt pathway by interfering with PSD-95 transport. Protein levels of the BDNF receptor TrkB, TrkB downstream signalling molecules in the PI3K-Akt-mTOR pathway, mTOR effectors such as p70S6K and 4E-BP1, as well as the excitatory post-synaptic marker PSD-95 were examined by Western blotting in post-mortem fusiform gyrus tissue from 11 subjects with idiopathic autism and 13 control subjects. A similar analysis was carried out on cortical tissue from rats exhibiting autism-like behaviour whose mothers were treated with VPA at embryonic day 12.5. Genetic studies on single-gene developmental disorders such as fragile X syndrome, tuberous sclerosis and Rett syndrome have shown that both up- and down-regulation of the Akt/mTOR pathway are associated with de-regulation of spine protein synthesis and changes in brain connectivity. Here we show that protein products of genes in the Akt/mTOR pathway are ***down***-regulated in subjects with idiopathic autism and in rats exposed to VPA. Our results demonstrate for the first time that disruptions of this pathway are not limited to genetic forms of autism, but are widespread in the autism population. Furthermore, we show that down-regulation of the mTOR pathway affects spine protein translation not via the expected 4E-BP pathway, but, similarly to Rett syndrome, specifically via the p70S6K/eIF4B pathway, which controls translation of mRNAs coding for translational machinery components. Also, full-length TrkB protein expression is decreased in idiopathic autism and is associated with reduced PI3K, Akt and PSD-95. TrkB is involved in transport of PSD-95 via PI3K-Akt, and thus, TrkB may disrupt brain connectivity by interfering with PSD-95 trafficking. Lastly, we propose an epigenetic mechanism, supported by our animal data showing that prenatal exposure of rats to VPA, an HDAC inhibitor, is also associated with down-regulation of the Akt-mTOR pathway. Our results implicate epigenetic down-regulation of PI3K-Akt-mTOR pathways in idiopathic autism.

Disclosures: **M. Fahnestock:** None. **C. Nicolini:** None. **Y. Ahn:** None. **B. Michalski:** None. **J.M. Rho:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.19/J9

Topic: C.06. Developmental Disorders

Support: NIH Grant AG042804

Indiana University Collaborative Research Grant (IUCRG)

Title: Role for the secreted amyloid-β precursor protein (sAPPα) in Autism as early neurodevelopmental disorder

Authors: *D. K. LAHIRI[1], D. K. SOKOL[2], B. RAY[1];
[1]Dept. of Psychiatry, [2]Dept. of Neurol., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstracts: Autism is a neurodevelopmental disorder marked by deficits in social skills and communication and by repetitive behavior. Intellectual disability often accompanies autism. In addition to behavioral deficits, autism is characterized by aberrant neuronal connections and brain overgrowth. We previously reported that the Alzheimer's disease (AD) associated amyloid-β precursor protein (APP), especially its neuroprotective processing product, sAPPα, is elevated in persons with autism (Ray et al, 2011).This has led to the "anabolic hypothesis" of autism (and possibly fragile X) etiology, in which neuronal overgrowth in the brain results in interneuronal misconnections that may underlie multiple autism symptoms (Sokol et al, 2011). APP, a large membrane spanning glycoprotein, is produced in oligodendrocytes and neurons. A sequential cleavage of APP via specific secretase enzymes generates either to amyloid-beta (Aβ) peptides seen in AD amyloid plaques, or to the nonamyloidgenic sAPPα associated with neurotrophic properties. Our group first reported higher levels of sAPPα and lower Aβ peptides in plasma from children with severe autism later replicated independently. We further validated the proposed nonamyloidgenic "anabolic hypothesis" for autism (Lahiri et al, 2013) in autopsied brain tissue samples. Brain tissue specimens (from the Autism Tissue Program and University of Maryland Brain Bank) were homogenized, fractionated, and levels of sAPPα and sAPPtotal were assayed by sensitive sandwich ELISA and Western blotting. Data were analyzed statistically after normalization to the concentration of the total sample. Brain samples from 7 subjects with

autism were compared to 8 age matched controls. Children with autism appeared to be severely affected: incidence of seizures (42.9%), intellectual (mean 57.3, SD 21.01) and social (mean 47.7, SD 23.9) quotients available for 3/7 subjects. Brain extracts from frozen, left temporal lobe showed significant elevation in sAPPα levels for children with autism compared to controls ($p<0.02$). Levels of other APP pathway metabolites, including Aβ peptides, are being analyzed. This novel work warrants further work with a large number of subjects . Our work suggests the relationship of APP to other proteins and pathways that have already been directly associated with autism, such as fragile X mental retardation protein, Ras/ERK and PI3K/Akt/mTOR. Thus, elevated levels of sAPPα detected in autism brain tissue support the sAPPα-driven anabolic pathway for neurodevelopmental disorders, including autism, and open up new therapeutic drug targets.

Disclosures: D.K. Lahiri: None. **D.K. Sokol:** None. **B. Ray:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.20/J10

Topic: C.06. Developmental Disorders

Title: Prenatal exposure to histone deacetylase inhibitors delays neuronal maturation by regulating gene expression of morphogenesis-related molecules

Authors: *T. KAWANAI[1], R. WATANABE[1], A. INOUE[1], Y. AGO[1], K. TAKUMA[1], T. MTSUDA[1,2];
[1]Lab. of Medicinal Pharmacol., Grad. Sch. of Pharmaceut. Sci., Osaka Univ., Osaka, Japan;
[2]Unit-Grad. Sch. of Child Dev., Osaka Univ., Osaka, Japan

Abstracts: We have recently demonstrated that prenatal exposure to a histone deacetylase (HDAC) inhibitor valproic acid (VPA) at embryonic day 12.5 (E12.5) causes autism-like behavioral abnormalities, such as social interaction deficits, anxiety-like behavior and spatial learning disabilities, in male mouse offspring (Int. J. Neuropsychopharamacol., 2013). We have also found that the prenatal VPA exposure causes a transient increase in acetylated histone levels in the embryonic brain, followed by an increase in apoptotic cell death in the neocortex and a decrease in cell proliferation in the ganglionic eminence, and it decreases Nissl-positive cell numbers in the prefrontal and somatosensory cortices after birth. In this study, we examined the effect of prenatal exposure to HDAC inhibitors on neuronal maturation. VPA (500 mg/kg, i.p.),

trichostatin A (TSA; 500 μg/kg, i.p.) or vehicle was injected into pregnant mice on gestational days 12.5 or 14.5, and primary neurons were prepared from the cerebral cortex at E16. Cells were stained with MAP-2 antibody and quantitative morphological analysis was carried out using a Neurolucida software (MBF Bioscience). Prenatal exposure to VPA at E12.5, but not at E14.5, caused decreases in total numbers and length of neuronal dendrites at 14 days *in vitro* (14 DIV). The differences disappeared at 21 DIV. Similar delay of cellular maturation was observed in cortical neurons from embryos exposed to TSA at E12.5. Furthermore, the present study demonstrated that the prenatal exposure to HDAC inhibitors at E12.5 decreased neuroligin-1 mRNA level and increased CNTNAP2 and Shank3 mRNA levels. These findings suggested that prenatal exposure to HDAC inhibitors at E12.5 delays neuronal maturation by regulating gene expression of morphogenesis-related molecules.

Disclosures: **T. Kawanai:** None. **R. Watanabe:** None. **A. Inoue:** None. **Y. Ago:** None. **K. Takuma:** None. **T. Mtsuda:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

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Program#/Poster: 604.21/J11

Topic: C.06. Developmental Disorders

Support: McKnight Endowment Fund for Neuroscience award (AB)

Training grant from the China Scholarship Council (LZ)

NINDS MSTP T32 NS07224 (C.M.B)

Title: Blocking MEK-ERK1/2 activity in TSC rescues defects in neuronal dendritic trees

Authors: *C. M. BARTLEY[1], L. ZHANG[2], X. GONG[2], L. S. HSIEH[2], T. V. LIN[2], A. BORDEY[2];
[1]Neurobio. and MSTP Program, Yale Univ., New Haven, CT; [2]Neurosurgery, and Cell. & Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT

Abstracts: Tuberous sclerosis complex (TSC) is a monogenic disorder due to mutations in TSC1 or TSC2. Most patients display cortical malformations called tubers associated with seizure activity. One of the hallmarks of tubers is an abnormal morphology of cortical neurons. In particular, tuberous neurons display severe dysmorphogenesis of their dendritic trees. Because

the dendritic tree determines the synaptic connectivity and input integration that is necessary for proper network function, alterations in dendritic morphology affect neuronal network activity. Recent studies reported that ERK1/2 activity is increased in both human TSC patients and mouse models of TSC cortical malformations (i.e., tubers). Here we explored whether ERK1/2 activity was increased in Tsc1null neurons and contributed to their abnormal dendritic development. We found that ERK1/2 activity was indeed increased in Tsc1null neurons *in vivo*. Increasing MEK-ERK1/2 activity with expression of a constitutively active MEK1 vector in developing neurons led to increased dendritic complexity. Expressing a dominant negative MEK1 vector in Tsc1null neurons normalized their abnormal dendritic morphology. Finally, pharmacologically decreasing MEK-ERK1/2 activity in Tsc1null neurons significantly rescued dendritic defects. These data demonstrate that altered MEK-ERK1/2 activity increases dendritic complexity and contributes to pathologic dendritic patterning in TSC thus providing a potential additional therapeutic option.

Disclosures: C.M. Bartley: None. **L. Zhang:** None. **X. Gong:** None. **L.S. Hsieh:** None. **T.V. Lin:** None. **A. Bordey:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.22/J12

Topic: C.06. Developmental Disorders

Support: K99/R00 Grant MH102244

Title: Dynamic autism-associated protein interaction networks engaged by glutamate activity at the synapse

Authors: *S. E. SMITH, S. C. NEIER, T. R. DAVIS, A. G. SCHRUM;
Mayo Clin., Rochester, MN

Abstracts: A key limitation in the field of autism research is that despite knowing the identity of tens or even hundreds of risk factors for autism, we do not understand how (or if) these diverse genetic and environmental insults converge on common cellular or molecular processes to cause the core symptoms of the disorder- impaired social interaction, reduced communicative behavior and increased repetitive, stereotyped behaviors. There is strong evidence that many different autism risk factors disrupt glutamatergic signaling, leading to the current “synaptic hypothesis of autism”, but the shared mechanisms, if any, remain unclear. To begin to address this problem, we

have developed a novel multiplex immunoassay to quantify dynamic protein-protein interaction networks at the glutamate synapse, Quantitative Multiplex Immunoprecipitation (QMI). The QMI platform currently measures the relative abundance of 16 autism-associated proteins expressed at the glutamate synapse, and the degree of interaction among them (240 interactions). To validate the assay, we observed several known interactions among synaptic proteins, for example Fyn-GRM5 and GRIA1-Homer1. We also observed several previously unreported protein complexes that we are working to confirm using traditional methods (e.g., IP-Western blot). Upon glutamate stimulation or pharmacological blockade of glutamate receptors, we observed dynamic changes in 15-30% of the observed protein co-associations. We are now beginning to analyze mouse models of autism on the QMI platform (e.g. Shank3 KO, Fragile X mouse, Ube3a mouse) to identify structural changes in the protein interactome at the synapse of these mice, both under baseline conditions and following neuronal activity modulation. Our goal is to better understand the network-scale alterations in protein associations during glutamatergic signaling, and to define how different autism-associated risk factors may play important, perhaps convergent, roles in these synaptic network processes.

Disclosures: **S.E. Smith:** None. **S.C. Neier:** None. **T.R. Davis:** None. **A.G. Schrum:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Topic: C.06. Developmental Disorders

Support: NIH grant R01 NS076860 (MZL)

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Stanford Pediatric Research Fund (YG)

Title: Role of FMRP in activity-dependent expression of synaptic proteins revealed by TimeSTAMP-tagging of PSD95

Authors: *Y. GENG[1], Y. YANG[1], M. Z. LIN[2];
[1]Pediatrics, [2]Pediatrics, Bioengineering, Stanford Univ., Stanford, CA

Abstracts: Protein synthesis is highly regulated throughout nervous system development, plasticity, and regeneration. Studies in Fragile X Fragile X mental retardation have indicated that protein synthesis, especially local protein regulation at synaptic level, has been significantly disturbed. However, tracking the distributions of specific new protein species has not been possible in living neurons or at the ultrastructural level. Previously we created TimeSTAMP epitope tags, drug-controlled tags for immunohistochemical detection of specific new proteins synthesized at defined times. We extend TimeSTAMP to label new protein copies by fluorescence. Using TimeSTAMP:YFP, we demonstrate that copies of the synaptic protein PSD95 are synthesized in response to local activation of growth factor and neurotransmitter receptors, and preferentially localize to stimulated synapses. Our preliminary data suggests that local PSD95 synthesis is significantly impaired in a mouse model of Fragile X mental retardation.

Disclosures: **Y. Geng:** None. **Y. Yang:** None. **M.Z. Lin:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.24/K2

Topic: C.06. Developmental Disorders

Title: Loss of Cntnap4 differentially alters GABAergic and dopaminergic synaptic transmission and triggers behavioral endophenotypes

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Abstracts: Although considerable evidence suggests that the chemical synapse is a lynchpin underlying neurodevelopmental disorders, how molecular insults differentially affect specific synaptic connections remains poorly understood. For instance, Neurexin 1a and 2 (NRXN1 and

2) and CNTNAP2 (aka. CASPR2), all members of the neurexin superfamily of transmembrane molecules, have been implicated in such brain disorders. However, their loss leads to deficits that have been best characterized with regard to their impact on excitatory cells. Notably, other disease-associated genes such as BDNF and ErBb4 implicate specific interneuron synapses in neurodevelopmental disorders. Consistent with this, cortical interneuron dysfunction has been linked to epilepsy, schizophrenia, and autism. Using a microarray screen that focused upon synapse-associated molecules, we identified Cntnap4 (contactin-associated protein 4, also known as Caspr4) as highly enriched in developing interneurons. In this study we show that Cntnap4 is localized presynaptically and its loss leads to a reduction in the output of cortical PV- positive GABAergic basket cells. Paradoxically, the loss of Cntnap4 augments dopamine release in the nucleus accumbens. Consistent with a small number of CNTNAP4 human gene deletions we identified in neuropsychiatric patients, Cntnap4 mutant mice exhibit sensory-motor gating and grooming endophenotypes. These behavioral abnormalities could be pharmacologically reversed in adult animals by functionally normalizing the observed synaptic defects in the two systems, providing some promise for possible therapeutic intervention in neurodevelopmental disorders.

Disclosures: **G.J. Fishell:** None. **T. Karayannis:** None. **E. Au:** None. **J.C. Patel:** None. **M.E. Rice:** None. **I. Kruglikov:** None. **B. Rudy:** None. **R.W. Tsien:** None. **C. Hoeffer:** None. **S. Restituito:** None. **E. Peles:** None. **A. Gordon:** None. **T. Bourgeron:** None. **R. Delorme:** None. **G. Huguet:** None. **H. Hakonarson:** None. **J. Glessner:** None. **D. Salomon:** None. **N. Roy:** None. **S. Markx:** None. **J. Gogos:** None. **M. Karayiorgou:** None. **L. rodriguez-murillo:** None. **D. Heron:** None. **B. Keren:** None.

Poster

604. Autism Synaptic and Cellular Mechanisms

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 604.25/K3

Topic: C.06. Developmental Disorders

Support: NIH/NINDS 5R01NS057819-08

5 P30 HD024064-25

Howard Hughes Medical Institute

Title: Neural mechanisms of circuit homeostasis failure in MeCP2 disorders

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Abstracts: Background: Loss-of-function mutations in methyl CpG binding protein 2 (MeCP2), as occurs in Rett syndrome, and duplication of the genomic region including *MECP2* (Xq28), as occurs in *MECP2* duplication syndrome, lead to overlapping clinical features including autism, epilepsy, and intellectual disability. Similarly, mouse models lacking versus overexpressing MeCP2 exhibit partially overlapping autism-associated behavioral phenotypes even though they demonstrate opposite patterns of global gene expression, LTP induction, synapse strength/number, and dendritic arborization. How opposite changes at the levels of transcription, synaptic function, and neuronal morphology lead to autism-like and other phenotypes remains unexplained. We hypothesize that similar impairment of circuit function contributes to the overlapping behavioral phenotypes of Rett and *MECP2* duplication mice. **Methods:** To study the state of neural circuit homeostasis in *MECP2* disorders, Rett (*Mecp2* null, 129 background) and *MECP2* duplication (Tg1, FVB background) mice were crossed to transgenic animals expressing the genetically encoded calcium indicator GCaMP3 in excitatory neurons (thy1-gcamp3, C57Bl6J background). Coronal slices were prepared from F1 experimental animals at 8 weeks of age, and calcium events in hippocampal CA1 neurons were imaged by ex-vivo 2-photon microscopy. **Results:** Change of MeCP2 expression in either direction led to circuit hypersynchrony both at baseline and in response to small perturbations in the balance of excitation and inhibition. Experiments in conditional *Mecp2* mutants revealed that MeCP2 function in excitatory neurons enables the desynchronized network state at baseline, while MeCP2 function in inhibitory neurons is critical for maintaining this desynchronized state in response to perturbation. Pathological hypersynchrony appeared to be driven by decreased recurrent inhibition in both mutants, in addition to a selective increase in local excitatory connectivity in Tg1 mice and abnormal cellular excitability in *Mecp2* null mice. **Conclusions:** Opposite deviations of MeCP2 expression from normal level lead to similar CA1 circuit functional phenotypes characterized by hypersynchrony and impairment in CA1 circuit homeostasis, suggesting that impaired neuronal circuit homeostasis may be a common theme underlying the similar behavioral abnormalities seen in *Mecp2* mutants.

Disclosures: H. Lu: None. **R.T. Ash:** None. **W. Wang:** None. **D. Yu:** None. **B.R. Arenkiel:** None. **S.M. Smirnakis:** None. **H.Y. Zoghbi:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.01/K4

Topic: C.07. Epilepsy

Support: UVA Departmental Startup Funds

Title: Fn14-TWEAK mediate an inflammatory response in Status Epilepticus

Authors: *Z. POTTANAT;
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Abstracts: Objective: Status Epilepticus (SE) is a life threatening disorder characterized by continuous seizure activity and neuronal death. There is current interest in neuroprotective agents that can be given to reduce disease complications. FN14, a member of the TNF-α receptor superfamily, and its ligand, TWEAK, have been shown to be upregulated in other models of neuronal injury. We tested the hypothesis that FN14 is similarly up regulated during SE and is detrimental to neuronal health. Methods: SE was induced via intraperitoneal injections of pilocarpine using published methods. Control mice were given saline injections (n=5) only. Hippocampal and cortical brain tissue was collected at 6 (n=5) or 24 (n=7) hours after SE onset and RNA/protein prepared. We used quantitative PCR and western blots to assess levels of FN14, TWEAK, and downstream effector RNA and protein, respectively. Results: Fn-14 was upregulated in SE in a time dependent manner. At 6 and 24 hours, there were a 38-fold and 188-fold increase in FN14 RNA respectively ($p = <0.001$).Western blots confirmed an upregulation in protein levels as well. In accordance with published records in other neuronal injury models, TWEAK ligand levels remained stable. Upregulation of FN14 corresponded with activation of the inflammatory NFκB pathway. Interpretation and future studies: Upregulation of the FN14/TWEAK pathway has been postulated to mediate neuronal damage in acute neurologic disease. Here we show that the pathway is highly upregulated in SE. Experiments are underway to determine if genetic loss of FN14 or antibody-mediated abrogation of the pathway reduces neuronal loss.

Disclosures: Z. Pottanat: None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.02/K5

Topic: C.07. Epilepsy

Support: PAPIIT (IN 211913-3)

CONACyT (152613)

Title: Alterations of amino acids involved in threonine metabolism in a rat model of acute seizures induced by lithium-pilocarpine

Authors: *V. ARRIAGA-AVILA, J. LANDGRAVE-GÓMEZ, O. MERCADO-GÓMEZ, R. GUEVARA-GUZMÁN;
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Abstracts: To date, some reports suggest that changes in the amino acid metabolism are involved in a variety of neurological disorders such as epilepsy. In this sense, a nutritional challenge such as calorie restriction (CR) or ketogenic diet (KD) that modulates the amino acid metabolism, has been observed that has anticonvulsant activity. Environmental inputs, such as nutrition, can modulate cell metabolism, and the relationship between epigenetic modifications and metabolism regulation is beginning to emerge. Because CR and KD can induce changes in metabolism, the aims of the present work were to study whether acute seizures induce alterations involved on threonine metabolism and if the feeding restriction model(FR)would reverts those changes on amino acids, which may contribute to the anticonvulsant effect observed in other studies. The model of acute seizures consisted in a pre-treatment of lithium chloride (3 mEq/kg) followed by pilocarpine administration (60 mg/kg) with a previous injection of scopolamine nitrate (1 mg/Kg) 30 min before. The FR was to allow rats to fed for two hours daily for 21 days; control and pilocarpine animals consisted of feeding ad libitum. Animals were divided into four groups (i.e.ad libitum, ad libitum+pilocarpine, FR, and FR+pilocarpine) and sacrificed 24 hrs after drug administration; serum samples were collected afterwards. For amino acid analysis, high-performance liquid chromatography (HPLC) was performed. In order to remove proteins by precipitation, plasma sample was mixed 1:1 with acetonitrile. The mixture was centrifuged at 14,000 RPM for 8 min. The supernatant was filtered and diluted 1:20 with Krebs-Ringer solution (pH=3.0). Twenty μL were transferred into a Microtube insert and placed in vials with screw caps, and then stored in a refrigerated sampler of the HPLC system at 4o C. The analysis was done with an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a fluorescence detector, a binary pump, an automatic injector sampler and a column thermostat.Our results shown a significant decrease of threonine, methionine and glycine concentration on plasma in ad libitum + pilocarpine group compared with that of the ad libitum control group ($n=5$, $p<0.005$). Moreover, the FR + pilocarpine group showed an increase of threonine compared with that of the ad libitum + pilocarpine group ($n=5$, $p<0.005$). Conclusions:

Seizures per se decrease the concentration of amino acids involved in threonine metabolism (i.e. threonine, glycine, and methionine). However, this alteration was only partially reverted in some of them, but fully reverted in threonine concentration in animals that followed the FR model.

Disclosures: V. Arriaga-Avila: None. **J. Landgrave-Gómez:** None. **O. Mercado-Gómez:** None. **R. Guevara-Guzmán:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.03/K6

Topic: C.07. Epilepsy

Title: Characterization of chloride homeostais alteration in a rodent model of temporal lobe epilepsy

Authors: *N. KOURDOUGLI, C. PELLEGRINO, G. CHAZAL, J.-L. GAIARSA, V. CRÉPEL, C. RIVERA;
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Abstracts: Epilepsy is one of the most common neurological disorders, with a 0.5% prevalence. The seizure focus can be the entire brain or a particular region; the most commonly affected is the temporal lobe. This pathology is characterized by an initial generalized seizure, followed by a clinical quiescent period. Within this period functional and morphological network reorganization presumably occur that leads to spontaneously recurring seizures at later stages of the disease. In this context, the working hypothesis for seizures genesis have been linked to altered GABAergic signaling and excitotoxicity-induced cell-death. Gamma-Amino Butyric Acid (GABA) is the main inhibitory neurotransmitter in the central nervous system, but under physio-pathological conditions it leads to hyperexcitabilty, through a dysregulation of chloride homeostasis in neurons. This phenomenon is controlled by Cation Chloride Co-transporters (CCCs), like the neuronal specfic K+-Cl- co-transporter KCC2 and the widely distributed Na+-K+-2Cl- co-ctransporter NKCC1. In many pathophysiological conditions, it has been shown that KCC2 is down-regulated and NKCC1 up-regulated, result in a depolarizing effect of chloride permeable GABAA receptors. Using a rodent model of Temporal Lobe Epilepsy (TLE) we aim at studying the network reorganization within the latent phase following the status epilepticus (SE) in the hippocampus. To assess those questions we analyzed by histological, molecular and biochemical techniques the expression of both KCC2 and NKCC1 transporters, as well as the

time course of cell death. Our results show an increase cell death right after the SE with concomitant changes in expression intensity and distribution of KCC2 that correlates with the post SE changes in GABAergic neurotransmission. These preliminary results support the idea of an altered GABAergic signaling during the latent phase due to compromised chloride regulation. Further investigate is required to assess if these changes lead to network reorganization and recurrent seizures in this model of TLE.

Disclosures: **N. Kourdougli:** None. **C. PELlegrino:** None. **G. Chazal:** None. **J. Gaiarsa:** None. **V. Crépel:** None. **C. Rivera:** None.

Poster

605. Status Epilepticus-Induced Changes

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Program#/Poster: 605.04/K7

Topic: C.07. Epilepsy

Support: CONACYT grant 106402 (MLLM)

CONACYT scholarship 249772 (DMAC)

Title: Interleukin-1β and IL-1RI expression in the developing rat thalamus and amygdala following status epilepticus

Authors: *D.-M. ALVAREZ-CRODA[1,2], L. BELTRÁN-PARRAZAL[1], C. MORGADO-VALLE[1], C. A. PEREZ-ESTUDILLO[1], M. L. LÓPEZ-MERAZ[1];
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Abstracts: Status epilepticus (SE) induces age-dependent neuronal injury in specific rat amygdaloid and thalamic nuclei; however, the cell death mechanisms involved in this process are not completely understood in the developing rat brain. SE induced in twenty-days-old rats (P20) causes neuronal cell death in ventral lateral, posteromedial and basomedial amygdala nuclei, whereas in P15 rats, SE induces injury mainly in the medial amygdaloid nucleus. Regarding the thalamus, SE induced in P12 rats produces neuronal cell death in the lateral and medial nuclei. It is known that inflammation can contribute to SE-induced neuronal injury in the immature brain. Interleukin-1β (IL-1β) gene and protein expression is up regulated following seizures and this cytokine has been associated with neuronal cell death following epileptic activity. The aim of

this study was to evaluate the temporal pattern of neuronal cell death in the amygdala and thalamus from P14 rats after SE and to determine whether IL-1β and its type I receptor (IL-1RI) are expressed in those brain areas. Rat pups were given 3 mEq/kg lithium chloride i.p. on the day before the induction of SE, which was carried out at P14 by subcutaneous injection of 100 mg/kg pilocarpine hydrochloride; control animals (n=7) were given an equal volume of saline subcutaneously. Six (n=7) and 24 h (n=9) following SE, rats were anesthetized and transcardially perfused with 4% phosphate-buffered paraformaldehyde. Subsequently, brains were dehydrated, embedded in paraffin and cut into 10-μm-thick coronal sections at the level of dorsal hippocampus. Fluoro-Jade B staining was carried out in order to detect neuronal cell death, whereas IL-1β and IL-1RI were detected by immunohistochemical procedures. Neither neuronal cell death nor IL-1β/IL-1RI expression was observed in control animals. Injured neurons were detected in the dorsomedial thalamus 6 and 24 h after SE; IL-1β was expressed only 6 h following SE, while no expression of IL-1RI was observed in this thalamic nucleus. No F-JB positive cells or IL-1RI expression was detected in the lateral thalamic nucleus, but IL-1β was expressed 6 h after SE. Respecting amygdala, neuronal injury was detected 24 h after SE in the medial nucleus, whereas IL-1β and IL-1RI were observed 6 but not 24 h after SE in this brain region. Results suggest that the IL-1β/IL-1RI system could be involved in region-specific neuronal cell death mechanisms following SE in the immature rat brain.

Disclosures: D. Alvarez-Croda: None. **L. Beltrán-Parrazal:** None. **C. Morgado-Valle:** None. **C.A. Perez-Estudillo:** None. **M.L. López-Meraz:** None.

Poster

605. Status Epilepticus-Induced Changes

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Topic: C.07. Epilepsy

Support: Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs (Grant number A111313) funded by the Korean government

National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2012R1A2A1A01001775)

Title: Cellular and regional specific changes in multidrug efflux transporter expression during recovery of vasogenic edema in the rat hippocampus and piriform cortex

Authors: *T.-C. KANG, J.-Y. KIM, A.-R. KO, Y.-J. KIM, J.-E. KIM;
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Abstracts: Since ATP-cassette-binding protein (ABC) family plays a role as drug efflux transporters, ABC family expression is an important factor in the therapeutic failure of antiepileptic drugs (AEDs). We investigated the correlation between ABC family alterations and vasogenic edema formation following status epilepticus (SE) to understand the characteristics of seizure-induced over-expression of drug efflux transporters. Vasogenic edema was peaked 3 - 4 days after SE in the hippocampus and piriform cortex (PC). In the hippocampus, the expressions of breast cancer resistance protein (BCRP), multidrug resistance protein-4 (MRP4) and p-glycoprotein (p-GP) were decreased 4 days after SE, but subsequently increased at 4 weeks after SE. Multidrug resistance protein-1 (MRP1) expression gradually decreased in endothelial cells until 4 weeks after SE. Enhancements of BCRP, MRP4 and p-GP expressions were mainly detected in astrocytes, neuropils and reactive astrocytes, respectively. In the PC, BCRP, MRP4 and p-GP expressions were transiently decreased and subsequently increased after SE. MRP1 expression was gradually decreased in the PC following SE. Up-regulation of BCRP expression was detected in palisade astrocytes around the vasogenic edema lesion. MRP4 expression was increased in neuropils. p-GP expression was up-regulated in endothelial cells. Our findings indicate that SE-induced vasogenic edema formation transiently reduced drug efflux pump expressions in endothelial cells. Subsequently, during recovery of vasogenic edema drug efflux pump expressions were differentially up-regulated in astrocytes, neuropils and endothelial cells. Therefore, we suggest that vasogenic edema formation may be a risk factor in pharmacoresistent epilepsy.

Disclosures: **T. Kang:** None. **J. Kim:** None. **Y. Kim:** None. **J. Kim:** None. **A. Ko:** None.

Poster

605. Status Epilepticus-Induced Changes

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Program#/Poster: 605.06/K9

Topic: C.07. Epilepsy

Support: PAPIIT Grant IN211913

CONACyT Grant 152613

Title: Balance in mitochondrial fission and fusion in neuronal injury in pilocarpine-induced epileptic rats

Authors: D. CARRERA-CALVO[1], J. SOLÍS-NAVARRETE[1], *L. CÓRDOVA-DÁVALOS[1], O. MERCADO-GÓMEZ[1], V. ARRIAGA-ÁVILA[1], E. MARTÍNEZ-ABUNDIS[2], R. GUEVARA-GUZMÁN[1];
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Abstracts: Epilepsy is a neurological disorder that is characterized by recurrent and spontaneous seizures this causes neuronal damage and loss in several regions from hippocampus. There are some reports showing that mitochondrial fission is involved in oxidative stress and apoptosis of several neurodegenerative diseases. Recently, it has been reported the role of mitochondrial fission in a pharmacological animal model; the results show that mitochondrial fission increased after seizures. However, the information about the balance of mitochondrial fission and fusion events for cell survival is scarce and was not fully appreciated until fairly recently. The aim of this study is to show that there is a balance between mitochondrial fission and fusion events in epileptic seizures development in Wistar male rats. Our data show that fission protein Drp1 expression increases at short time in cytoplasm, but in mitochondria there are no changes in level expression. Therefore, there is no increase translocation Drp1 protein to outer membrane mitochondria, and Fis1 did not change in level expression. Moreover, mitochondrial fusion, which is mediated by Mfn1, an outer membrane mitochondrial protein, and Opa1, an inner membrane mitochondrial protein, show an increase in level expression for a short time. In summary, this study demonstrated that mitochondrial fusion is up-regulated after seizures during three days and Drp1 fission protein is expressed but not translocated to outer membrane mitochondria during seizures.

Disclosures: D. Carrera-Calvo: None. **J. Solís-Navarrete:** None. **L. Córdova-Dávalos:** None. **O. Mercado-Gómez:** None. **V. Arriaga-Ávila:** None. **E. Martínez-Abundis:** None. **R. Guevara-Guzmán:** None.

Poster

605. Status Epilepticus-Induced Changes

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National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012R1A2A1A01001775)

Title: PARP1 activation/expression modulates regional specific neuronal and glial responses to seizure in a hemodynamic-independent manner

Authors: *J.-E. KIM, J.-Y. KIM, Y.-J. KIM, A.-R. KO, T.-C. KANG;
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Abstracts: In the present study, we investigated the role of poly(ADP-ribose) polymerase-1 (PARP1) activity in the differential neuronal/glial responses to pilocarpine (PILO)-induced status epilepticus (SE) within the rat hippocampus and piriform cortex (PC). CA1- and CA3 pyramidal cells showed PARP1 hyperactivation-dependent neuronal death pathway following SE. PC neurons and hilar neurons exhibited PARP1 degradation-mediated neurodegeneration following SE. PARP1 degradation was also observed in astrocytes within the molecular layer of the dentate gyrus. PARP1 induction was detected in CA1-3 reactive astrocytes, as well as in reactive microglia within the PC. PARP1 inhibitors deteriorated the astroglial death in the molecular layer of the dentate gyrus, and induced astroglial death in the stratum lucidum of the CA3 region. *Ex vivo* study showed that these regional and cellular specific patterns of PARP1 activation/degradation were hemodynamic-independent responses. Taken together, our findings indicate that PARP1 hyperactivation, degradation and induction may distinctly involve neuronal damage, astroglial death and reactive gliosis in response to SE. Therefore, the present study suggests that the selective modulation of PARP1 activation/expression may be a considerable strategy for therapy in various neurological diseases.

Disclosures: J. Kim: None. **J. Kim:** None. **Y. Kim:** None. **A. Ko:** None. **T. Kang:** None.

Poster

605. Status Epilepticus-Induced Changes

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.08/L2

Topic: C.07. Epilepsy

Support: Pre-doctoral award, American Heart Association

Albert J. Ryan Foundation

Title: Clonal analysis of newborn hippocampal dentate granule cell proliferation and development in temporal lobe epilepsy

Authors: *S. P. SINGH, S. C. DANZER;
Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstracts: Hippocampal dentate granule cells (DGCs) are among the few neuronal cell types generated throughout adult life in mammals. In the normal brain, adult neurogenesis gives rise to DGCs neurons which integrate into the granule cell layer of the dentate gyrus in a stereotypical fashion. By contrast, in the epileptic brain, DGC neurogenesis is profoundly altered. Specifically, granule cell progenitors exposed to status epilepticus (SE) exhibit increased proliferation and integrate abnormally. The factors regulating these responses remain uncertain and could involve changes in systemic cues, regional cues or cell-intrinsic properties. Here, we used clonal analysis of DGC progenitors to determine whether increased proliferation and abnormal integration of newborn DGCs appears uniformly across DGC progenitors or whether progenitors respond heterogeneously to the insult, exhibiting progenitor-specific or region-specific differences. To this end, we used Gli1-CreERT2-Brainbow reporter mice to differentially label progenitor cells and their clonal progeny following pilocarpine-induced SE. Hippocampi were isolated and rendered translucent using Scale reagent. Confocal microscopy was used to generate 3-dimensional reconstructions of the entire dentate. Clonal DGC clusters from epileptic animals were compared to clusters from controls. This study is designed to determine whether there are differences among hippocampal progenitors following SE-induced DGC proliferation and integration. The results of this study will provide novel insights into the potential mechanisms regulating DGC dysmorphogenesis in epilepsy.

Disclosures: S.P. Singh: None. **S.C. Danzer:** None.

Poster

605. Status Epilepticus-Induced Changes

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Program#/Poster: 605.09/L3

Topic: C.07. Epilepsy

Support: TT startup funds, ISU, Ames, USA

Title: Breaking the bottleneck of using kainate-resistant C57BL/6J mice in epilepsy research: Developing an advantageous early-onset mouse model of epilepsy

Authors: *S. PUTTACHARY[1], S. SHARMA[1], K. TSE[2], J. CRUTISON[1], A. SEXTON[1], T. THIPPESWAMY[1];
[1]Biomed. Sci., Iowa State Univ., Ames, IA; [2]Univ. of Liverpool, Liverpool, United Kingdom

Abstracts: Temporal lobe epilepsy (TLE) is the most common form of acquired human epilepsy. A robust preclinical model to boost new and effective antiepileptic drugs (AEDs) is crucial as, current drugs are not responsive in 1/3rd of epileptic patients. Hitherto, rat models of TLE have been used to mimic some aspects of human TLE. Currently, the epilepsy research has gained momentum by using of transgenic mouse models bred on C57BL/6J genetic background. Lack of reliable critical control in using C57BL/6J wild-type mouse model has let-down many researchers due to specific disadvantages such as, a well-documented kainate (KA) resistance to neurotoxicity, unreliable seizure response and high mortality (>20%). We have recently addressed these issues by administering KA in repeated low doses (RLD) intraperitoneally (i.p, 5mg/kg at 30min intervals) in C57BL/6J mice (7wk age), until they achieved first convulsive motor seizure (CMS) stage-5 (severe seizure group, SSG) and stage ≤3 (Mild seizure group, MSG) during 2h status epilepticus (SE) (PLoS ONE, 2014, 9(5): e96622). The SE was later terminated with diazepam (10mg/kg, i.p). Experiments were carried out in accordance with IACUC (ISU, USA) and at end of experiment animals were euthanized by an overdose of pentobarbital (100mg/kg, i.p). For the first time, with continuous video-EEG monitoring (EEG acquired through Dataquest® and analyzed with Neuroscore DSI®) for 18 weeks, we demonstrate that C57BL/6J mouse indeed become epileptic as early as 48-72h and show numerous classical spontaneous recurrent seizures (SRS) later (31±4.8 CMS episodes in SSG, 5±1.8 CMS episodes in MSG during 18wks, $p<0.05$, Mann-Whitney test, n=18). The key to SRS onset is the severity and duration of electrographic CMS episodes (>10min) during SE and RLD method of KA administration. We characterized all the patterns of SRS episodes. Five EEG patterns of spontaneous recurrent CMS consisted of stage-3 to -5 with a minimum duration of 30s for each episode. Three patterns of EEG non-convulsive seizure (NCS) episodes (>12s) consisting of stage 1 and 2 spikes were identified. Two patterns of inter-ictal spikes (0.2-4Hz) consisted of stage-1 or -2 spikes or a mixture of both were observed before the first onset of a CMS episode. All types of SRS episodes observed peaked in the first 2 months and subsequently declined. These findings provide evidence for a more robust and early onset C57BL6 mouse model of epileptogenesis that can be widely used for drug screening and as a wild-type control in experiments involving transgenic mice. Because of very early onset of SRS in our model, compared to rat models, it reduces time and amount of drugs required and overall cost-effective.

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Poster

605. Status Epilepticus-Induced Changes

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Topic: C.07. Epilepsy

Support: European Science Founfation (EUROCORES Program EuroEPINOMICS)

Austrian Science Funds (FWF) I6440, P26680

Title: Changes in the expression of histone deacetylases 1-11 mRNAs in the hippocampus in mouse models of temporal lobe epilepsy

Authors: *G. SPERK, R. JAGIRDAR, M. DREXEL, R. TASAN;
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Abstracts: Epigenetic histone modifications involve acetylation and deacetylation of histone proteins contributing to transcriptional silencing of gene expression. Deacetylation of histone proteins is catalyzed by histone deacetylases (HDAC) comprising four different classes with 11 isoforms. We investigated changes in the expression of HDAC mRNAs in an animal model of temporal lobe epilepsy. In this model local injection of kainic acid (KA; 0.350 nmol/70 nl) into the hippocampus of mice induced a status epilepticus (lasting for 3 d) and thereafter in average 2 spontaneous seizures per day. In the injected hippocampus, losses in pyramidal neurons and granule cell dispersion (after 14 d) were seen. Mice were killed after different intervals (4 to 28 d) and HDAC 1-11 mRNAs were determined by *in situ* hybridization. In the dentate gyrus, expression of HDAC 1, 2, 7 and 11 mRNAs was significantly decreased 4 h after KA ipsi- and contralaterally to the injection, recovering after 48 h. In contrast, HDAC5 mRNA levels were significantly increased 4 and 12 h after KA injection, remaining increased in the injected hippocampus at later intervals. There was also a pronounced increase in HDAC9 mRNA expression 14 and 28 d after KA in granule cells of the injected dentate gyrus. To investigate this further we determined HDAC mRNA expression also in pilocarpine injected mice (300 mg/kg, i.p.). Also these mice develop a status epilepticus with subsequent spontaneous seizures, but expose no granule cell dispersion. They showed decreased HDAC 1 to 3 mRNA levels but no increases in HDAC 5 and 9 mRNAs in the granule cells at the late intervals. Our data show distinct and specific expression patterns for the different HDAC mRNAs in both animal models for TLE indicating rather specific changes in the expression of numerous genes after seizure induction. The early bilateral decreases in HDAC 1, 2, 7 and 11 mRNAs may be caused by the

initial status epilepticus and may be related to the rapid expression of various genes. Subsequently increased expression of HDACs 2 and 3 may counteract this reaction. Overexpression of HDAC9 mRNA may be associated with the granule cell dispersion developing at later time points.

Disclosures: G. Sperk: None. **R. Jagirdar:** None. **M. Drexel:** None. **R. Tasan:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.11/L5

Topic: C.07. Epilepsy

Support: INSERM

Title: Predicting and reversing depression and cognitive deficits in experimental temporal lobe epilepsy

Authors: *C. BERNARD[1], A. GHESTEM[1], F. BARTOLOMEI[1], S. SIYOUCEF[1], E. BOUVIER[2], C. BECKER[2], J.-J. BENOLIEL[2];
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Abstracts: Depression and cognitive disorders are common and severe comorbidities in TLE, with a major negative impact on the quality of patients' life. Among TLE patients, 14-31% develop depression. Several hypotheses have been proposed to explain these comorbidities, including the deleterious consequences of the treatment, a direct consequence of the circuit reorganization associated with epilepsy, and factors predisposing certain individuals; epilepsy then acting as a revelator of depression and/or cognitive deficits. The latter hypothesis remains to be tested. Predisposition could be triggered by a first "hit", e.g. a non-epileptogenic brain insult, which would leave permanent imprints in neuronal circuits. A subsequent "hit", an epileptogenic insult, would then result in comorbidities. In this scheme, either hit would not be sufficient in isolation to trigger comorbidities, a double hit is necessary. The goal of this study is to provide the proof-of-concept that past events may prime individuals for comorbidities once they become epileptic, and to identify biomarkers predictive of such vulnerability. We used Social Defeat (SD) in rats as a first hit. Eleven days after SD, 40% of animals were characterized by low levels of serum BDNF. Status epilepticus was used as a second hit. Once animals were epileptic, only

the low BDNF group presented a depressive phenotype and severe cognitive deficits. Infusion of 7,8-DHF, a BDNF mimetic, before the second hit, abolished the vulnerability phenotype. Epilepsy still developed but none of the animals displayed a depressive phenotype and severe cognitive deficits. We conclude that a past history of stress can sensitize some individuals to depression, which is revealed after an epileptogenic insult. Serum BDNF is a biomarker identifying this vulnerable population. A treatment with a BDNF mimetic can reverse the phenotype.

Disclosures: **C. Bernard:** None. **A. Ghestem:** None. **F. Bartolomei:** None. **S. Siyoucef:** None. **E. Bouvier:** None. **C. Becker:** None. **J. Benoliel:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.12/L6

Topic: C.07. Epilepsy

Support: Academy of Finland

Finnish Cultural Foundation

Title: Diffusion tensor imaging detects progressive changes in hippocampal subfields during epileptogenesis

Authors: ***A. SIERRA LOPEZ**[1], T. MIETTINEN[1], R. SALO[1], T. LAITINEN[1], A. PITKÄNEN[1,2], O. GRÖHN[1];
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Abstracts: Lack of imaging biomarkers for monitoring disease progression, recovery and treatment efficacy is a major unmet need in many neurological diseases, including epilepsy. It has been recently shown that diffusion tensor imaging (DTI) provides a high microstructural contrast also outside major white matter tracts. We hypothesized that *in vivo* DTI detects progressive changes in the hippocampus as well as in other brain areas during early stages of epileptogenesis. To test the hypothesis, we induced status epilepticus (SE) with systemic kainic acid or pilocarpine in adult male Wistar rats. Animals were scanned using *in vivo* DTI in a 7T/30cm magnet before and at 10, 20, 34 and 79 days after SE. Data were acquired using a

diffusion-weighted segmented spin echo echo-planar imaging pulse sequence (TR = 2.5 s and TE = 30 ms, 4 segments), 21 diffusion weighting directions (= 4 ms, Δ = 11 ms, b-value = 1000 s/mm^2), FOV of 21.12 x 14.08 mm^2 and in-plane spatial resolution of 110 x 110 μm^2. Number of slices was 14, slice thickness 500 μm, and number of averages 32, resulting in 140 min scan time. Analysis of the dentate gyrus (DG) showed increased fractional anisotropy (FA) (day 34, $p<0.01$; day 79, $p<0.001$) along with increased axial diffusivity (day 34, $p<0.001$; day 79, $p<0.001$) during the 79-d follow-up. In the CA3bc, we also found increased FA (day 79, $p<0.001$), and a progressive change in the main diffusion orientation from rostral-caudal to more dorsal-ventral (day 34, $p<0.001$; day 79, $p<0.001$). In order to validate the DTI findings, Fourier analysis of images captured from myelin-stained histological preparations and from GFAP immunohistochemistry was performed. The analysis revealed that in the DG only myelinated axons showed increased anisotropy ($p<0.001$) and orientation changes ($p<0.001$), consistent with DTI findings. In the CA3bc, both axons and astrocytes showed increased anisotropy ($p<0.01$) and orientation changes ($p<0.001$). Additionally, we performed tract-based spatial statistics (TBSS) analysis on DTI data to find statistical differences throughout the brain. TBSS analysis showed increased FA ($p<0.05$, FWE corrected) in the thalamus, whereas in the fimbria, external capsule and optic tract FA was decreased in rats with SE as compared to that in controls at the latest time point. Increased FA correlated to axonal alterations or on-going inflammation. Our findings demonstrate that *in vivo* DTI is able to detect progressive microstructural alterations in the brain during epileptogenesis, which suggest that DTI provides a tool for identification of imaging biomarkers for epileptogenesis and the progression of epilepsy.

Disclosures: A. Sierra Lopez: None. **T. Miettinen:** None. **R. Salo:** None. **T. Laitinen:** None. **A. Pitkänen:** None. **O. Gröhn:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.13/L7

Topic: C.07. Epilepsy

Title: MicroRNAs as biomarkers of epilepsy: expression profiling in the pilocarpine model

Authors: *P. RONCON[1], M. SOUKUPOVÀ[1], A. BINASCHI[1], C. FALCICCHIA[1], S. ZUCCHINI[1,2], M. FERRACIN[3,2], E. PETRETTO[4], M. R. JOHNSON[5], S. R. LANGLEY[5], M. SIMONATO[1,2];

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Abstracts: Temporal lobe epilepsy (TLE) often occurs secondary to other pathologies like tumors, traumas or stroke after a latent period that can last from months to years. However, preventive treatments in at-risk individuals are not currently available. The identification of biomarkers of the transformation of a normal tissue in epileptic (epileptogenesis) would be very useful to identify and validate new treatment strategies. MicroRNAs (miRNAs) represent an attractive option. miRNAs play important roles in the nervous system and their expression is altered in many neurological disorders. To better understand miRNA expression changes associated with the development of epilepsy, miRNA arrays were performed on laser-microdissected dentate granule cells and on plasma in different stages of epilepsy development in a rat model. An epileptogenic insult (status epilepticus, SE) was induced by pilocarpine administration (370 mg/kg i.p.) and rats were sacrificed at different time points: 4 days after SE (early latency), 8 days after SE (late latency), within 12 hours from the 1st spontaneous seizure and 50 days after the 1st seizure (chronic phase). We identified a profile of miRNAs differentially expressed in the different phases of the pathology, both in dentate gyrus and in plasma samples. miRNAs whose expression is altered before the first seizure are the best candidates to become biomarkers of epileptogenesis and may also be therapeutic targets useful to prevent the development of the pathology. In contrast, miRNAs identified in the late phases might correlate with particular histologic subtypes and different prognoses. They may therefore be useful to stratify patients with different prognoses, in particular post-surgery patients from whom bioptic tissue is available.

Disclosures: P. Roncon: None. **M. Soukupovà:** None. **A. Binaschi:** None. **C. Falcicchia:** None. **S. Zucchini:** None. **M. Ferracin:** None. **E. Petretto:** None. **M.R. Johnson:** None. **S.R. Langley:** None. **M. Simonato:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.14/L8

Topic: C.07. Epilepsy

Support: GlaxoSmithKline

Title: Loss of GluR1 and NeuN labeling within the dysgranular retrosplenial cortex after status epilepticus is not associated with cell death

Authors: *S. HU, A. M. SLOMKO, D. I. GREENTREE, J. WONGVGRAVIT, N. W. MACKLIN, L. K. FRIEDMAN;
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Abstracts: The hippocampus is highly sensitive to seizure-induced damage and is therefore a major region examined by numerous investigators whereas other limbic structures such as the retrosplenial and cingulate cortex; regions involved with seizure activity, memory, and other cognitive functions have received much less attention. One study showed that neuronal loss and volume reduction within the densely packed retrosplenial granular cortex (Rgb) occurred in response to pilocarpine-induced status epilepticus whereas the dysgranular adjacent retrosplenial region (Rdg) was spared. While examining the efficacy of retigabine on the seizure threshold and hippocampal injury in adult rats at times following kainate (KA) -induced status epilepticus, obvious depletions of the GluR1 subunit were observed in large patches within the Rgb and Rdg. Therefore, histological and NeuN staining were used to assess the morphology of the retrosplenial cortex to determine whether reduction in GluR1 was associated with cell loss. NeuN immunohistochemical labeling decreased markedly in Rgb and Rdg subregions in animals treated with KA relative to controls which corresponded to the same regions of GluR1 depletion at both acute (72 h) and delayed (28 d) time points. KA+retigabine treated rats exhibited the same pattern but with increased delay. Despite NeuN depletion, Nissl staining revealed loss of cortical neurons was either absent or restricted to the Rgb. Retrospective horseradish peroxidase transport (HRP) studies illustrated these neurons receive input from the cingulate cortex (Cg1 and Cg2) and project to adjacent Rdg neurons as well as basolateral amygdala and entorhinal cortex, significant circuitry of the limbic/ autonomic loop involved with emotion and memory. Reduced fast synaptic transmission mediated by GluR1 subunits with simultaneous depletion of NeuN staining of nuclear elements in populations of limbic cortical neurons in the absence of cell loss may indicate a “circuitry break” due to lack of neurotransmission and transcriptional regulation within critical regions responsible for memory deficits and other cognitive comorbidities associated with sustained seizures.

Disclosures: S. Hu: None. **A.M. Slomko:** None. **D.I. Greentree:** None. **J. Wongvgravit:** None. **L.K. Friedman:** None. **N.W. Macklin:** None.

Poster

605. Status Epilepticus-Induced Changes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 605.15/L9

Topic: C.07. Epilepsy

Support: FAPEMIG (Programa Primeiros Projetos - number CBB –APQ- 04389-10)

PRPq - UFMG (number 08/2010)

Title: Role of phosphatidylinositol 3-kinase in excitotoxicity induced by intrahippocampal microinjection of pilocarpine in C57BL/6 mice

Authors: *I. V. LIMA[1], A. C. CAMPOS[2], L. B. VIEIRA[3], M. F. D. MORAES[4], A. L. TEIXEIRA[2], A. C. P. DE OLIVEIRA[3];
[1]Pharmacol., Federal Univ. of Minas Gerais, Brazil, Brazil; [2]Clin. Med., [3]Pharmacol., [4]Physiol. and Biophysics, Federal Univ. of Minas Gerais, Belo Horizonte, Brazil

Abstracts: Introduction: Phosphatidylinositol 3-kinase (PI3K) is an enzyme involved in different pathophysiological processes, including neurological disorders. However, its role in seizures and epilepsy is still not fully understood. We investigated the role of PI3Kγ in seizures, glutamate release and neuronal death in mice subjected to intrahippocampal microinjection of pilocarpine (PILO). Methods: Adult male WT and PI3Kγ-/- were submitted to stereotaxic surgery for bilateral cannulae implantation guided to the hippocampus. After 5 days of recovery, the Status Epilepticus (SE) was induced by a bilateral intrahippocampal injection of PILO (20 µg per side/200 nL) or saline as control. Behavioral activity was recorded during 90 min after injections, followed by an injection of diazepam (10 mg/kg, i.p.) for seizures interruption. Seizures were classified according to the scale established by Racine (1972). Twenty-four hours later, the animals underwent thoracotomy and intracardiac perfusion and then were decapitated. Hippocampal brain slices were prepared and stained with Fluoro Jade C to evaluate hippocampal neuronal death. Another group of WT and PI3Kγ-/- mice were decapitated and had their hippocampi removed for synaptosomes preparation. Synaptosomes from WT mice were pre-incubated with a PI3Kγ inhibitor (AS605240 - 1, 10 or 100 nM) or vehicle to evaluate glutamate release induced by KCl. All experiments were approved by the Institutional Ethics Committee (CEUA-UFMG Protocol 068-2011). Data were analyzed by ANOVA and expressed as mean ± SEM ($P<0.05$). Results: PI3Kγ-/- mice exhibited more susceptibility to PILO-induced SE. Moreover, the latencies to trigger the seizures and the number of 4 and 5 seizures' scores of Racine Scale were higher in PI3Kγ-/- group than in WT group. Furthermore, glutamate release was enhanced in synaptosomes from PI3Kγ-/- and WT incubated with AS605240 (10-100nM) in comparison with non-treated WT. There was a neuronal death in CA1, dentate gyrus and CA3 hippocampal regions in both groups after PILO injection. However, neuronal death was more

pronounced in PI3Kγ-/- mice in CA1 region. Conclusions: We have observed that the absence of PI3Kγ enhances the susceptibility of the animals to seizures induced by PILO. There was an increase of glutamate release in PI3Kγ-/- and WT hippocampal synaptosomes pre-incubated with AS605240. Moreover, there was an increase of neuronal death in PI3Kγ-/- animals. Genetic ablation of PI3Kγ-/- enhanced the pathological pattern in an animal model of seizures induced by pilocarpine, suggesting that the enzyme PI3Kγ can play a neuroprotective role during the seizures.

Disclosures: I.V. Lima: None. **A.C. Campos:** None. **L.B. Vieira:** None. **M.F.D. Moraes:** None. **A.L. Teixeira:** None. **A.C.P. de Oliveira:** None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.01/L10

Topic: C.07. Epilepsy

Support: UNAM PAPIIT RN304112-3

Title: Memory and forgetting in epileptics and controls: Testing the free-recall of emotional and neutral stimuli across three trials

Authors: *V. M. SOLIS, SR;
Psychology Dept. Natl. Univ. of Mexico, Mexico City, Mexico

Abstracts: Numerous studies document various kinds of memory loss in epileptic patients. The present investigation addresses the following questions. First, is there any evidence of such losses for the free-recall of emotional and neutral verbal stimuli across trials? Second, would a control group with matched normal participants exhibit better retention than that of epileptic patients? Third, incremental memory (hypermnesia) usually emerges when free-recall is tested across successive trials; therefore, it is likely that our control group will display the phenomenon. More importantly still, can hypermnesia be observed in epileptic patients? To our knowledge, this questions has never been asked before, therefore, it is important to ask whether a generalised memory decrement in epileptic patients will also be accompanied by a lack of incremental retrieval across trials. We used a mixed factorial design; Group (epileptic and control, n=10 per group) varied between-participants. Stimuli (emotional and neutral) as well as Trials (R1, R2 and R3) varied within-participants. Our main results suggest: Firstly, net level of recall was indeed

better for controls than epileptics ($p < .008$). Secondly, recall was also significantly better for emotional than for neutral stimuli ($p < .0001$). More importantly, however, is the fact that we observed hypermnesia for epileptic patients. There was a significant Trials effect in both groups, ($p < .003$ for controls; $p < .02$ for epileptic patients). Thus, although somewhat attenuated relative to controls, epileptic patients still managed to recall significantly more items across three trials. We conclude that although the memory of epileptic patients shows several deficits compared to controls, the mechanisms responsible for incremental item sampling across trials are still operational in spite of their neurological condition.

Disclosures: V.M. Solis: None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.02/L11

Topic: C.07. Epilepsy

Support: NIH Grant R01-NS074450

Title: A virtual t-maze to assess oscillations during a working memory task in patients with temporal lobe epilepsy

Authors: *A. A. ROBBINS[1], A. S. TITIZ[1], G. L. HOLMES[2], B. C. JOBST[3];
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Abstracts: Spatial navigation tasks are often used in experimental neuroscience to investigate the mechanisms of learning and memory. These tasks also allow for insight into alterations in memory processes that occur in disease states. Working memory deficits have been shown previously in rodent models of early life seizures. In the present study, we investigate if patients with epilepsy demonstrate similar deficits in working memory by utilizing a using a virtual T-maze task (vTMT) that we developed using the "Source Engine" (Valve™). The vTMT is an alternation task where the subject is instructed to navigate hallways to find a reward at either end of a T-shaped arena. The vTMT task included three levels. The first level was a training level where directional markers indicate the location of a reward. The second and third levels had no directional markers, but the location of the reward alternated between sides as with the first level. The final two levels differed by the number of cues, where the second level had objects in the

destination rooms to differentiate the two sides. All of the rooms in the third level of the vTMT were visually identical. Patients with TLE showed marked impairments in this task where they had a lower average percentage of correct trials in levels 2 and 3 when compared to normal control subjects (66.71±8.94% vs 92.6±2.11% $p<0.05$). Local field potential (LFP) recordings were made from both hippocampal depth and cortical grid electrodes while the patients performed the task. In this study, we present results from the analyses of coherence of LFP in two regions of the environment: the central corridor and the junction of the central corridor and the arms.

Disclosures: **A.A. Robbins:** None. **G.L. Holmes:** None. **B.C. Jobst:** None. **A.S. Titiz:** None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.03/L12

Topic: C.07. Epilepsy

Title: Real-time cortical language mapping during spontaneous conversation with children

Authors: ***J. A. WILSON**[1], R. ARYA[1], H. FUJIWARA[1], F. T. MANGANO[2], D. ROSE[1]; [2]Neurol., [1]Cincinnati Children's Hosp., Cincinnati, OH

Abstracts: Invasive pre-surgical evaluation with subdural electrodes in a subgroup of people with drug resistant epilepsy has 2 core objectives: localization of the seizure-onset zone and defining its anatomic relationship to functionally essential cortex. The conventional standard-of-care is based on direct current electrical cortical stimulation (ECS) and use of task-response paradigm to map language and motor cortical areas. ECS testing requires extensive patient cooperation, which is often difficult for young children. Here we present a method utilizing real-time signal processing and visualization during spontaneous conversation for rapid identification of receptive and productive cortical language areas in pediatric epilepsy patients with implanted subdural ECoG grids. Up to 128 ECoG channels were acquired during a 5 min. baseline and 5-10 min. conversation period. BCI2000 and SIGFRIED were used for signal processing, classification, and visualization. A baseline resting model was created from the baseline data using the SIGFRIED procedure (Brunner et al, Epilepsy Behav. 2009;15(3):278-286). A natural conversation was recorded from two cardioid microphones. During the procedure, the power from each microphone was calculated every 50ms, and signals that exceeded a threshold triggered either a speech production or reception state if detected on the patient's or researcher's

microphone, respectively. During these states, the high-gamma (70-115 Hz) ECoG power was compared to the baseline model on each channel, producing a composite score indicating whether the value was significantly different from baseline. Finally, a Bonferonni-corrected t-test was used to determine whether individual channels were significantly different during silent, talking, and listening periods. Results were compared with picture naming during ECS, where only sites tested with ECS were compared. In all patients, 100% of facial sensorimotor and 83% of all naming sites were correctly identified, with false-positive rates of 31% and 37%, respectively. All naming sites were in left-hemisphere implant patients. False-positive sites were generally concentrated in cortical areas related to language, including Broca's and Wernekie's areas, and auditory cortex. This procedure had excellent sensitivity and selectivity, and has the potential to address major shortcomings of existing functional mapping techniques, particularly in children. In the near future, this method may be used to inform the decision-making progress prior to resection surgery, and provide regions of interest for ECS testing.

Disclosures: J.A. Wilson: None. **D. Rose:** None. **R. Arya:** None. **F.T. Mangano:** None. **H. Fujiwara:** None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.04/M1

Topic: C.07. Epilepsy

Title: Effects of subclinical epileptiform discharges on driving performance in people with epilepsy

Authors: *Y. SI[1], E. GUDBRANSON[1], W. C. CHEN[1], M. MIDURA[1], R. WU[1], B. GENG[1], P. VITKOVSKIY[1], A. SIVARAJU[1], R. SAINJU[1], A. FERNANDEZ[1], A. ALAREDDY[1], I. QURAISHI[1], R. B. DUCKROW[2], L. J. HIRSCH[2], H. BLUMENFELD[3];
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Abstracts: Public safety is one of the critical concerns among drivers with epilepsy. Although people with epilepsy are permitted to drive under certain legal restrictions if their overt seizures are controlled, it is still unclear whether interictal spikes and other subclinical epileptiform discharges (SEDs) might affect driving performance. Previous studies suggest that SEDs have an additional and independent mild effect on transient cognitive processes (alertness, mental speed

etc). To investigate this further the present study explores the effects of SEDs on driving safety. A computer-based video driving simulator was prospectively provided for inpatients with epilepsy who underwent continuous video-electroencephalography (VEEG) monitoring in our epilepsy center. EEG recordings of SEDs, video/behavioral recordings and synchronized driving data were extracted and collected. Clinical relevance with regard to driving behavior during SEDs was evaluated using crashing the car as a terminal impairment criterion. 641 SEDs during driving were found in 16 patients. 132 (20%) of SEDs were isolated spikes, 299 (47%) were isolated sharp waves, 195 (30%) were spike/sharp-slow wave complexes lasting up to 10 seconds, and 15 (2%) were longer-lasting subclinical epileptiform events (duration 10 to 30 seconds). By comparing the baseline of driving performance for each participant with SED epochs, no significant impairment of driving performance was found during isolated spikes or sharp waves. The longer-lasting subclinical epileptiform events were associated with collisions in a few cases, but we are unable to verify statistical significance of this finding so far due to low sample size. Analysis of SED characteristics such as morphology, and localization (temporal, extemporal, generalized) revealed no obvious relationship to impaired driving, however there seems to be a slight trend that longer lasting generalized SEDs were more likely associated with crash. In conclusion, SEDs might generally have a lower risk of traffic accident than overt clinical seizures. However, whether collisions were associated with some sustained subclinical epileptiform events is unclear, and considering the limited sample of different SED subgroups, additional work should be done to determine whether certain types of SEDs might produce transient cognitive impairment during driving. In addition, more subtle behavioral changes aside from collisions could be detected until using larger cohort in future investigations.

Disclosures: **Y. Si:** None. **E. Gudbranson:** None. **W.C. Chen:** None. **M. Midura:** None. **R. Wu:** None. **B. Geng:** None. **P. Vitkovskiy:** None. **A. Sivaraju:** None. **R. Sainju:** None. **A. Fernandez:** None. **A. Alareddy:** None. **I. Quraishi:** None. **R.B. Duckrow:** None. **L.J. Hirsch:** None. **H. Blumenfeld:** None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.05/M2

Topic: C.07. Epilepsy

Title: A portable handheld device for prospective driving evaluation in the epilepsy monitoring unit

Authors: N. LI[1], J. THOMSON[1], W. CHEN[1], D. KLUGER[1], C. CUNNINGHAM[1], R. GEBRE[1], Y. SI[1], J. BLUMENFELD[1], E. CHEN[1], M. JOHNSON[1], P. VITKOVSKIY[1], Y. BAYKARA[1], E. GUDBRANSON[1], A. MORAWO[1], *H. BLUMENFELD[2];
[1]Neurol., [2]Neurology, Neurobiology, & Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

Abstracts: Patients with epilepsy often have impaired consciousness during seizures and lose the ability to interact with their surroundings. How impaired consciousness in epilepsy affects driving abilities is particularly relevant because driving is an essential part of daily life. Prior work on this subject has been limited by both the transient nature of seizure occurrence and the lack of standardized easy-to-use behavioral testing instruments to evaluate patients during seizures. We partly overcame these obstacles in previous work with computer-based driving simulators. However desktop or laptop computers and external controllers such as steering wheel and gas/brake pedals are cumbersome in the inpatient video/EEG monitoring unit. With the recent development of handheld devices with high-definition graphics and powerful processing capabilities, we have introduced tablet computers as behavioral testing instruments that are lightweight, easy-to-pick-up, and absorbing. We customized a commercially available driving game that can be used on handheld tablets to prospectively evaluate ictal and interictal driving performance. We also developed specialized apps to acquire and save specific driving performance metrics including car position, car velocity, acceleration/braking, steering wheel movement, and collision events using the devices' built-in sensors. The driving data acquisition was synchronized with continuous EEG and video data obtained from the epilepsy patients during their hospital stay, although in principle the same approach could be used in the outpatient setting. Preliminary testing in 12 patients demonstrated the feasibility of examining driving performance prospectively in patients with epilepsy using a handheld device with good patient tolerability and convenience. Data analysis enabled complete reconstruction of all driving performance parameters, simultaneous with video/EEG results. With ongoing data collection and analyses, we hope to elucidate the relationship between specific seizure variables and driving risk in the ictal and interictal periods, with the objective of providing more informed advice regarding driving to legislators, physicians and patients with epilepsy.

Disclosures: **N. Li:** None. **J. Thomson:** None. **W. Chen:** None. **D. Kluger:** None. **C. Cunningham:** None. **R. Gebre:** None. **Y. Si:** None. **J. Blumenfeld:** None. **E. Chen:** None. **M. Johnson:** None. **P. Vitkovskiy:** None. **Y. Baykara:** None. **E. Gudbranson:** None. **A. Morawo:** None. **H. Blumenfeld:** None.

Poster

606. Human Studies of Epilepsy

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.06/M3

Topic: C.07. Epilepsy

Support: OBI grant IDS-11-05

Title: Examining white matter abnormalities in patients with Temporal Lobe Epilepsy using diffusion MRI

Authors: *A. J. BARNETT[1], M. P. MCANDREWS[2];
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Abstracts: Temporal lobe epilepsy (TLE) is a neurological disorder that has been shown to cause widespread structural and functional changes in the brain. These changes are largely seen in the medial temporal lobes, specifically the hippocampus (HC), where seizures are thought to originate. Diffusion tensor imaging (DTI) studies have also shown white matter abnormalities within this population. The aim of the current study was to examine how white matter abnormalities in patients with TLE interact with cognitive performance and disease characteristics. We collected diffusion weighted MRI data for 15 patients with left TLE (LTLE), 9 patients with right TLE (RTLE), and 19 healthy controls. DTI data was analyzed in a voxel-wise fashion using tract-based spatial statistics to examine differences in fractional anisotropy (FA), mean diffusivity (MD) and radial diffusivity (RD) which are considered to be indirect measures of white matter integrity. We also ran an automated segmentation to calculate HC volume for patients and controls. Using these measures from DTI, we examined the relationship between white matter integrity with cognitive performance and disease characteristics (disease duration, age of onset). Results demonstrated that the LTLE group had reduced white matter integrity in the left temporal lobes, along with reduced left HC volume. The RTLE group showed bilateral white matter integrity reductions in the temporal lobes compared to healthy controls and reduced right HC volume. We found in our patient groups that smaller right HC volume was associated with reduced FA in the right temporal lobes. In patients with LTLE, performance on the Boston Naming Test was positively correlated with left temporal FA and negatively correlated with RD. Duration of disease was also associated with reduced FA in the left temporal lobes. These results suggest that there are local and distal white matter alterations in TLE and these alterations are associated with disease characteristics and, in LTLE, language ability.

Disclosures: A.J. Barnett: None. **M.P. McAndrews:** None.

Poster

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Topic: C.07. Epilepsy

Support: NIH Grant MH55687

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Title: Improving seizure detection with single/multiunit information in the MTL requires the extraction of specific subpopulations

Authors: *X. LONG[1], A. MISRA[1], M. R. SPERLING[2], A. D. SHARAN[3], K. A. MOXON[1]; [1]Sch. of Biomed. Engin., Drexel Univ., Philadelphia, PA; [2]Neurol., [3]Neurolog. Surgery, Thomas Jefferson Univ., Philadelphia, PA

Abstracts: Over the past several decades, numerous studies have attempted to investigate the behavior of single neurons and multiunit during and immediately prior to seizure. The majority of these studies report a heterogeneity in firing behavior of neurons, complicating the use of single/multiunit information in automated detection or prediction algorithms. Here we present an approach to subdividing this heterogeneous population (specific to neurons of the mesial temporal lobe MTL) to obtain reliable information to aid in the detection of seizure events. Firing rate and unit-field coherence were extracted from A) multiunit recordings and B) putative interneuron recordings from 35 seizure recordings (and corresponding interictal recordings) in 7 patients with seizures that spread to the MTL. These variables were used in a parametric threshold based algorithm and their ability to detect seizures along with the latency to seizure detection were assessed in comparison to a more traditional LFP-amplitude based detection algorithm. While both multiunit and interneuron specific information appeared to improve the detection latency, multiunit information was associated with a loss in sensitivity and a greater than 20 fold increase in false detection rate. In contrast, interneuron specific information retained a high sensitivity while the relative increase in false detection rate was comparatively reduced (4x). Most importantly, when using interneuron specific information, the latency to detection was consistently negative (i.e.) the algorithm predicted electrographic seizure onset, despite recordings occurring outside of the clinically identified seizure focus. Thus the clinical utility of

single/multiunit information is highly dependent on which subpopulation is extracted for analysis, and this information may complement existing seizure detection efforts, as well as challenge our interpretation of the focal seizure onset zone.

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Poster

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Support: Romanian UEFISCDI PN-II-ID-PCE-2011-3-0240

Title: Single-unit activity evoked by electrical stimulation of human epileptogenic cortex

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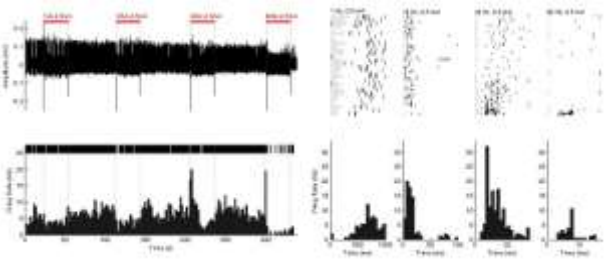
Abstracts: Background: Intracranial direct electrical stimulation (DES) during presurgical stereoelectroencephalograpic (SEEG) evaluation of patients with drug-resistent epilepsy is a powerful method for mapping the epileptogenicity of various brain areas. In order to elucidate the basic neural mechanisms underlying electrographic responses to DES, we aim at investigating human single unit firing during intraoperative DES of epileptogenic areas for different stimulation amplitudes and frequencies. Methods: We performed SEEG presurgical evaluation of 8 patients with drug-resistant focal epilepsy to locate the seizure-onset zone (SOZ) and delineate the area to be resected. Prior to the resective surgery, we are stereotactically inserting three microelectrodes, spaced 2mm apart, in a linear configuration, following a trajectory targeting SOZ. We use standard clinical microelectrodes and equipment used in functional mapping for deep brain stimulation implantations. Bipolar electrical stimulation is applied between the two outer macro contacts of the electrodes while recording the unit activity on the center microelectrode. Constant current 0.5 to 1 mA biphasic pulses, 0.3 ms pulse width, frequency 1, 10, 30, 60 and 130 Hz were applied for 30 s using a clinical stimulator (Guideline LP+, FHC Inc, Bowdoin, ME). Results: We have recorded to date 14 neurons in SOZ and

adjacent areas. We were able to find typical patterns for inter-ictal spiking and single-pulse stimulation: burst only, burst-suppression, suppression-only, no-change. In addition, repetitive stimulation resulted in a buildup of the firing rate over the stimulation duration in about 28% of the neurons. Higher stimulation frequency had a suppressive effect on the neuronal firing. Conclusions: SOZ neurons exhibit heterogenous responses to repetitive stimulation. Evidence for increased plasticity of the epileptogenic cortex was supported by the activity of a subpopulation of neurons. Detailed analysis of the firing patterns may provide an insight on the network mechanisms underlying epileptogenicity.



Disclosures: **A. Barborica:** A. Employment/Salary (full or part-time):; FHC Inc, Bowdoin ME. **C. Donos:** None. **I. Mindruta:** None. **J. Ciurea:** None.

Poster

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Topic: C.07. Epilepsy

Support: H.L.M. grant sponsor : CONACYT Grant 203881

Title: Glutamate-mediated up-regulation of the multidrug resistance protein 2 in porcine and human brain capillaries

Authors: *H. L. MUNGUÌA[1,2], J. D. SALVAMOSER[2], B. PASCHER[3], T. PIEPER[3], T. GETZINGER[3], M. KUDERNATSCH[4], H. POTSCHKA[2];
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Abstracts: As a member of the multidrug-resistance associated protein family MRP2 (ABCC2) affects the brain entry of different endogenous and exogenous compounds including toxicants and drugs. Considering the functional role of this transporter at the blood-brain barrier the regulation of its expression and function is of particular interest. However, so far there is only limited knowledge regarding the factors that might regulate MRP2 in neurological disease states. Thus, we addressed the hypothesis that MRP2 might be affected by a glutamate-induced signaling pathway that we previously identified as one key mechanism in the regulation of the transporter P-glycoprotein. Studies in isolated porcine brain capillaries confirmed that glutamate and NMDA exposure up-regulates expression and function of MPR2. The involvement of the NMDA receptor was further confirmed by the fact that the NMDA receptor antagonist MK-801 abolished the impact of glutamate. In addition, co-incubation of porcine capillaries with the cyclooxygenase-2 inhibitor celecoxib efficaciously prevented a glutamate-induced up-regulation of MRP2. Translational studies in human capillaries from surgical specimen demonstrated a relevant MRP2 efflux function, and confirmed the effect of glutamate exposure as well as its prevention by cyclooxygenase-2 inhibition. Taken together the findings revealed a role of a glutamate-induced NMDA receptor/cyclooxygenase-2 signaling pathway in the regulation of MRP2 expression and function. The response to excessive glutamate concentrations might contribute to overexpression of MRP2, which has been reported in neurological diseases such as epilepsy. The resulting overexpression might have implications for brain access of endogenous and exogenous compounds including therapeutic drugs.

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Poster

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Program#/Poster: 606.10/M7

Topic: C.07. Epilepsy

Title: Impaired cerebrovascular reactivity in patients with mesial temporal lobe epilepsy

Authors: *K. ALHADID[1,2], O. SOBCYK[2], J. POUBLANC[3], A. CRAWLEY[3], L. VENKATRAGHAVAN[4,5], J. A. FISHER[5,2,6], D. J. MIKULIS[7,1,4,2], T. A. VALIANTE[8,1,4,2]; [1]Toronto Western Res. Inst., Toronto, ON, Canada; [2]Inst. of Med. Science, Univ. of Toronto, Toronto, ON, Canada; [3]Joint Dept. of Med. Imaging, Univ. Hlth. Network, Toronto, ON,

Canada; [4]Univ. Hlth. Network, Toronto, ON, Canada; [5]Dept. of Anaesthesia and Pain Management, Univ. Hlth. Network, Toronto, ON, Canada; [6]Dept. of Physiology,University of Toronto, Toronto, ON, Canada; [7]Joint department of Med. Imaging, Univ. Hlth. Network, Toronto, ON, Canada; [8]Dept. of Surgery, Div. of Neurosurgery, Univ. of Toronto, Toronto, ON, Canada

Abstracts: Purpose: Epilepsy surgery can achieve excellent outcomes in mesial temporal lobe epilepsy (mTLE) patients when the epileptogenic zone is accurately localized. Ancillary tests such as FluoroDeoxyGlucose Positron Emission Tomography (FDG-PET) are used for this task when standard tests are inconclusive, or discordant. Although decreased inter-ictal metabolism and perfusion to the epileptogenic temporal lobe has been demonstrated, the underlying mechanism for these alterations remain poorly understood. Cerebrovascular reactivity (CVR) is the change in a measure of cerebral blood flow (CBF), such as the BOLD MRI signal, to a vasoactive stimulus, such as hypercapnia. We hypothesized that the reduced inter-ictal hypometabolism in mTLE patients is also associated with vascular dysfunction detectable on CVR studies, and thus would help delineate the epileptogenic zone. Methods: A homogenous group of unilateral mTLE patients who had undergone standard pre-operative investigations for seizure localization were included (n=5) in this study. We used BOLD MRI as the high-resolution surrogate for CBF and a standardized CO2 stimulus sequence targeting arterial partial pressure of CO2 (PaCO2) (RespirAct™). The experimental protocol consisted of a one step 10-mmHg increase in PaCO2 from baseline levels for 2 minutes, followed by a gradual increase in PaCO2 from 30 mmHg to 55 mmHg over 4 minutes. CVR was calculated as the best-fit regression for the BOLD signal vs. PaCO2. CVR was scored as both the amplitude and time constant of response, voxel-by-voxel, according to the mean and variance of the corresponding voxel from a previously generated reference cohort of healthy volunteers who had undergone the same protocol. Inter-hemispheric discrepancies in CVR values were also evaluated. Results: Preliminary findings demonstrate a marked increase in CVR in the epileptogenic temporal lobe compared to normal controls, and when compared to the contralateral non-epileptogenic temporal lobe in the same patient. Discussion: The observed increase in CVR in unilateral mTLE patients ipsilateral to the epileptogenic side might represent a pathophysiological increase in vascular capacity required to support the sustained increase in neuronal activity during a seizure. CVR imaging might represent a non-invasive test similar to FDG-PET for lateralization of the epileptogenic zone, without the requirement of radioactive tracers.

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Poster

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Topic: C.07. Epilepsy

Support: CNPq 162233/2011-6

Title: Hippocampal newborn cells in infantile rasmussen encephalitis patients

Authors: *E. D. MAGNO[1], E. A. CAVALHEIRO[1], R. CORAS[2], I. BLÜMCKE[2]; [1]Neurology/Neuroscience, UNIFESP, São Paulo, Brazil; [2]Inst. of Neuropathology, Hosp. Univ. of Erlangen, Erlangen, Germany

Abstracts: Aim: Determine the neurogenesis rate in hippocampal tissue from Rasmussen Encephalitis patients and compare with tissue obtained from human autopsy. Methods and Results: Five hippocampal specimens were obtained from surgery to treatment of drug-resistant Rasmussen Encephalitis (RE) (age range from 3 to 15 years old). All patients included in this study were adequately informed and gave their written consent for the scientific project, which was approved by the local ethics committees of the University of Erlangen (Germany) and the Universidade Federal de São Paulo (Brazil). All procedures were conducted in accordance with the Declaration of Helsinki (1964). Hippocampal tissue obtained from three human autopsy cases (age range from 5 to 27 years old) were used as control, whose causes of death were unrelated to reports of neurological disease. All specimens were submitted to immunohistochemical and immunofluorescence techniques for neuronal counting (NeuN, H&E) and the verification of neurogenesis (Ki67, vimentin and Sox2) in the hippocampal dentate gyrus. After quantification in an optic microscope, comparisons were performed by analysis of variance followed by Bonferroni´s posthoc test (ANOVA-Bonferroni) and non-parametric t-test, with Mann-Withney posttest. The number of neurons in the dentate gyrus, but not in the CA1-4 hippocampal subfields, was significantly decreased in RE patients when compared to control specimens. In contrast to autopsy specimens, the dentate gyrus of RE patients showed an expressive increase in neurogenesis when the marker Ki-67 was used, but no significant changes could be detected with vimentin or SOX-2. Conclusion: These results suggest that Rasmussen Encephalitis is accompanied by increased neurogenic rates in the dentate gyrus when compared to specimens obtained from non-epilepsy cases. Besides, the neuronal cell loss observed in the dentate gyrus of these patients was not accompanied by similar changes in other hippocampal areas. Studies aiming at investigating the relation of these changes with the severe inflammatory process observed in RE are now in progress.

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Poster

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Support: KAKEN 21300134

KAKEN 22700376

Title: Epileptogenic mechanisms in mesial temporal lobe epilepsy: an *in vitro* optical imaging study of resected human hippocampus specimens

Authors: ***H. KITAURA**[1], H. SHIROZU[2], M. SONODA[2], H. SHIMIZU[1], H. MASUDA[2], H. TAKAHASHI[1], S. KAMEYAMA[2], A. KAKITA[1];
[1]Pathology, Brain Res. Inst, Niigata Univ., Niigata, Japan; [2]Neurosurg., Nishi-Niigata Chuo Natl. Hosp., Niigata, Japan

Abstracts: Mesial temporal lobe epilepsy (MTLE) is the most frequent focal epilepsy syndrome, and the vast majority of seizures originate from the mesial structures of the temporal lobe. Tissues surgically resected from the hippocampus usually exhibit various degrees of neuronal loss, a condition that is referred to as hippocampal sclerosis. The epileptogenic mechanisms operating in the sclerotic hippocampi of patients with MTLE remain uncertain. To investigate this issue, we performed flavoprotein fluorescence imaging *in vitro* using surgically removed human hippocampus specimens. As a control, we retrieved hippocampus specimens showing no histopathologic abnormalities removed surgically from two patients with no MTLE symptoms, but harboring either cortical tubers or focal cortical dysplasia nearby. According to the severity of hippocampal sclerosis in the patients with MTLE, we defined cases with no apparent neuronal loss, restricted neuronal loss in the CA1 subfield, and severe loss in the CA1/3/4 subfields as having no, mild, and severe HS, respectively. Flavoprotein fluorescence responses in the subiculum and CA1 subfield in the no HS group were significantly more marked than in the controls. In the mild and severe HS groups, the responses in each subfield were markedly diminished where neuronal loss was evident; however, similar enhanced responses were observed in the subiculum. Interestingly, stimulation of the CA4 subfield elicited recurrent activity in the dentate gyrus (DG) only in the severe HS group, and the activity was abolished by

application of CNQX. These findings indicated mossy fiber sprouting in the DG, as supported by our histological observations of TIMM-positive fibers. In the subiculum in all HS groups, recording of local field potential demonstrated spontaneous discharges with a clear high-frequency oscillation component. In the DG in the severe HS group, distinct spontaneous rhythmic activities lasting 10 s or more were observed. Application of an adenosine blocker clearly attenuated these spontaneous activities in the subiculum, but not those in the DG. Thus, intrinsic spontaneous activity, i.e. ictogenesis, may develop initially in the subiculum of patients with MTLE before neuronal loss occurs in the CA subfields of the hippocampus. Such abnormal discharges may then induce neuronal loss in the CA subfields, resulting in loss of target neurons in the dentate granule cell projection pathway. Therefore, mossy fiber sprouting may be induced in the DG. We speculate that the mechanism of epileptogenesis in MTLE specifically involves individual subicular neurons, and subsequently the dentate granular circuits.

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Poster

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Title: Pyramidal cell morphology in human cortical dysplasia

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

Abstracts: Malformations of cortical development underlie a significant portion of adult and pediatric forms of medically intractable epilepsy. Severe forms of focal cortical dysplasia are characterized by dysmorphic/cytomegalic neurons and balloon cells in the context of a pervasive laminar disorganization. To date, direct measures of the morphology of human dysplastic neurons have not been possible on a large scale. Here, we present detailed, high-resolution structure of dysplastic pyramidal neurons in the seizure onset zone (SOZ) of the inferior parietal

lobule of two adult patients with epilepsy. Pyramidal neurons of presumably normal cortical area around the seizure onset zone as determined by intracranial EEG and cortical mapping were used as control. The null hypothesis of no significant morphological changes in the synaptic connectivity of the SOZ and each spine type (mushroom and thin) were tested, as measured by spine length, spine volume, and spine head diameter. Detailed structural data for layer II/III and layer V dysplastic pyramidal neurons of the SOZ were obtained through morphometric analysis of Lucifer Yellow filled neurons in brain slices prepared from surgical resections (300 μm thick). High quality dye-loaded neurons were selected and imaged at high magnification (63X, 1.4 N.A. 100 nm cubic voxel) using confocal laser scanning microscopy. The neuronal morphology, dendritic arbor and dendritic spine morphology were 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. Basal thin spines were found to be significantly longer in the control than in the dysplastic cells ($p < 0.001$, corrected), with differences in mean lengths differing by as much as 0.2 micrometers at 95% confidence. The mean volume of basal mushroom spines, however, differd by as much as 0.04 cubic micrometers (95% confidence bound), nearly 30% of the mean volume of dysplastic basal mushroom spines ($p < 0.01$, corrected), with the control being larger. Thin spine head diameter also varied by a difference in means of 0.1 micrometers and 0.12 micrometers (apical, basal) between the dysplastic cells and control cell ($p < 0.001$, corrected), with control being larger. The null hypothesis was therefore rejected, alluding to significant changes in the dysplastic pyramidal neurons of the SOZ, such as significant loss of synaptic input to stable mushroom spines. These findings provide morphological evidence on how and why the dysplastic tissue is more prone to seizures in this common form of medically intractable epilepsy.

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

Poster

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Topic: C.07. Epilepsy

Support: NIH RO1DC011805

Title: The functional connectome of high frequency networks during epileptic seizures

Authors: *S. FUERTINGER[1], F. HAMZEI-SICHANI[2,3], J. C. ZINN[1], M. R. SPERLING[4], A. D. SHARAN[3], K. SIMONYAN[1];
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Abstracts: High frequency oscillations (HFO, 100-500 Hz) or ripples in cortical networks have been shown to underlie seizure generation in human intracranial electroencephalographic (iEEG) recordings as well as a in *in vitro* recordings from brain slices of rodents in various animal models of hippocampal and neocortical epilepsy. HFOs in contrast to other epileptiform discharges such as sharp waves or spikes are the most reliable marker of the epileptogenic area leading to the best surgical outcome when used to define the extent of surgical resections. However, a rigorous analysis of the interplay of different cortical generators of HFOs during ictal and interictal periods in a graph theoretical context has been lacking. Here, we have applied such scheme to iEEG recordings (1000-2000 Hz) of patients with epilepsy during two well-defined cortical states, namely the awake-resting (1 hour) and awake-seizure (peri-ictal 10 minutes) states. Normalized mutual information (NMI) coefficients were calculated for each pair of intracranial electrode time courses in each cortical state after band-pass filtering for ripple frequency. Graphs were constructed by interpreting electrodes as network nodes and associated NMI coefficients as weights of the graph's edges. The resulting weighted undirected networks were analyzed to assess topological changes between rest and seizure. Differences in network segregation were estimated by calculating the clustering coefficient. We used local efficiency to assess variations in network integration. Deviations in nodal influence were quantified by estimating nodal connectivity in terms of degree and strength. Variations in graph metrics of the rest and seizure networks were tested for statistical significance using a two-sample t-test at a corrected $p \leq 0.01$. The strength of network connectivity significantly increased in the seizure period compared to the resting state ($p < 0.001$) with highly interconnected nodes (hubs) moved from the area adjacent to the seizure onset zone (SOZ) into the SOZ. Local efficiency, which estimates a node's average communication performance, was significantly increased in the seizure period than during rest ($p < 0.001$). Nodal clustering coefficients showed a trend towards higher values during seizure ($p = 0.052$). Remarkably, changes in nodal strength revealed silent zones in the networks corresponding to HFO sources adjacent to the SOZ. The observed increase in efficiency during seizure was indicative of a sudden parallel activation of spatially distributed nodes. Simultaneously, decreased clustering during seizure implied a loss of functional organization characteristic of cortical networks.

Disclosures: S. Fuertinger: None. **J.C. Zinn:** None. **F. Hamzei-Sichani:** None. **M.R. Sperling:** None. **A.D. Sharan:** None. **K. Simonyan:** None.

Poster

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Topic: C.07. Epilepsy

Support: NIH Grant R37-AG06647

Title: Pyramidal cell morphology in seizure onset zone, seizure spread zone and silent cortical areas in patients with parietal lobe epilepsy

Authors: *F. HAMZEI-SICHANI[1], J. C. ZINN[2], W. G. M. JANSSEN[3], J. EVANS[4], A. D. SHARAN[4], M. R. SPERLING[5], K. SIMONYAN[2], P. R. HOF[3], J. H. MORRISON[3];
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[4]Neurosurg., [5]Neurol., Thomas Jefferson Univ., Philadelphia, PA

Abstracts: Genetic and acquired abnormalities of neuronal connectivity are thought to underlie many types of human epilepsy. However, direct measures of the morphology of cortical neurons in the disordered microcircuit in human epileptic tissue have not been possible on a large scale. Here, we present detailed, high-resolution structure of human neocortical pyramidal neurons in three salient cortical areas associated with seizures, namely the seizure onset zone (SOZ), the seizure spread zone (SSZ) and the silent area with no seizure propagation, all within the inferior parietal lobule of two patients with epilepsy. The type of seizure locus was determined based on intracranial EEG recording and mapping by stimulation of each subdural electrode. The null hypothesis of no significant morphological changes in the synaptic connectivity of each area and each spine type (mushroom and thin) was tested, as measured by three spine morphological features (spine length, spine volume, and spine head diameter). Detailed structural data for layer II/III and layer V pyramidal neurons of each area were obtained through morphometric analysis of Lucifer Yellow filled neurons in brain slices prepared from surgical resections (300 μm thick). High quality dye loaded neurons were selected and imaged at high magnification (63X, 1.4 N.A. 100 nm cubic voxel) using confocal laser scanning microscopy. The neuronal morphology, dendritic arbor and dendritic spine morphology were 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. We found the head diameter of thin and the length of mushroom dendritic spines to be significantly smaller in the SOZ and SSZ compared to the silent area. The head diameter of mushroom dendritic spines was significantly higher in the SOZ compared to SSZ and the silent area. The volume of mushroom dendritic spines was significantly higher in SOZ compared to SSZ and in SSZ compared to the

silent area. The length of thin dendritic spines was significantly lower in the SOZ compared to SSZ and the silent area. Corrected p-values were <0.05. The null hypothesis was therefore rejected, alluding to significant changes in SOZ, such as strengthened synaptic input to stable mushroom spines. These findings provide morphological evidence on how and why the SOZ is more prone to seizures which is a necessary step to understand the pathophysiology of this common disorder of the nervous system.

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

Poster

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CEI BioTic at Universidad de Granada

Euskampus at UPV/EHU

Title: Redundancy and synergetic circuits in inter-ictal activity of human temporal lobe epilepsy

Authors: ***S. STRAMAGLIA**[1,2,3], A. ERRAMUZPE[3], G. J. ORTEGA[4], J. PASTOR[4], R. G. DE SOLA[4], D. MARINAZZO[5], J. M. CORTES[2,3];
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Abstracts: The use of information theory in neuroscience have allowed to evaluate the interaction between groups of correlated variables, revealing their functional role and underlying

circuits capable of processing information (Borst and Theunissen, 1999, Nat Neurosci; Panzeri et al., 1999, Proc Biol Sci; Quiroga and Panzeri, 2009, Nat Rev). In addition to information storage/coding/decoding, information theory can address whether the interactions between the correlated variables are mutually redundant or synergetic (Schneidman, Bialek and Berry, 2003, J Neurosci; Bettencourt et al., 2007, Phys Rev E). In general, synergy occurs if the knowledge of some variables contributes to predict another variable with more information than the sum of the information provided individually by the variables; redundancy corresponds to situations with the same information being shared by the variables. In particular, the well-known interaction information (McGill, 1954, Psychometrika) applied to triplets quantifies the amount of either redundant (positive interaction) or synergetic information (negative interaction) contained in the triplet; notice that unlike the mutual information, the interaction information can be either positive or negative. We calculated the interaction information in electricorticography data that have a clinical validation: after resection, no further seizures occurred. Using the pre-surgery data recorded from the epileptic patient and knowing the resection area, we addressed both the functional organization and dynamics of redundancy and synergy near the epileptogenic network (the circuit triggering epileptic seizures). Regarding functional organization, redundancy (corresponding to positive values of interaction information) matched with the resection area, while synergy (negative values) emerged in its surroundings. In relation with dynamics, redundancy had the biggest contribution at higher frequency bands (14-100Hz) whilst synergy was more expressed at very slow frequencies (1-7Hz). Thus, the application of the interaction information to this clinical data unveils new aspects of epileptogenic structure in terms of interaction nature (redundancy vs synergy) and dynamics (fast vs slow rhythms).

Disclosures: **S. Stramaglia:** None. **A. Erramuzpe:** None. **G.J. Ortega:** None. **J. Pastor:** None. **R.G. de Sola:** None. **D. Marinazzo:** None. **J.M. Cortes:** None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.17/N2

Topic: C.07. Epilepsy

Title: Ultrafast oscillations and cognitive processes in epileptic drug-resistant patients

Authors: *M. MONTES DE OCA BASURTO[1], F. VELASCO CAMPOS[2], R. J. STABA[3], D. VÁZQUEZ-BARRÓN[2], V.-M. ANA LUISA[2], A. NUCHE-BRICAIRE[2], M. CUÉLLAR-

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Abstracts: BACKGROUND The ultrafast oscillations measured through the depth EEG in patients with refractory TLE function as biological markers of pathology in epileptic models , but so far have not been used to measure their relationship to cognitive aspects . Quick frequencies are related to the behavior and memory consolidation , however the ultrafast oscillations are considered pathological .

Disclosures: M. Montes De Oca Basurto: None. **F. Velasco Campos:** None. **R.J. Staba:** None. **D. Vázquez-Barrón:** None. **V. Ana Luisa:** None. **A. Nuche-Bricaire:** None. **M. Cuéllar-Herrera:** None.

Poster

606. Human Studies of Epilepsy

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.18/N3

Topic: C.07. Epilepsy

Title: Alterations of 5-HT1A receptor-induced G-protein functional activation and relationship to memory deficits in patients with pharmacoresistant temporal lobe epilepsy

Authors: *M. CUÉLLAR-HERRERA[1], L. ROCHA[3], F. VELASCO[2], D. TREJO-MARTINEZ[2], A. NUCHE-BRICAIRE[2], M. ALONSO-VENEGAS[4], A. L. VELASCO[2];
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Abstracts: The 5-hydroxytryptamine-1A (5-HT1A) receptors are known to be involved in the inhibition of seizures in epilepsy. Moreover, studies propose a role for the 5-HT1A receptor in memory function; it is believed that the higher density of this receptor in the hippocampus plays an important role in its regulation. Positron emission tomography studies in patients with mesial temporal lobe epilepsy (mTLE) have demonstrated a decrease in 5-HT1A receptor binding in temporal regions may play a role in memory impairments of these patients. The evidences lead us to speculate whether this decrease in receptor binding is associated with a reduced receptor number or if the functionality of the 5-HT1A receptor-induced G-protein activation and/or the

second messenger cascade is modified. The purpose of the present study is to determine 5-HT1A receptor-induced G-protein functional activation by 8-OH- DPAT-stimulated [35S]GTPγS binding assay in hippocampal tissue of surgical patients with mTLE. We correlate functional activity with epilepsy history and neuropsychological assessment of memory. We found that maximum functional activation stimulation values (Emax) of [35S]GTPγS binding were significantly increased in mTLE group when compared to autopsy samples. Furthermore, significant correlations were found: 1) positive coefficients between the Emax with the age of patient and frequency of seizures; 2) negative coefficients between the Emax and working memory, immediate recall and delayed recall memory tasks. Our data suggest that the epileptic hippocampus of patients with mTLE presents an increase in 5-HT1A receptor-induced G-protein functional activation, and that this altered activity is related to age and seizure frequency, as well as to memory consolidation deficit.

Disclosures: **M. Cuéllar-Herrera:** None. **L. Rocha:** None. **F. Velasco:** None. **D. Trejo-Martinez:** None. **A. Nuche-Bricaire:** None. **M. Alonso-Venegas:** None. **A.L. Velasco:** None.

Poster

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Program#/Poster: 606.19/N4

Topic: C.07. Epilepsy

Support: NIH Grant NS062092

Epilepsy Foundation Grant 222178

Title: Changes in neuronal activity outside of the seizure focus in patients with temporal lobe epilepsy

Authors: *J. NAFTULIN, O. AHMED, S. CASH;
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Abstracts: Epilepsy affects approximately one percent of the population and epileptic seizures can be divided into two broad categories: primarily generalized and focal seizures. Focal seizures start in a localized brain region and then can either remain focal or spread to other brain regions (secondary generalization). However, our concept of the focus, and even the existence of a true focus, is being increasingly questioned due to a variety of imaging and physiological data. Here,

we examined how activity outside the seizure focus changes at the time of seizure onset. Stereotactic depth electrodes were implanted in patients with longstanding pharmaco-resistant epilepsy for stage II lateralization. We compared focal temporal lobe seizures that remained focal and those that secondarily generalized. Spectral analyses were used to quantify changes in power over delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), gamma (30-70 Hz), and high gamma (70-120 Hz) frequency bands in regions inside, close to, and well outside the area of seizure onset. We examined a total of 70 seizures in 12 patients. In focal seizures that remained focal, we found only 20 out of 464 (4.3%) electrodes outside the seizure focus showed significant increases in any of the frequency bands at the time of seizure onset. However, 44 out of 272 (16.2%) electrodes outside the seizure focus showed significant increases at the moment of onset in focal seizures that would go on to secondarily generalize. Thus, we find that at the moment of seizure onset, changes outside the focus are rare during focal seizures that remain focal, but more frequent during focal seizures that will later go on to secondarily generalize. These observations may help to better understand the neuronal dynamics underlying the initiation of focal seizures, and to design better brain-machine-interface algorithms to distinguish between subtypes of focal seizures.

Disclosures: **J. Naftulin:** None. **O. Ahmed:** None. **S. Cash:** None.

Poster

606. Human Studies of Epilepsy

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Topic: C.07. Epilepsy

Support: ABRC grant: ADHS13-031259

Title: The role of bdnf-trkb signaling in epileptogenesis in human hypothalamic hamartoma

Authors: Y. HUANG[1], S. SEMAAN[1], Q. LIU[2], *Y. CHANG[2], J. WU[2];
[1]St. Joseph's Hosp. and Med. Ctr., Phoenix, AZ; [2]Barrow Neurolog. Inst., St. Joseph's Hosp. & Med. Ctr., PHOENIX, AZ

Abstracts: Human hypothalamic hamartoma (HH) is a developmental malformation that occurs in the region of the tuber cinereum and inferior hypothalamus. This lesion is associated with a range of neurological and endocrine disorders including intractable seizures, cognitive impairment, behavioral disturbances, and central precocious puberty. HH is often characterized

by gelastic seizures, which are usually refractory to classical anti-epileptic drugs and alternative therapies. The most effective treatment of gelastic seizures associated with HH is surgical resection of the lesion. Evidence indicates that the source of gelastic seizures in patients with HH lies within the lesion itself. Our previous studies using surgically resected HH tissues have revealed that a proportion of HH neurons are non-GABAergic neurons with an immature phenotype (Wu et al., 2005, 2007, and 2008). This suggests that these neurons may have a relatively low expression level of the outwardly-directed K^+-Cl^- co-transporter (KCC2) and a relatively high intracellular Cl^- concentration. As a result, the inhibitory neurotransmitter GABA rather plays an excitatory role in these immature HH neurons, potentially leading to gelastic seizures. However, the signaling mechanisms underlying KCC2 downregulation and GABA-induced neuronal excitation and epileptogenesis in HH remain to be elucidated. In the present study, we examined the effects of pharmacological manipulations of BDNF-TrkB signaling in seizure-like discharges and neuronal firing of HH neurons using surgically resected HH tissues. We found that exposure of recorded HH neurons to BDNF increased neuronal firing rate. Pharmacological blockade of BDNF-TrkB signaling using a selective inhibitor for Trk kinase, K252a, reduced large-sized HH neuron firing, but minimally affected small-sized HH neuron firing rate. In fresh HH slices, although BDNF did not show clear effect on spontaneous inhibitory post synaptic potential (sIPSCs), it reduced GABA-induced inward current under patch-clamp whole-cell recording configuration in acutely dissociated single large HH neurons. Finally, we found that in HH tissue, the BDNF mRNA expression was significantly enhanced, whereas the anti-KCC2 immunostaining signal was weaker in HH tissue compared to normal human hypothalamic control tissue. In conclusion, our data suggests that the BDNF-TrkB signaling plays an important role in downregulation of KCC2 expression, which in turn alters $GABA_A$ receptor function and leads to epileptogenesis in HH lesion.

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Poster

606. Human Studies of Epilepsy

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Program#/Poster: 606.21/N6

Topic: C.07. Epilepsy

Title: Yield of linear pairwise granger causality in seizure onset identification versus visual EEG evaluation in pharmaco-resistant non-lesional partial epilepsy

Authors: *E. ANDRADE, Z. LIU;
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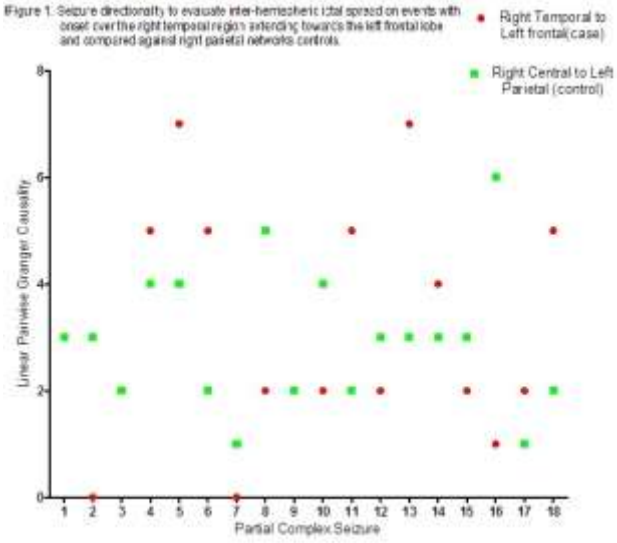
Abstracts: Several studies have revealed the presence of at least three well defined ictal neuronal networks as the cause of partial complex seizures. Evidence includes *in vivo* animal and human electrophysiological recording. Several papers have postulated the role of autoregressive models such as linear pairwise granger causality (LPGC) as a noninvasive method to detect ictal onset. To test the effectiveness of LPGC, we hypothesize that the ictal onset (IO) in patients with partial epilepsy refractory to medical treatment (PERT) originate in a single network and propagates within the same hemisphere. The feasibility of this hypothesis was retrospectively analyzed on 90 clinical seizures from 23 subjects admitted to the epilepsy monitoring unit for phase I (pre surgical) evaluation. The visual assessment of the ictal electroencephalogram EEG was used as a control and compared to that of the validated LPGC. First, ictal EEG was defined by an experienced board certified pediatric epileptologist blind to the study. Second, the EEG raw data were analyzed using PGC. Boot-strapping methodologies were used to address the statistical significance of the network interactions defined with PGC. Time frequency distribution was plotted for each seizure and compared versus controls. PGC showed no statistically significant difference for intra-hemispheric directionality in subjects with partial complex seizures when the IO was over the left temporal (p=0.0548) and right frontal lobe (p=0.0558). Furthermore, there were no significant differences when looking at the inter-hemispheric PGC measures obtained in subjects with seizures with an IO over the left frontal (p=0.2882), left temporal (p=0.1572), right frontal (p=0.6772) and right temporal networks (p=0.7795) when compared against the parietal lobe (Figure 1). The findings of our pilot study indicate no actual ipsilateral ictal propagation. Alternatively, ipsilateral ictal propagation may not identifiable with PGC. To answer this last question, we now proposed further evaluation using segmentation and surrogate analysis.



Disclosures: **E. Andrade:** None. **Z. Liu:** None.

Poster

606. Human Studies of Epilepsy

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Program#/Poster: 606.22/N7

Topic: C.07. Epilepsy

Support: CAPES

CNPq

FAEPA

Title: Differential neuronal loss in the hippocampus of patients with temporal lobe epilepsy and psychiatric comorbidities

Authors: ***J. B. DE ROSS**, L. KANDRATAVICIUS, R. C. SCANDIUZZI, C. G. CARLOTTI JR, J. A. ASSIRATI JR, J. E. C. HALLAK, J. P. LEITE, J. A. S. CRIPPA;
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Abstracts: Despite the high prevalence of psychiatric comorbidities in pharmacoresistant mesial temporal lobe epilepsy (MTLE) patients, the mechanisms underlying this association are poorly understood. Recent studies have shown distinct neuropathological features in patients with MTLE and psychiatric comorbidities, like differential neuronal loss among the hippocampal formation subfields. Examining a new series of patients, our goal was to evaluate neuronal density in these cases and correlate possible differences with clinical data. Retrospectively, 43 cases were selected from patients with pharmacoresistant MTLE who underwent surgery to epilepsy control. Specimens were divided into three groups according to psychiatric diagnosis: interictal psychosis (MTLE + P), major depression (MTLE + D) and without psychiatric symptoms (MTLE). Tissue from autopsies were used as controls (CTRL). Neuronal density was calculated in hippocampal formation subfields in sections immunostained with NeuN antibody. Compared to CTRL, MTLE specimens showed significant neuronal loss in all hippocampal subfields, with exception of parasubiculum and entorhinal cortex. Among patients with psychiatric comorbidities, MTLE + P group showed lower neuronal density in CA2 when compared to MTLE + D group ($p=0.024$). No effect of haloperidol in neuronal density was seen in MTLE + P group. However, MTLE + D patients taking selective serotonin reuptake inhibitors

(SSRI) showed increased neuronal density in granular layer of dentate gyrus (p=0.032) when compared to those not taking SSRI. Our results indicated that MTLE + P patients present decreased neuronal density in the CA2 subfield. Likewise, a postmortem study of schizophrenic individuals also demonstrated decreased neuronal density of non-pyramidal neurons in this same region. A recent study showed that CA2 subfield is essential for social memory processing in rodents, behavioral parameter usually altered in subjects with psychiatric disorders. In addition, our results indicated that MTLE + D patients taking SSRI have greater neuronal density in the granular layer of dentate gyrus. Several studies indicate hippocampal neurogenesis as a necessary mechanism for SSRI antidepressants action. However, since we performed mature neuron count, neurogenesis detection techniques are needed to confirm and expand this finding.

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Poster

606. Human Studies of Epilepsy

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Program#/Poster: 606.23/N8

Topic: C.07. Epilepsy

Title: Brain connectivity analysis can identify primary epileptogenic zone in pediatric epilepsy patients

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Abstracts: Resective surgery can be an effective treatment in intractable epilepsy and patients can be freed of seizure by removing the epileptogenic zone. It is critical to accurately localize the epileptogenic zone for successful resective surgery. Many models of source localization analysis such as equivalent current dipole, sequential dipole fitting, and independent component analysis have been applied to the issue. More recently, functional brain connectivity analysis such as Granger causality methods has been proposed to better explain causality and directionality of epileptic network. This analysis method can identify the causality between different cortical areas and it can be used to identify the source of the abnormal electrical signals. However, sensitivity and specificity of this approach has not been fully evaluated yet. We propose to test

the validity of brain connective analysis as a mean to identify true epileptogenic zone. We used focal epilepsy patients who underwent resective surgery with good outcome as a reference. We compared surgical sites and the site that was identified by connectivity analysis to see how close they matched. We analyzed sharp and wave discharge in preoperative electroencephalography to identify the primary epileptogenic zones using direct directed transfer function (dDTF) based on a multivariate autoregressive model. We compared the areas identified by dDTF with the resection areas in the patients.

Disclosures: D. Lee: None. **R. Yu:** None. **H. Kim:** None. **Y. Hur:** None.

Poster

606. Human Studies of Epilepsy

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 606.24/N9

Topic: C.07. Epilepsy

Title: Genetics markers associated with refractory epilepsy in mexican patients

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Abstracts: More than 30% of patients develop drug refractory epilepsy (DRE). The Temporal Lobe Epilepsy (TLE) with or without Hippocampal Sclerosis (HS) tends to be refractory. For many years DRE has been related with inherited or acquired alterations in multidrug transporters, but these haven't been adequate to explain the molecular and cellular mechanisms of drug-resistance. In recent years, it has been suggested that the influence of some microtubule-associated proteins (MAPs) and molecules of inflammation may contribute with recurring neuronal excitability episodes. Tau protein is a MAP that in increased levels or in the hyperphosphorylated state has been associated with the probability of seizure attacks. In the same way, Tumor Necrosis Factor-α (TNF-α) enhances neuronal excitability and the Cyclooxygenase-2 (COX-2) has been related with antiepileptic drug transport. Objective: Due to the impact of this molecules in seizures and because DRE could be a polygenic disorder, we analyzed the risk effect of 14 single nucleotide polymorphisms (SNPs) in Tau, GSK-3β, HSP-70, TNF-α, COX-2 and complement receptor 1 (CR1) genes Method: Genotyping was performed by

qPCR, in 35 clinically diagnosed patients with DRE (23 HS cases and 12 TLE without HS) and 47 controls of Mexican population. Allelic frequencies were analyzed with a Chi-square test. Results: The SNP located in CR1 gene could be linked with a risk increased of DRE (p=0.03). When we analyzed allele frequencies separating patients with or without HS, we found a possible association of CR1 and HSP-70 SNPs that would be exclusive in patients with HS (p=0.007, p=0.03). Also, in TLE group without HS, we found one SNP in Tau gene and another in GSK-3β gene that may be associated as differential markers between patients with HS and without.

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Poster

606. Human Studies of Epilepsy

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Program#/Poster: 606.25/N10

Topic: C.07. Epilepsy

Title: Empirical mode decomposition as a seizure detection tool

Authors: *K. R. ASHMONT[1], R. WAHNOUN[2,1], P. ADELSON[2,1], S. HELMS TILLERY[1], B. GREGER[1];
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Abstracts: Focal resection surgery can offer long term control of seizures for people with drug resistant epilepsy. Successful surgery relies on the accurate identification of where and when the patient's seizures start. The current identification "gold standard" involves the implantation of electrocorticography (ECoG) grids below the skull, followed by continual monitoring of cortical activity for up to two weeks. During this time, all of the information collected is visually reviewed by highly trained epileptologists. This process requires extensive time and is highly subjective. Here, we propose the use of empirical mode decomposition (EMD) as a seizure detection tool to supplement the visual review process. EMD is an adaptive sifting algorithm that breaks complex waveform data into intrinsic mode functions (IMF). Sifting begins by connecting local maxima and local minima with two cubic spline interpolations, generating maxima and minima envelopes of the data x_i (t). The mean of these envelopes m_j is then calculated and subtracted from the data h_j=x_i (t)-m_j. h_j is considered an IMF if: (1) the number of extrema

and zero crossings are equal or differ at most by one, and (2) m_j is zero at any point. If h_j does not meet the IMF criteria, h_j is re-sifted h_j=h_((j-1))-m_j with j = j+1 until it does, then ⟦IMF⟧_k=h_j. If h_j does meet the IMF criteria, then ⟦IMF⟧_k is subtracted from the data, leaving residual data x_(i+1) (t)=x_i (t)-⟦IMF⟧_k. The residual data is then reintroduced to the sifting process. The sifting process continued until the peak to peak amplitude of x_(i+1) (t) is less than 20 μV and its frequency is below 2 Hz. We applied EMD to recordings performed using clinical ECoG grids and sampled at 500 Hz. EMD was applied to 5 s windows of the ECoG data, and this window was moved through the data with a step size of 3 s. We marked a seizure as detected when an increase in the number of IMFs lasted for at least four consecutive windows, with the seizure onset time taken as the median of the first of these windows. EMD seizure detection within 1 min of the seizure onset time as noted by the epileptologist was considered a true-positive. We were able to successfully detect six of six seizures from one patient with the EMD seizure onset time an average of 8.67 s from the epileptologist noted onset time. Three of the EMD detections occurred prior to, while three occurred after the seizure onset time noted by the epileptologist. Further, the post-ictal state was indicated by a decrease in the number of IMFs and was useful in excluding to false-positive EMD seizure detections. In future work we will optimize the EMD parameters and expand testing to all electrodes in the grid and to additional patients.

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Poster

606. Human Studies of Epilepsy

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Program#/Poster: 606.26/N11

Topic: C.07. Epilepsy

Support: Grant-in-Aid for Specially Promoted Research (No.20001008)

Title: Changes in extracellular metabolites during cooling of the epileptogenic cortex in human epilepsy; an intraoperative microdialysis study

Authors: *T. INOUE[1], S. NOMURA[1], M. FUJII[1], Y. HE[1], Y. MARUTA[1], H. KOIZUMI[1], E. SUEHIRO[1], H. IMOTO[1], T. YAMAKAWA[2], M. SUZUKI[1];
[1]Dept. of Neurosurg., Yamaguchi Univ., Ube, Japan; [2]Fuzzy Logic Syst. Inst., Kitakyushu, Japan

Abstracts: Introduction: Although focal brain cooling has been a candidate for epilepsy treatment, the therapeutic feasibility remains to be elucidated. Of particular importance in this regard is to clarify cooling-induced neurovascular and metabolic changes. To address this issue, cerebral blood flow (CBF) and microdialysis measurements were performed during focal brain cooling of epileptogenic zone in epileptic patients. Methods: A PID-controlled, thermoelectrically-driven cooling device (cooling area: 40×40 mm for cortex, 10x10mm for hippocampus) was intraoperatively applied on the cortical or hippocampal epileptogenic foci in intractably epileptic patients, and CBF measurement (n=6) and microdialysis (n=9) were performed. Findings: Although CBF in the epileptic foci was transiently reduced during 15°C cooling, it was reversible and immediately recovered following rewarming. Cooling-induced metabolic changes were qualitatively similar across patients. Of glycolytic metabolism, lactate levels were significantly reduced ($p<0.01$) during cooling and rewarming period, while glucose and pyruvate levels remained relatively unaffected. Extracellular levels of glutamate, gamma-aminobtyric acid (GABA) and grycerol were significantly decreased during cooling ($p<0.05$) with the effect remained even after rewarming ($p<0.05$). Interpretation: It has been reported that autoregulation and metabolic coupling in epilepsy are impaired. This study showed, for the first time, coupling of CBF and metabolism with cooling of the epileptogenic zone to 15°C. Decreased lactate, grycerol and glutamate levels suggested neuroprotective effects of cooling. Together with the previous our investigations in animals, the results suggested a feasibility of hypothermal device-based therapy.

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Poster

606. Human Studies of Epilepsy

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Topic: C.07. Epilepsy

Support: O. A. is funded by a grant from the Israeli Ministry of Science, Technology and Space

Title: Deviations from critical dynamics in inter-ictal epileptiform activity

Authors: *O. ARVIV[1], L. SHEINTUCH[2,3], M. MEDVEDOVSKY[5], A. GOLDSTEIN[1,6], A. FRIEDMAN[2,3,4], O. SHRIKI[4,3];

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Abstracts: Epilepsy is associated with dynamic changes in the excitation-inhibition balance (EIB). Characterizing and monitoring these changes is of high importance for diagnosis and treatment of the disease. Recently, several studies demonstrated that a healthy cortex displays critical dynamics, namely, it maintains a continuous balance of excitatory and inhibitory forces, preventing premature termination or explosive growth of neuronal activity. This critical state is characterized by scale-free cascades of activity, termed neuronal avalanches. Here we quantitatively assessed deviations from critical dynamics by applying the neuronal avalanche metrics to resting-state electroencephalography (EEG) and magnetoencephalography (MEG) recordings from epilepsy patients and healthy control subjects. We found that periods of inter-ictal epileptiform activity (IEA) display super-critical dynamics. This was manifested in the shape of the cascade size distribution, which deviated from the expected power law and displayed a prominent 'bump' over large avalanches, as well as by an increase in the 'branching parameter', which represents the gain of the system. In some patients, deviation from critical dynamics was evident from the full recording. In others, it was evident only after separating the data into periods containing IEA, as compared with quiet periods, which displayed normal critical dynamics. Furthermore, in some patients the avalanche metrics were predictive of the level of IEA, as measured by the number of inter-ictal spikes in a given time window. In such cases, periods with high incidence of IEA can be detected without resorting to spike detection. In addition to looking for differences in time, we also looked for difference in space. Applying source analysis allowed us to compare the known epileptic foci (identified by other methods) to other cortical regions. This comparison revealed clear differences in terms of the avalanches metrics, again indicating super-critical dynamics in the epileptic foci. These findings demonstrate a novel approach for assessing deviations from EIB in both space and time based on non-invasive recordings in epilepsy patients.

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Poster

606. Human Studies of Epilepsy

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Program#/Poster: 606.28/O1

Topic: C.07. Epilepsy

Title: Brain metals: Methods of analysis and their role in epilepsy

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Abstracts: The human brain is abundant in metals which are an essential part of brain biochemistry, structure and, by extension, cognitive functions. Changes to the level of metals in the brain occur in typical brain development and aging, but may also result in, or be the consequence of, neurological conditions. Some conditions are well known to involve etiologies of large shifts in brain metal distribution and concentrations (both increases and deficiencies). Examples include: Menkes disease (Cu deficiency), Wilson's disease (Cu accumulation) and neuroferritinopathy (Fe accumulation). However, it is not always recognized that many common conditions may have forms that are linked to changes in brain metals and metal-metabolism. In this presentation we focus on the possible roles of metals in epilepsy. These include idiopathic and pharmacoresistant epilepsies. We survey historical and recent evidence of "deficiencies" and "surplus" in various metals, as (i) possible causal mechanisms and (ii) the results of seizures. We further describe the historical advancements in methodologies used to examine brain metals, from histology to neuroimaging and novel optical and x-ray techniques. These include recent examples of synchrotron x-ray fluorescence imaging from our laboratory. Finally, we suggest that multi-scale, spatial and temporal characterizations of metal distributions in the brain may be critical for developing treatments for certain forms of epilepsy as well as for a deeper understanding of cognitive function in the healthy brain.conditions.

Disclosures: **L.K. Frutos:** None. **L.K. Frutos:** None. **E.L. Ohayon:** None. **A. Lam:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.01/O2

Topic: C.10. Trauma

Support: NIH R01NS075162

Title: Mild traumatic brain injury in adult mice induces marked neurogenesis in the dentate gyrus

Authors: *T. L. NIEDZIELKO[1], P. C. PUGH[2], J. H. CHANCEY[2], L. OVERSTREET-WADICHE[2], C. L. FLOYD[1];
[1]Physical Med. & Rehab, [2]Neurobio., Univ. Alabama, Birmingham, BIRMINGHAM, AL

Abstracts: The United States Centers for Disease Control and Prevention estimate that 1.7 million persons per year sustain a traumatic brain injury (TBI) and approximately 75% of these TBIs are forms of mild TBI (mTBI). In moderate/severe models of TBI, neurons throughout the hippocampal formation are particularly vulnerable to injury. The dentate gyrus (DG) of the hippocampus undergoes granule cell (GCs) neurogenesis throughout adulthood; however, the contribution of neurogenesis to DG function is poorly understood. As pathophysiological stimuli such as seizures and brain lesions can alter GC neurogenesis, we hypothesize that mTBI alters neurogenesis and these alterations in the hippocampus may have significant consequences for acute and long-term hippocampal network activity. Thus, this study utilized adult male transgenic mice with enhanced green florescent protein expressed under transcriptional control of the mouse proopimelanocortin gene promoter (POMC-GFP) to identified newborn GCs. Age matched adult male mice were randomly assigned to mTBI or sham control groups. Animals received mTBI via a 100g weight dropped from 60cm onto a 1.25cm metal disk attached to the skull. Sham animals received all surgical procedures except the weight drop. Animals were euthanized on post-TBI day 2, 6, or 10 and brains were removed for serial sectioning and histological analysis including cresyl violet (nissel substance), expression of GFP, Ki-67 and doublecortin expression. Expression of histological markers was quantified using unbiased stereology. Acute hippocampal slices were taken from separate animals for electrophysiological assessment of intrinsic properties of mature GCs in whole-cell patch clamp. We found no differences between mTBI and sham groups in neuronal number as indicated by cresyl violet histology in the dentate gyrus. However, the number of GFP-positive cells was significantly increased in the mTBI groups versus sham at all post-TBI time points, with corresponding increases in the proliferative markers Ki-67 and doublecortin. There was also an increase in the total dendrite length of newborn GCs in the mTBI group at the 2 day time point. Whole-cell patch clamp recording revealed that mTBI alters the intrinsic properties of mature GCs. Our results demonstrate that mild TBI induces negligible DG cell loss yet profoundly enhances neurogenesis and alters the physiology of mature DG neurons. These alterations could represent a novel mechanism that could contribute to the persistent behavioral impairments seen after mTBI. Supported by R01NS075162.

Disclosures: **T.L. Niedzielko:** None. **C.L. Floyd:** None. **L. Overstreet-Wadiche:** None. **P.C. Pugh:** None. **J.H. Chancey:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.02/O3

Topic: C.10. Trauma

Support: Savo Cultural Foundation, Finland

Academy of Finland

Title: T cell infiltration into the brain after traumatic brain injury is associated with injury severity

Authors: *X. E. NDODE-EKANE, L. MATTHIESEN, A. PITKÄNEN;
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Abstracts: Traumatic brain injury (TBI) is the most common cause of permanent disability and death in people under the age of 40. About 11% of TBI patients develop posttraumatic epilepsy (PTE). However, the mechanisms that underlie the development of PTE remain unclear. T cell infiltration into the brain parenchyma is a prominent inflammatory response following TBI. Reports suggest that T cells may contribute to the progression of epileptogenesis and the development of post-traumatic epilepsy (PTE). Our objective here is to understand the dynamics of T cell infiltration into the brain after TBI, and the functional significance of T cell invasion to post-traumatic epileptogenesis and progression of neurological deficits. To achieve this, TBI was induced in adult rats using lateral fluid-percussion injury. A composite neuroscore test was performed to assess the severity of somato-motor deficits, at 24 h, 2 d, 4 d, and 7 d after injury. Thereafter, animals were sacrificed by transcardiac perfusion with 4% paraformaldehyde. T cells were identified in brain sections by immunohistochemical staining with an antibody against the T cell antigen CD3. The number of T cells was stereollogically assessed in the ipsilateral cortex, hippocampus and thalamus. Analysis of sham-operated animals revealed very low T cells counts in all areas. However, the number of T cells in all regions increased dramatically at 24 h post-injury. At 2 d the number was even higher, being 154% of that at 24 h post-TBI. At 4 d and 7 d, their number dropped to 77% and 10%, respectively, of that at 24 h. The highest number of T cells was observed in the cortex when compared with other areas ($p<0.05$). Visual analysis of brain sections at the chronic phase (3 months post-TBI) showed a resurgence of T cells particularly in the ipsilateral thalamus and cortex. There was a strong inverse correlation between the total number of T cells at the acute phase (24 h, 2 d, 4 d and 7 d time-points) and the neuroscore at 24 h or 2 d post-injury reflecting the severity of injury ($p<0.05$). This data suggest

that the severity of post-traumatic neurological deficits can predict the level of post-traumatic T cell response. The increase in T cell number in the early days following TBI and in the chronic phase suggest a time window during which T cells can influence epileptogenesis.

Disclosures: X.E. Ndode-Ekane: None. **L. Matthiesen:** None. **A. Pitkänen:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.03/O4

Topic: C.10. Trauma

Support: Supported by the Department of Neurosurgery, Medical College of Wisconsin and VA Medical Research.

Title: Neuronal death and glial activation in rat organotypic hippocampal slice cultures exposed to a blast injury

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Abstracts: Blast induced traumatic brain injury (bTBI) is a substantial and significant injury that has escalated amongst military personnel. Understanding the molecular events and cascades underlying cell death and glial response are essential to elucidate potential future therapeutic strategies. The aims of this investigation are to characterize the blast response in organotypic hippocampal cultures (OHCs) and to illuminate the microglia and astrocyte activation in response to shock wave damage. The hippocampi were isolated from 7-10 day old rats, dissected into 400 μm sections, and grown using the standard interface method. At 8 days *in vitro* OHCs were exposed to a shock wave overpressure of 150 kPa (low) or 270 kPa (high) using a vertical open-ended helium driven shock tube. Cell death was analyzed at various time points post injury via propidium iodide (PI) uptake. PI staining was quantified by measuring the mean pixel intensity (MPI) of cornu Ammonis 1 (CA1) and dentate gyrus (DG) hippocampal regions using MATLAB software (Mathworks, Inc., Natick, Massachusetts). At 3 or 7 days post injury OHCs were fixed with 4% paraformaldehyde and processed for immunohistochemistry against neuronal (beta III Tubulin; Tuj1), microglia (ionized-calcium-binding adapter molecule 1; IBA-1), and

astrocyte (glial fibrillary acidic protein; GFAP) markers. Following immunostaining, microglia activation was assessed based on changes from resident ramified to rounded amoeboid-like appearance, while astrocyte activation was analyzed by intensity of GFAP fluorescence. Compared to control sections the shock wave-exposed OHCs exhibited significant increase in PI uptake both in high and low groups at all time points post injury. Cell death gradually increased in the low blast group over 7 days. OHCs exposed to high blast exhibited an initial dramatic increase in cell death until it plateaued at approximately 24 hours post-injury. MPI was significantly different between high and low groups only at earlier time points. Tuj1 immunostaining indicated that the majority of blast-evoked cell death was restricted to neurons which co-expressed PI and Tuj1. Preliminary data suggest that at 3 days post injury the percentage of activated microglia was significantly higher in the high blast group compared to control OHCs. Our preliminary data also imply significant astrocyte activation in OHCs exposed to a high blast at 7 days post injury. Together our data suggest that our novel *in vitro* bTBI model is useful tool to study cellular and molecular changes in neuronal tissue following blast exposure.

Disclosures: **A.P. Miller:** None. **A. Shah:** None. **B.V. Aperi:** None. **M.D. Budde:** None. **F.A. Pintar:** None. **S. Kurpad:** None. **B.D. Stemper:** None. **A. Glavaski-Joksimovic:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Program#/Poster: 607.04/O5

Topic: C.10. Trauma

Support: NIH R21 AG042016

Alzheimer's Association IIRG-11-202064

Title: Effect of aging on cognitive outcome and neuroinflammatory response after traumatic brain injury

Authors: *A. CHOU, J. M. MORGANTI, S. ROSI;
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Abstracts: Traumatic Brain Injury (TBI) can alter neuronal function and inflammatory responses even beyond the site of injury. TBI outcomes are worse in elderly patients including higher fatality rates and greater severity of TBI-related disabilities. In various animal models

recapitulating TBI, aging predisposes exacerbated neuronal loss, inflammation, and motor function acutely. However, no mechanism for age-related exacerbation of TBI has been identified. In the current study we investigated the effect of aging on TBI-induced cognitive deficits and neuroinflammatory response. TBI was induced by controlled cortical impact over the right parietal cortex in 3 and 18 month old male mice. Thirty days after injury, hippocampal-dependent learning and memory functions were measured using the radial arm water maze (RAWM) which consists of eight arms with an escape platform located at the end of one arm. Our data demonstrates that both age and TBI increases the number of errors that the animal commits to locate the escape platform when compared to their respective young and sham controls. We further characterized changes in synaptic plasticity markers in the ipsilateral hippocampus. Many studies have shown that reducing the pro-inflammatory response can alleviate TBI-induced outcomes. While acute pro- and anti-inflammatory responses are exacerbated by age, it is unclear if the pro-inflammatory response is prolonged or if there is diminished anti-inflammatory response over time in old animals as compared to young animals. We characterized the inflammatory response of the injured brain in both young and old animals by flow cytometry and quantitative PCR on isolated microglia/macrophages from the injured hemisphere at a sub-acute time point 7 days after injury. Our results demonstrate that 7 days after injury there was a significant decrease in anti-inflammatory cytokine and M2 macrophage expression in old animals compared to the young. These data suggest an imbalance in the regulation of inflammation in the aging brain which may sustain a proinflammatory environment after injury.

Disclosures: **A. Chou:** None. **J.M. Morganti:** None. **S. Rosi:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Program#/Poster: 607.05/O6

Topic: C.10. Trauma

Support: New Jersey Commission Brain Injury Research-CBIR12MIG011

Title: Effects of PTEN inhibition on neuronal morphological changes and survival after traumatic brain injury

Authors: ***C. LIANG**[1], K. ELMORSHEDY[1], P. SWIATKOWSKI[1], T. M. KAZDOBA[1], I. NIKOLAEVA[1], M. BEAMER[2], D. F. MEANEY[2], G. D'ARCANGELO[1], B. L. FIRESTEIN[1];

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Abstracts: In response to traumatic brain injury (TBI), neuronal tissues are damaged by rapid and transient mechanical deformation, followed by secondary injury due to excess glutamate release, which causes morphological changes in neurons. These changes include focal swellings in dendrites, or varicosity formation, and spine retraction and re-emergence. Previous studies in the Firestein laboratory showed that a large number of small varicosities are neuroprotective, while large varicosities are detrimental to neuronal survival. In this study, we aim to test how alteration of the mTOR pathway, by deleting or inhibiting PTEN, contributes to changes in neuronal morphology, neuronal survival, and neural circuitry after TBI. We mimic the primary mechanical injury and secondary excitotoxicity by utilizing an *in vitro* stretch injury model or NMDA treatment. We first examined varicosity formation in hippocampal neuronal cultures from NEX-PTEN mice after NMDA treatment, and we observed a decrease in the percentage of neurons with varicosities in PTEN knockout mice compared to littermate controls. In contrast, we did not see a significant difference in varicosity size between each genotype. In parallel, we used an *in vitro* stretch injury model to induce mechanical injury to rat hippocampal neurons. We compared the percentage of neurons that formed varicosities at different time points after stretch and varicosity size in cultures pre-treated with bpV(phen), a PTEN inhibitor, and control cultures. We found that PTEN inhibition causes a time-dependent two-phase change in varicosity formation: during the initial phase after stretch injury, neurons with PTEN inhibited had a larger number of smaller varicosities than observed in control neurons, while in the latter phase during our observation, PTEN inhibition caused a higher percentage of neurons with varicosities, which were larger in size than observed in control cultures. Together, our studies provide new evidence for a role of PTEN and the mTOR pathway in neuronal recovery from TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

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Topic: C.10. Trauma

Support: the research fund of the State Key Laboratory of Trauma, Burn and Combined Injury of China SKLZZ200805

Title: Expression and effects of c-Ski in mice brain after traumatic brain injury

Authors: *Y. ZHOU, P. LI, X. CHEN, Y. PENG, Z.-A. ZHAO, Y.-L. NING, N. YANG, Y. ZHAO, X.-Y. CHEN, R.-P. XIONG;
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Abstracts: Traumatic brain injury (TBI) causes neuronal apoptosis, inflammation, and reactive astrogliosis, which contribute to secondary tissue loss, impaired regeneration, and the leading cause of death and disability. c-Ski, the cellular homologue of v-Ski, was a multifunctional modulator that involved in proliferation, apoptosis, inflammation, fibrosis, and cell differentiation. However its distribution and function in the central nervous system (CNS) during TBI are not well understood. In this study, using immunohistochemistry in a moderate controlled cortical impact model of mice, we found that the expression of c-Ski was observed mainly in the cortex surrounding the injury site and reached its peak on day 3 and then significantly decreased. And c-Ski was also expressed at relatively low levels in the ipsilateral hippocampus, thalamus and contralateral cerebral cortex. Immunofluorescence double-labeling showed that c-Ski was localized almost exclusively in neuronal cells. To further investigate the function of c-Ski, we found that increasing local Ski expression by gene transfer in the above model reduced lesion volume by increasing quantity of neurons and decreasing inflammation by histological assessments. In addition, Consistent with the histological changes, the neurological deficit scores in the gene-transfer mice were significantly lower than that of control on day 3. Together with our data, we speculate that c-Ski play an important role in CNS pathophysiology after TBI and is a prospective new target for therapeutic approaches to TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

Support: CDMRP Grant W81XWH-11-2-0127

CDMRP Grant W81XWH-08-2-0018

Title: Phosphorylation of Tau after blast exposure: A potential predisposition to Alzheimer's-like pathology

Authors: *P. ARUN, D. M. WILDER, A. A. EDWARDS, Y. WANG, I. D. GIST, J. B. LONG; Ctr. for Military Psychiatry and Neurosci., Walter Reed Army Inst. of Res., Silver Spring, MD

Abstracts: Blast-induced traumatic brain injury (TBI) is one of the major disabilities in service members returning from recent military operations. Blast-induced TBI is associated with acute and chronic neuropathological and neurobehavioral deficits. Epidemiological studies indicate that brains of 30% of victims who die acutely following TBI have Aβ plaques, a pathological feature of Alzheimer's disease (AD), which suggests that TBI may predispose to AD, although to date this notion remains somewhat speculative. *Tau* protein, phosphorylated at serine396 (S396), is rich in paired helical filaments which form neurofibrillary tangles (NFTs) observed in the brains of patients with AD. The number of NFTs is tightly linked to the degree of dementia, indicating that the formation of NFTs may underlie and contribute to neuronal dysfunction. In this study, rats were exposed to single or double blasts using a shock tube. Brain regions were collected at different intervals for Western blotting. Preliminary results indicate that phosphorylation of *Tau* protein occurs preferentially at S396. S396 phosphorylation of *Tau* varied in different regions of the brain and the degree of phosphorylation increased with number of blast exposures. Increased S396 phosphorylation occurred acutely after blast exposures and subsequently returned towards normal levels by day 28, which at this stage did not positively correlate with the accumulation of amyloid precursor protein (APP). These results indicate that acute *Tau* protein phosphorylation at S396 and chronic accumulation of APP in the brain after blast exposure may predispose to Alzheimer's-like disease.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

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Program#/Poster: 607.08/O9

Topic: C.10. Trauma

Support: Academy of Finland

Center for International Mobility

Finnish Graduate School of Neuroscience

Title: Quantitative microscopic analysis of morphology of individual neuronal mitochondria in the cortex of anesthetized mice under injury

Authors: *M. KISLIN[1], E. PRYAZHNIKOV[1], E. LIHAVAINEN[2], R. AFZALOV[1], D. TOPTUNOV[1], A. S. RIBEIRO[2], L. KHIROUG[1];
[1]Univ. of Helsinki, Helsinki, Finland; [2]Tampere Univ. of Technol., Tampere, Finland

Abstracts: Functions of mitochondria are directly related to their morphology, and morphological abnormalities such as fragmentation have been frequently reported in disease models *in vitro*. Using intravital two-photon microscopy, we imaged the dynamics of neuronal mitochondrial morphology under traumatic conditions in the brain of anesthetized Thy1-mitoCFP mice and quantified these dynamics utilizing an automated analysis method based on supervised learning. To test the sensitivity of mitochondrial morphology within cortical layer 1 to injury, we measured the degree of mitochondrial fragmentation in three models: i) mild photodamage (MPD); ii) focal laser-lesion (FLL); and iii) Rose Bengal photosensitization (RBPS). To access the time course, changes in mitochondrial morphology were analyzed during acute (3 hours), subchronic (2, 4, 7 days) and chronic (3 weeks) phases. MPD was induced in a small volume by exposing the tissue to approximately 50-fold higher light energy than during imaging; FLL was produced by targeted laser light eliminating individual branches of the apical dendrites in a controlled volume of somatosensory cortex; RBPS was achieved by i.v. injection of Rose Bengal followed by exposure to green light. We found that MPD resulted in a rapid mitochondrial fragmentation in the high-exposure region. Surprisingly, the damage did not extend to the surrounding tissue and recovery of mitochondria morphology occurred only 4 days after MPD. FLL resulted in a complete loss of the CFP fluorescence at the lesion site and induced mitochondria fragmentation at the perilesion site that recovered during one week. Spontaneous dendritic regrowth into the lesion site occurred during 7-14 days and was accompanied by recovery in mitochondrial morphology. RBPS rapidly produced a core with severe mitochondrial fragmentation that did not show any recovery over 3 weeks. We observed some recovery of mitochondrial morphology after 3 weeks but only in relatively small parts of the remote area. Finally, we used dual TG mouse line to evaluate the temporal relationship between mitochondrial morphology and the dendritic structure during the first 3h after RBPS. Interestingly, structural changes in neurons, such as blebbing, were always observed with some delay after the mitochondrial fragmentation occurred first in response to RBPS. We propose that microscopic imaging with supervised learning-based analysis of neuronal mitochondrial morphology *in vivo* reveals a pivotal role of mitochondria in neuronal survival or death, and can therefore be used as a sensitive readout suitable for assessing the effectiveness of neuroprotective therapies in preclinical studies.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

Support: Center for Neuroscience and Regenerative Medicine

Title: Progression of myelin pathology in TBI with traumatic axonal injury of the corpus callosum

Authors: *A. J. MIERZWA[1,2], C. M. MARION[1,2], G. M. SULLIVAN[1,2], D. MCDANIEL[1,2], R. C. ARMSTRONG[1,2];
[1]Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]Ctr. for Neurosci. and Regenerative Med., Bethesda, MD

Abstracts: Traumatic brain injury (TBI) from impact-acceleration forces often results in post-concussive symptoms that persist into a chronic disease phase, even in patients diagnosed initially as mild TBI. To better understand the progression to chronic disease, we examined acute through chronic white matter pathology in mice using a concussive TBI model of traumatic axonal injury. TBI was produced in adult male C57BL/6J mice by controlled impact onto the skull at bregma. Animals were perfused at multiple time points between 3d-6wks post-TBI and tissues were processed for either electron microscopy or immunohistochemistry. At all times post-TBI, degenerating axons were evident mainly in the corpus callosum, particularly over the lateral ventricles. Degenerating axons were distributed among intact fibers-- modeling the diffuse pattern of traumatic axonal injury in TBI. Axon diameters were reduced among the overall population of remaining axons. Demyelination may contribute to functional deficits and potentially leave denuded axons vulnerable to further damage. After TBI, demyelination of intact axons significantly increased at 3d followed by remyelination evident at 1 wk. In addition, abnormal myelin figures were prevalent at all post-TBI time points, yet extremely rare after sham surgery. Redundant myelin sheaths formed from myelin that collapsed back onto itself as the axon degenerated or from excessively long outfoldings of myelin,which may be indicative of myelin synthesis. For comparison with an example of demyelination and remyelination, we examined the number of redundant myelin figures in the cuprizone model of widespread corpus

callosum demyelination. Surprisingly, TBI mice had 10-fold more redundant myelin figures than observed during either the demyelination or remyelination phase of the cuprizone disease course. Due to the dispersed nature of demyelination and axon degeneration in TBI, oligodendrocytes may increase myelin synthesis to remyelinate intact axons or the excessive myelin formed by reflect dysregulation in maintaining multiple sheaths among a cohort including intact and degenerating axons. Excessive myelin outfoldings may increase myelin debris, which can stimulate microglial activation. Immunohistochemistry demonstrated microglial activation and reactive astrogliosis that persisted 6 wks post-TBI. Further studies will be important to determine the contribution of this myelin pathology to persistent white matter neurodegeneration and neuroinflammation after TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

Support: KSCHIRT- 12-16A

Title: Early M1 and M2 response in hippocampus following traumatic brain injury

Authors: *M. A. ANSARI[1], K. N. ROBBERTS[2], T. L. SUDDUTH[3], D. M. WILCOCK[3], S. W. SCHEFF[2];
[1]Sanders-Brown Ctr. on Aging, Univ. Kentucky, Lexington, KY; [2]Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY; [3]Sanders-Brown Ctr. on Aging, Uneversity of Kentucky, Lexington, KY

Abstracts: Traumatic brain injury (TBI) triggers a complex series of inflammatory responses that are categorized into two phases, M1 (pro-inflammatory) and M2 (anti-inflammatory). M1 participates in multiple secondary injury cascades (SIC), while M2 maintains cell/tissue homeostasis and contributes to repair/regenerative assets. The terms M1 and M2 are derived from subsequent classical and alternative activation of macrophages, including microglia, astrocytes, and neutrophils. Following TBI, the blood brain barrier is breached allowing blood born immune cells to infiltrate the brain. Modulating the neuroinflammatory response may be

part of a rational therapy to enhance recovery following TBI. The present study was undertaken to investigate the very early neuroinflammatory response in the hippocampus, a brain area that is involved in a variety of cognitive functions and is affected after TBI. Young adult rats were killed at 2, 4, 6, 10, & 24h following a moderate unilateral cortical impact injury. The hippocampus was analyzed for both the phenotype (gene expression) and protein level of cytokines of M1 (IFNγ, TNFα, IL-1β, & IL-6) and M2 (IL-4, IL-10, & IL-13). Phenotypic expression of M1 cytokines (IFNγ, IL-6, IL-1β, & TNFα) was significantly elevated as early as 2h. Maximum expression of IFNγ, TNFα, & IL-1β genes occurred at 2-6h, while in IL-6, it was between 4-10h. Although values of M1 phenotype began to decline at 24h, they were elevated compared to sham operated animals. The level of IFNγ protein was not significantly altered while other M1 cytokines were significantly elevated within 2h post injury. The maximum surge of various M1 cytokines was between 2 and 6h, although not identical [TNFα (2h), IL-1β (6h), & IL-6 (4-6h)]. Levels of M1 proteins remained significantly higher than sham animals during the initial 24h post trauma. The M2 response was unlike the M1 response, with maximum levels of M2 observed at 24h post injury. Level of IL-10 was the only one that initially elevated at 6h. Like the protein levels, maximum gene expression in all the M2 cytokines was at 24h. The M1 response arises rapidly after TBI and it might overwhelm the early, relatively smaller, and transient M2 response. Our data suggests that a treatment which is able to enhance the M2 response, should be initiated early (before 10h), which would not only minimize inflammation mediated SIC, but also improve recovery after TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

Support: NJCBIR grant CBIR12MIG011

Title: MTORC1 signal activation in the mouse hippocampus after traumatic brain injury

Authors: *I. K. NIKOLAEVA[1], B. CROWELL[2], J. VALENZIANO[4], D. MEANEY[4], G. D'ARCANGELO[3];

[1]Grad. Program for Mol. Biosci., [3]Cell Biol. & Neurosci., [2]Rutgers, the State Univ. of New Jersey, Piscataway, NJ; [4]Univ. of Pennsylvania, Philadelphia, PA

Abstracts: The mTORC1 signaling pathway mediates many aspects of cell growth and regeneration, and is upregulated after moderate to severe traumatic brain injury (TBI). The significance of this increased signaling event for recovery of brain function is presently unclear. We hypothesized that different cell types experience increased mTORC1 signaling after TBI, and that cell type determines whether this event is beneficial or detrimental to recovery. To test this hypothesis, in this study we established the time course and the cell specificity of mTORC1 signaling activation in the mouse hippocampus after moderate controlled cortical impact (CCI). We then blocked the peak of mTORC1 signaling by injecting rapamycin 1 hour after CCI. Behavioral studies indicated that this treatment improves performance in cognitive tests several weeks after injury. Our work suggests that the early peak of mTORC1 signaling after TBI occurs predominantly in neurons and it is deleterious to brain function. Suppression of this biochemical event, achieved shortly after injury has occurred, may represent an effective form of intervention to improve recovery in human patients. This work is supported by multiprogrammatic grant CBIR12MIG011 from the New Jersey Commission on Brain Injury Research (G.D. and D.M.).

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.12/P1

Topic: C.10. Trauma

Support: NIH Grant NS038079

UCSF/BASIC pilot funds

Title: TBI influences systemic inflammation by mediating differentiation of myeloid cells

Authors: *S. LEE, N. SINGHAL, J. SACRAMENTO, A. LIN, J. C. BRESNAHAN, M. S. BEATTIE;
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Abstracts: Traumatic brain injury (TBI) leads to pro-inflammatory responses in both CNS and peripheral organs. The inflammatory responses may be involved in the subsequent development of clinical systemic inflammatory response syndrome, which eventually causes immune dysfunction and increases susceptibility to infection in chronic TBI patients. We examined the influence of TBI on systemic and CNS inflammation. Using a CCI-TBI model in C57BL/6 WT mice, we first examined the effects of TBI on the activation of peripheral immune cells. Flow cytometric analysis showed that 7 days after TBI, $CD11b^{high}$ cells were significantly increased in the circulation compared to $CD11b^{low}$ cells. Most of these cells were $Ly6C^{high}$, inflammatory monocytes, suggesting TBI stimulates the differentiation of myeloid cells.TBI also significantly induced mature myeloid cell populations ($CD11b^{+}F4/80^{+}$ macrophages and $CD11b^{+}CD11c^{+}$ dendritic cells) in the circulation at 7 days post injury. We also investigated the effects of TBI on trafficking of inflammatory myeloid cells in CNS. Consistently, we found that $CD45^{+}F4/80^{+}$ and $CD45^{+}CD11c^{+}$ cells were significantly increased in the injured brain at 7 days by immunostaining analysis. We also demonstrated significantly increased gene expression of proinflammatory mediators by real time quantitative RT-PCR in the spleen. Finally, *in vitro* studies using myeloid cells isolated from blood from WT mice demonstrated potent effects of LPS on differentiation of immature myeloid cells to mature myeloid inflammatory cells ($CD11b^{+}F4/80^{+}Ly6C^{high}$ cells). In addition, LPS also increased $Ly6C^{+}$ cells in splenocytes. Interestingly, LPS down-regulated CD3ζ in CD3 lymphocytes, suggesting proinflammatory response impedes T cell function. Together, our new findings suggest that TBI systemically induces pro-inflammatory responses by modulating myeloid cell differentiation in the circulation and subsequently affects leukocyte trafficking into the CNS. In addition, proinflammatory responses may contribute to T-cell dysfunction in spleen, and subsequent susceptibility to infections. Therefore, modulating immune responses may serve as an important therapeutic target for TBI patients.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

Support: NICHD Grant HD061963

Title: Decreased axonal transport following pediatric traumatic brain injury

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Abstracts: Axonal injury is a common occurrence in pediatric TBI and has been associated with long-term cognitive and behavioral deficits. In the acute post-traumatic period (hours to days), injured axons may have transport and/or conduction deficits, while in the chronic phase degeneration of axons has been observed. We have reported accumulation of amyloid precursor protein (APP) indicative of impaired axonal transport in the acute phase after closed head injury in an 11-day-old rat; immunoreactivity for APP is typically absent by 7 days after injury, thereby rendering it useless as a marker of transport deficits in the chronic phase (weeks to months). To determine the presence of axonal transport deficits in this post-traumatic period, 11-day-old rat pups were subjected to a moderate brain injury over the left parietal cortex, and received intracerebral injections of the retrograde tracer, fluorogold (FG, 2% in ddH2O) into either the left cortex (ipsilateral to the injury) or the right cortex (contralateral to impact site) at 2 weeks after injury; 5 days later, brains were analyzed for the presence of FG-labeled cells in the homotypic region of cortex contralateral to the site of injection. Compared to sham-injured animals, brain-injured animals had 79 % fewer labeled cells (when FG was injected into the un-injured cortex) and 64% fewer labeled cells when FG was injected into the injured cortex. Nissl staining did not reveal a loss of cells in the cortex in either hemisphere, suggesting that the decreased FG labeling was not due to a lack of cells available to take up the tracer. In previous studies, we have reported that the anti-inflammatory compound minocycline decreased the extent of traumatic axonal injury at 3 days after injury. To determine whether minocycline could also reverse axonal transport deficits in the chronic phase, a group of animals was treated with minocycline immediately following injury (90 mg/kg) and then every 12 hours for 3 days (45 mg/kg). The extent of FG-labeled cells in the cortex contralateral to the site of injection at 2 weeks post injury in minocycline-treated rats was similar to that in the vehicle-treated cohort (64 vs. 63 % compared to sham-injured animals), suggesting that acute attenuation of axonal injury may not affect transport deficits in the chronic post-traumatic period.

Disclosures: **L.A. Hanlon:** None. **J.W. Huh:** None. **D.P. Fox:** None. **R. Raghupathi:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

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Title: Decreased expression of wild-type alpha-synuclein in rat ipsilateral hippocampus after traumatic brain injury

Authors: H. Q. YAN[1,2], Y. LI[1,2], J. J. HENCHIR[1,2], X. MA[1,2], S. CARLSON[1,2], *C. DIXON[1,2]; [1]Neurosurg., Univ. of Pittsburgh, PITTSBURGH, PA; [2]VA Pittsburgh Healthcare Syst., Pittsburgh, PA

Abstracts: Traumatic brain injury (TBI) is the leading cause of death and injury-related disability among young adults. TBI can result in the disturbance of cognitive, behavioral, emotional, and physical functioning. Synucleins (Syn), a family of synaptic proteins, includes alpha-synuclein (α-Syn), which plays a pivotal role in Parkinson's disease and related neurodegenerative diseases. The native function of α-synuclein is not completely understood, but is thought to involve regulation of synaptic vesicle trafficking. While the pathological forms of α-syn are considered to be the primary targets of TBI-associated neurodegeneration, disruption of the native function of α-Syn may contribute to pathology by diminishing synaptic function. Thus, the goal of the project was to examine the effects of TBI produced on wild-type α-Syn expression at 6 hours to 8 weeks post injury. Male Sprague-Dawley rats were anesthetized and surgically prepared for controlled cortical impact (CCI) injury (4 m/sec, 2.6 mm) or sham surgery. Rats were randomly assigned TBI or sham surgery and sacrificed for Western blot analysis and immunofluorescence double labeling assay by using commercial available antibodies. Semiquantitative measurements of the hippocampal tissues from rats sacrificed at 6 hour, 1 day, 1 week, 2 weeks 4 weeks and 8 weeks after injury or sham operation (N= 6 per group per time point) that were assessed using Western blot analysis show that expression of α-Syn are decreased ipsilaterally from 6 h to 8weeks in the hippocampus ($P < 0.05$). Double-label immunofluorescent staining sacrificed at 1 week after TBI or sham for α-Syn, neuron marker NeuN and astrocytes marker glial fibrillary acidic protein (GFAP) confirmed the Western blot findings. There is no overt change of NeuN immunostaining after TBI in regions of α-Syn loss. The increased expression of GFAP represents concomitant astrogliosis. This study suggests that wild-type α-Syn protein in the ipsilateral hippocampus is decreased after TBI compared to the sham controls. Additional work is required to determine if this represents a shift toward more cytotoxic forms of α-Syn or a reorganization of synaptic vesicle trafficking after TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Program#/Poster: 607.15/P4

Topic: C.10. Trauma

Support: NJCBIR 09.003.BIR1

NJCBIR 11-3223-BIR-E-O

Title: Structural differences between granule cells and semilunar granule cells

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Abstracts: In earlier studies, we identified that semilunar granule cells (SGCs), glutamatergic neurons in the inner molecular layer with axonal projections to CA3 (Williams et al., 2007), show enhanced excitability after brain injury. SGCs receive significantly greater inhibitory inputs than granule cells and demonstrate a post-traumatic decrease in the frequency of inhibitory synaptic inputs rather than an increase observed in granule cells (Gupta et al., 2012). Here, we examined whether differences in dendritic morphology contribute to the divergent intrinsic pattern and post-traumatic plasticity of synaptic inputs between the two cell types. Whole-cell recordings from dentate neurons were obtained from acute hippocampal slices prepared 1 week after lateral fluid percussion injury (FPI) or sham-injury in young adult rats. Recorded neurons were filled with biocytin and processed for post-hoc cell identification. Neuronal reconstructions and morphometric analysis were performed on Neurolucida. Simulations were performed using NEURON. Both granule cells and SGCs showed an increase in the frequency spontaneous EPSCs one week after FPI. Frequency of sEPSCs in SGCs from sham-injured rats was significantly greater than in granule cells. Molecular layer interneurons demonstrated fewer spontaneous inhibitory inputs and a post-FPI increase in sIPSC frequency, indicating that location may not account for the differences in synaptic inputs between SGCs and granule cells. Morphometric analysis revealed a greater dendritic contraction angle in SGCs.

Although the total dendritic length was not different between the two cell types, SGCs had more numerous first and second order branches and greater dendritic length in low order branches than granule cells. However, granule cells had greater dendritic length than SGCs at locations distal to the somata. Furthermore, hilar axonal length was greater in SGCs than in GCs, as were branching order of SGCs, suggesting SGCs target more hilar neurons than do GCs. Detailed morphological simulations of granule cells and SGCs incorporating identical active and passive properties suggest that the morphological differences contribute to greater attenuation of proximal EPSCs in SGCs. These data reveal unique dendritic morphological characteristics of granule cells and SGCs that could contribute to the differences in their synaptic inputs and post-traumatic plasticity.

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Poster

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Topic: C.10. Trauma

Support: NS076511

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Title: Temporal changes in brain lipids after traumatic brain injury - A global lipidomics study

Authors: T. ANTHONYMUTHU[1,2,3], Y. Y. TYURINA[4,3], J. LEWIS[1,2], V. A. TYURIN[4,3], A. A. AMOSCATO[4,3], P. KOCHANEK[1,2], V. E. KAGAN[4,3], *H. BAYIR[1,2,3];
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Abstracts: Studies of the brain lipidome after traumatic brain injury (TBI) are one of the emerging research areas, essential for understanding the cellular responses and identification of

new drug targets. While lipid peroxidation has been associated with TBI induced neurological dysfunction, its role in the production of lipid mediators remains poorly understood. To experimentally address these issues, here, we used a global lipidomics approach to monitor the temporal changes in glycerophospho lipidome and fatty acyl lipidome of rat brain after controlled cortical impact. The left parietal cortex in 17 day old rat was impacted by a 6mm pneumatically driven metal impactor tip with 4±0.2 m/sec velocity. The depth of the penetration was 2.5 mm and the duration was 50 milliseconds. LC-MS/MS analysis of pericontusional lipid contents revealed time dependent changes in three glycerophospholipid classes and in all monolyso-glycerophospholipids at 1h, 4h, and 24h after injury vs naïve controls. The content of cardiolipin, a mitochondrial specific phospholipid, decreased by 30% at 1 and 24 hr time points and by 50% at 4hr time point compared to naive. The other two lipid classes, phosphatidylserine (PS) and phosphatidic acid (PA) were changed in the selected species containing docasahexaenoic acid (DHA). Stearyl-docasahexaenoyl and stearyl-docasapentaenoyl PS species were reduced to 83±4 % whereas both species in PA were increased to about 149±5%. A 3-5 fold increase in the levels of monolyso-phospholipid species was observed after TBI in all the phospholipid classes except lysophosphatidylglycereol and lysophosphatidic acid. Among fatty acid oxidation products, all octadecanoids, eicosanoids and docosanoids were increased more than 10-fold, whereby the changes in docosanoids correlated with the reduction in PS and increase in monolyso-PS species stoichiometrically (in all experiments and groups $n=4$, $p<0.05$). These results are consistent with a reduction in mitochondrial content after TBI, likely through mitophagy. Moreover, permanent activation of phospholipase A_2-driven mechanisms up to 24hr post injury may have mediated the phosphatidylserine specific production of DHA-derived mediators contributing to maintenance of cellular homeostasis after TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.17/P6

Topic: C.10. Trauma

Title: HB-GAM overcomes the CSPG-dependent inhibition of neurite growth and cell attachment

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Abstracts: Chondroitin sulfate proteoglycans (CSPG) are the glial scar components that inhibit posttraumatic nerve regeneration in the mammalian central nervous system. Here we demonstrate that the extracellular matrix-associated protein HB-GAM (also known as pleiotrophin) overcomes the CSPG-dependent inhibition of neurite growth and cell attachment in cortical and hippocampal neurons from neonatal mice and from rat embryos and in the PC12 cell line. Both substrate-precoated and soluble forms of HBGAM exhibit this effect. Moreover HB-GAM rescues neurite growth in neuronal cultures primed on CSPG for 1.5 days before the addition of HB-GAM. The HB-GAM receptor N-Syndecan is not required for the neurite growth-promoting effect of HBGAM in the presence of CSPG as the effect is preserved in N-Syndecan KO mice. Our results indicate that HBGAM may have therapeutic potential for the treatment of the regeneration failure in the injured human CNS. We are currently working on the *in vivo* proof of concept in the mouse traumatic brain injury model.

Disclosures: M.N. Paveliev: None. **H. Rauvala:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.10. Trauma

Support: China Natural Science Foundation 81300998

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Title: MicroRNAs regulate mitophagy induced by experimental traumatic brain injury

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Abstracts: Mitophagy is a kind of reaction of eukaryotic cells when under pressure . In previous research , interaction of cadiolipin and LC3 (microtubule-associated-protein-1 light chain 3) is important for mitophagy induced . But the mechanism for the regulation of these remains unknown . And MicroRNA have key roles in normal CNS development and function, as well as

in disease condition . Based on previous study, mitophagy can be detected after stretch in the primary cortical neurons . In the current report, we found that one hour after mechanical stretch in primary cortical neurons MiR-137, MiR-21, and MiR-10 significantly decreased compared with sham controls . And the mechanical stretch induced mitophagy can be suppressed by overexpression of MiR-137, MiR-21, and MiR-10 . When the neurons were transfected with cadiolipin synthase (CLS), mechanical stretch only caused significant decrease of MiR-137 but not MiR-21 and MiR-10 . Our results suggested that MiR-137 might regulate neuronal mitophagy via CLS-LC3 pathway. Further detailed mechanism about the miRNA and mitophagy after stretch needs to be clarified .

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

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Ministry of Science & Technology, Govt. of India DST-DBT-56-23155.69

Astra-Zeneca Mölndal, Sweden

Title: Cold environment exacerbates brain pathology and oxidative stress following traumatic brain injuries. Potential therapeutic effects of nanowired antioxidant H-290/51

Authors: *A. SHARMA[1], D. F. MURESANU[2], P.-O. SJÖQUIST[3], Z. TIAN[4], H. S. SHARMA[5];
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Abstracts: Traumatic brain injuries (TBI) are one of the most devastating causes of death and disability across the World. Our soldiers are the most vulnerable to TBI either during peacekeeping or combat operations even at extreme hot and cold environments. Some reports suggest that hyperthermia following TBI is harmful but so far no studies are conducted on the effects of cold environment on the pathophysiological outcomes of TBI. In present investigations we examined the effects of cold environment on TBI in our rat model with regard to generation of oxidative stress and brain pathophysiology. In addition, we also evaluated the effects of a potent antioxidant compound H-290/51 with or without TiO2 nanowired drug delivery on the pathophysiology of TBI in cold environment as compared to room temperature. TBI was inflicted under Equithesin anesthesia in Wistar Male rats over the right parietal cortex by making an incision of 2 mm deep and 4 mm long after opening of the skull bone (ca. 4 mm diameter, area 12.56 mm2). The animals were allowed to survive 48 h after TBI. To understand the effects of cold environment animals were exposed at 5°C for 3 h daily for 5 weeks before injury. The control groups were maintained at normal room temperature (21±1°C). In these animals oxidative stress parameters e.g., Leucigenin (LCG), Luminol (LUM), Malondialdehyde (MDA) and Glutathione (GTH) were measured in the brain along with blood-brain barrier (BBB) breakdown, brain edema formation and neuronal injuries using standard procedures. Our observations show that TBI in animals subjected to cold environments resulted in about 80 to 190 % increase in LCG, LUM and MDA and 220 % decrease in GTH in the brain as compared to rats subjected to TBI at room temperature. The magnitude and intensity of BBB breakdown to radioiodine and Evans blue albumin, edema formation and neuronal injuries were also exacerbated in TBI group in cold by 120 to 280 % from the injured group placed at room temperature. Nanowired delivery of H-290/51 (50 mg/kg) 6 to 8 h after TBI in cold group was able to significantly thwart brain pathology and oxidative stress whereas normal delivery of H-290/51 failed to achieve any reduction in oxidative stress or brain pathology following TBI in this cold exposed group. However, normal delivery of H-290/51 at identical periods significantly reduced the oxidative stress and brain pathology following TBI at room temperature. These observations are the first to demonstrate that cold aggravates the pathophysiology of TBI and this could be partially due to an enhanced production of oxidative stress in cold environment, not reported earlier.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Uppsala University, Sweden

IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain

Title: Tio2 nanowired dl-3-n-butylphthalide (dl-nbp) delivery, a chinese traditional medicine profoundly attenuates blood-brain barrier disruption, brain edema formation and neuronal injuries following concussive head injury

Authors: *L. FENG[1], A. SHARMA[2], H. YIN[3], J. V. LAFUENTE[4], H. S. SHARMA[2];
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Abstracts: The DL-NBP is an extract from Chinese celery and is used in stroke patients showing marked improvement in their cognitive and mental health. Since concussive head injury (CHI) is often fatal in more than 90% of cases involving our soldiers in the battlefield and the remaining 10 % of cases show lifetime disability there is an urgent need to find out better therapeutic use of novel medicine or use of available medicine in a more effective way. Keeping these views in mind we wanted to explore the role of DL-NBP in CHI using nanodrug delivery employing TiO2 nanowires in our rat model of CHI that induced profound brain pathology similar to that of clinical cases. The CHI was induced by dropping a weight of 114.6 g on the right parietal skull bone over a distance of 20 cm in anesthetized rats resulting an impact of 0.224 N on the skull surface. This impact induces severe brain pathology over 4 h to 24 h. In our model CHI inflicted arts were allowed to survive either 8 h or 24 h after trauma. In these CHI inflicted rats blood-brain barrier (BBB) disruption to Evans blue and radioiodine and brain edema formation was measured after 4 h, 8 h, 12 h and 24 h survival. The neuronal damages were also examined at each time point using histological techniques. We observed a progressive increase in the BBB breakdown from 4 h after CHI that continued to enhance by 200 % at 8 h, 400 & after 12 h and 600 & at 24 h after injury. The neuronal damages were also showed massive increase by 8 h after CHI (+4 to 6 fold) followed by 7 to 8 fold at 12 h and 9 to 10 fold at 24 h in the cortical and subcortical areas. There was a voluminous increase in brain swelling by 1 % at 4 h, 2 % at 8 h, 4% at 12 h and 6 % at 24 h as compared to intact controls. In separate group of rats with CHI, we administered NBP (40 or 60 mg/kg, i.p.) 2h and 4 h after injury in 8 h survival group and 8 h and 12 h after trauma in 24 h survival group. We found that NBP in 40 mg was able to reduce BBB

breakdown, brain edema formation and brain pathology after 8 h CHI, whereas, 60 mg dose of NBP was needed for identical neuroprotection in 24 h CHI group. Interestingly, TiO2 Nanowired NBP requires only 20 mg/kg in 8 h group and 40 mg/kg NBP in 24 h group for effective neuroprotection indicating a potential enhanced effectiveness of DL-NBP following TiO2 nanowired delivery in CHI. These observations are the first to point out a new role of DL-NBP in treating CHI with regard to pathophysiology of brain injury. Furthermore, the nanowired delivery of NBP is far superior to NBP alone, not reported earlier.

Disclosures: L. Feng: None. **A. Sharma:** None. **H. Yin:** None. **J.V. Lafuente:** None. **H.S. Sharma:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

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IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain

Title: Nanowired delivery of histamine receptor antagonists attenuate nitric oxide synthase upregulation and spinal cord pathology following trauma

Authors: *R. PATNAIK[1], A. SHARMA[2], J. V. LAFUENTE[3], D. F. MURESANU[4], A. NOZARI[5], H. S. SHARMA[2];
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Napoca, Romania; [5]Anesthesiol. & Critical Care Ctr., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstracts: The possibility that histamine receptors could modulate cell damage in spinal cord injury (SCI) is investigated in this study using nanowired delivery of selected Histamine H1, H2 and H3 receptor modulating drugs. There are reasons to believe that histamine receptors could influence SCI pathology either through direct mechanisms or via upregulation of nitric oxide synthase (NOS). In present investigation we explored whether drugs influencing histamine H1, H2 and H3 receptors could modify SCI induced cord pathology through modification of neuronal NOS expression in our rat model. A focal trauma to the rat spinal cord was made by making an incision into the right dorsal horn of the T10-11 segments and the animals were allowed to survive 5 h after injury. This SCI significantly increased the spinal cord edema formation, BSCB breakdown to protein tracers and induced a marked reduction in the SCBF in the T9 and T12 segments. Pronounced upregulation of nNOS is seen in these segments at 5 h together with cell death and cord pathology. TiO2-Nanowired delivery of histamine H1 receptor antagonist mepyramine (1 mg, 5 mg and 10 mg/kg, i.p,) did not attenuate spinal cord pathology or nNOS expression following SCI. On the other hand, blockade of histamine H2 receptors with nanowired cimetidine or ranitidine (5 mg) 30 min after trauma significantly reduced these early pathophysiological events and nNOS expression. However, normal drug require 10 mg/kg doses for similar neuroprotection. The effects of normal or nanowired ranitidine were far superior in attenuating SCI pathology and nNOS upregulation. Nanowired delivery of histamine H3 receptor agonist α-methylhistamine (1 mg/kg/i.p.), that inhibits histamine synthesis and release in the CNS is able to thwart SCI pathology and nNOS expression significantly in the non-injured T9 and T12 segments. Whereas normal dose of histamine H3 agonist (2 mg) is needed for the same effects. On the other hand, blockade of Histamine H3 receptors with nanowired Thioperamide (1 mg) or normal drug (at 1 or 5 mg/kg, i.p.) exacerbated the spinal cord pathology and NOS expression. These observations are the first to suggest that stimulation of H3 receptors and blockade of H2 receptors attenuates nNOS expression and thereby induce neuroprotection in SCI. Nanowired delivery of right drug in low doses are able to thwart SCI pathology whereas, higher dose of the normal drug is needed to have similar effects. Taken together, our results indicate that histamine induced pathophysiology of CNS injury is mediated via nNOS expression, not reported earlier.

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Poster

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Ministry of Science & Technology, Govt. of India

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Title: Superior neuroprotective effects of nanowired delivery of AP-713 as compared to AP-173 and AP-364 compounds on functional recovery and cellular injuries following spinal cord trauma

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Abstracts: In our laboratory drugs attached to TiO2 nanowires causes enhanced delivery of compounds resulting in increased therapeutic efficacy. This investigation was undertaken to know whether any drug attached to nanowires might have better therapeutic efficiency in a model of rat spinal cord injury (SCI). We used 3 different compounds AP-173, AP-713 and AP-364 (Acure Pharma, Sweden) with different neuroprotective efficacy. These compounds were TiÖ2-nanowired and delivered following SCI in rats and compared with normal compounds. The SCI was produced by making a longitudinal incision into the right dorsal horn of the T10-11 segments under Equithesin anesthesia. In separate group of rats these compounds either alone or tagged with nanowires were applied topically within 5 to 10 min after SCI and allowed to survive 12 h. In these animals, behavioral outcome, blood-spinal cord barrier (BSCB) permeability, edema formation and cell injury were examined. The SCI resulted in severe motor paralysis, widespread disruption of the BSCB to Evans blue albumin (EBA), [131] Iodine tracers and exhibited profound edema formation. Cell or tissue destruction was present around the lesion

site extending up to T4 and L2 levels. Topical application of normal compounds in high quantity (10 µg in 20 µl) markedly attenuated behavioral dysfunction that are prominent around 2-3 h after SCI. BSCB disruption, edema formation and nerve cell, glial cell and axonal injuries are less pronounced in drug treated injured animals at 12 h. These beneficial effects are most marked in animals that received AP-713. Interestingly, when these compounds were administered with nanowires, their beneficial effects on functional recovery and spinal cord pathology were further enhanced. Thus, nanowired AP-713 attenuated functional disturbances up to 5 h after SCI. Spinal cord cell and tissue destruction was minimal in this group at 12 h. The other nanowired compounds were not that effective. Topical administration of nanowires alone did not influence spinal cord pathology or motor function after SCI. Taken together, our results are the first to demonstrate that the drug-delivery and their therapeutic efficacy is enhanced when the compounds are administered with nanowires depending on their inherent property and not due to nanowiring alone.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.23/P12

Topic: C.10. Trauma

Support: NIH Grant R01HD061946

NIH Grant R01NS065842

Title: Perivascular basement membrane proteins changes long-term after a juvenile traumatic brain injury: Possible link with amyloid-beta accumulation

Authors: *A. JULLIENNE[1,2], J. BADAUT[2,3,4], J. ROBERTS[5], V. POP[2], M. P. MURPHY[5], E. HEAD[5], W. J. PEARCE[1], G. J. BIX[5];
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Abstracts: Traumatic brain injury (TBI) is a leading cause of death and disabilities and it induces several neurovascular unit (NVU) dysfunctions including BBB disruption. We previously showed that, even when the BBB is no longer disrupted, a juvenile TBI leads to long-term transformations of endothelial cells with a decrease of P-glycoprotein (P-gp), associated with amyloid-β (Aβ) accumulation 2 months after injury in a rat model of cortical impact. These results led us to the hypothesis that a juvenile TBI causes long-term transformations of the NVU, leading to neurodegeneration-like processes. Here, we studied P-gp expression and Aβ accumulation up to 6 months after injury but we also focused on post-TBI (2 and 6 months) changes of 2 basement membrane proteins, perlecan and fibronectin, known to be implicated in perivascular drainage of Aβ. We used a model of controlled cortical impact in P17 rat and immunohistochemistry led us to the observation that, even after 6 months, when cognitive dysfunctions are emerging, Aβ accumulation and P-gp decreased expression are still present. Meanwhile, perlecan and fibronectin immunostaining are increased around cerebral blood vessels in injured animals after 2 and 6 months, suggesting their possible implication in Aβ accumulation. Interestingly, perlecan and fibronectin have also been shown to have neuroprotective and pro-angiogenic properties. So this increase could be a compensatory mechanism aiming at protecting the NVU. We investigated whether there is angiogenesis but the number of vessels was not different between naïve and TBI animals at both time points. However, we observed a decrease in the diameter of cerebral blood vessels at 2 and 6 months after the impact. These results support the hypothesis that a juvenile TBI induces long-term modifications of the cerebrovasculature that could compromise cerebral blood flow and cerebral integrity in the case of a future injury.

Disclosures: **A. Jullienne:** None. **J. Badaut:** None. **J. Roberts:** None. **V. Pop:** None. **M.P. Murphy:** None. **E. Head:** None. **W.J. Pearce:** None. **G.J. Bix:** None.

Poster

607. Traumatic Brain Injury: Cellular Events and Repair

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 607.24/Q1

Topic: C.10. Trauma

Support: Centre for Neuroscience and Regenerative Medicine

Title: Serum Amyloid A1 is induced in the liver following traumatic brain injury

Authors: S. VILLAPOL[1], D. KRYNDUSHKIN[1], M. BALAREZO[1], A. CAMPBELL[1], J. SAAVEDRA[2], F. P. SHEWMAKER[1], *A. J. SYMES[1];
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Abstracts: Pathological events associated with traumatic brain injury (TBI) affect many peripheral tissues, with the potential that the peripheral response could influence recovery of the brain. The liver mounts an acute phase response to many different forms of trauma, infections and inflammation including in response to TBI. We have previously shown that angiotensin II AT1 receptor (AT1R) blockers (ARBs) ameliorate detrimental morphological and functional outcomes from TBI. As AT1Rs are highly expressed in hepatic parenchyma we hypothesized that angiotensin II, acting through the AT1R in hepatic tissue may influence the hepatic response to TBI. To test our hypothesis, we performed controlled cortical impact (CCI) injury on 9-week-old male mice and treated with the ARB, telmisartan (1 mg/kg/day, starting 1 hour after injury) or saline. Mice were sacrificed at 6 hours (hpi), 1, 3, 7, and 30 days post injury (dpi). We focused on the hepatic synthesis of Serum Amyloid A1 (SAA1), a protein that is strongly induced in the acute phase response, is released into the circulation and is a powerful proinflammatory mediator. Hepatic expression of SAA1, AT1R, and pro-inflammatory cytokines were analyzed by QPCR, western-blot or immunohistochemistry. Hepatic expression of AT1AR mRNA was increased at 3dpi, but not at earlier time points. SAA1 was rapidly induced after TBI, with SAA1 mRNA increasing over 50 fold by 6 hpi, reaching a maximum of 145 fold by 1dpi, and decreasing to 14 fold induction by 3dpi. Telmisartan treatment reduced hepatic SAA1 mRNA and protein in the liver only at 3dpi, but not at earlier time points. Increased SAA1 expression was also detected in the serum. Immunohistochemical analysis indicated that SAA1 was colocalized with a marker for cholangiocytes at 3 dpi. We also detected increased hepatic expression of mRNA encoding the pro-inflammatory cytokines CXCL1 and CXCL10 at 6 hpi, but not CCL2, IL-6 or IFN-gamma. Interestingly, there was increased neutrophil infiltration into the liver after TBI, detected by staining for myeloperoxidase. We also found increased numbers of apoptotic cells by TUNEL staining at 3 dpi. These effects were not altered by telmisartan treatment. Thus we have shown that there is a strong hepatic response to TBI that includes induction of specific cytokines and the acute phase protein SAA1. Telmisartan can modulate SAA1 expression at 3dpi, which coincides with the induction of AT1R mRNA suggesting that any modulation by angiotensin may occur at later time points. Hepatic induction of SAA1 after TBI may contribute to the neuroinflammation that is an important component of the response to TBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Program#/Poster: 607.25/Q2

Topic: C.10. Trauma

Support: Congressional Directed Medical Research Program (awards W81XWH-10-2-0091 and W81XWH-10-2-0091)

Title: The cerebral cortex proteome in rodent models of fear conditioning and repetitive blast

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Abstracts: Objective. Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) remain serious medical issues for active duty service members and veterans. However, little is known about their underlying mechanisms. This study characterizes changes in the rodent cerebral cortex proteome in a model of conditioned fear and/or blast over-pressure (BOP). Methods. Using male Sprague-Dawley rats, inescapable electric shock (IES) and repetitive blast over-pressure (75kPa BOP/day X 3 days) were used as models of stress or mTBI, respectively (Genovese et. al, 2013). Experimental groups: (1) sham IES + sham BOP (SS), (2) IES + sham BOP (IS), (3) sham IES + BOP (SB), and (4) IES + BOP (IB). Cerebral cortex peptides were generated by in-gel tryptic digest and analyzed by nLC-MS/MS on a Thermo LTQ Velos. Searches and peptide assembly were performed using Sequest/Scaffold (SQ-SCF) or Myrimatch/IDPicker (MM-IDP), FDR<0.05%. Spectral count was determined using Prism (t-Test, $p \leq 0.05$). Ontology was conducted with WebGestalt and is expressed as the ratio of enrichment (R-value,$p \leq 0.05$). Pathways were derived from Pathway Studios analysis. Results. We defined the cerebral cortex proteome using two platforms in order to increase protein identification fidelity and determine which proteins and ontological groups were affected in experimental groups compared to sham controls. Venn diagram analysis of proteins identified with MM-IDP and SQ-SCF database searching identified 1101 unique proteins that were common to all experimental groups. Gene ontology analysis revealed that, overall, these proteins mapped to platelet-activating factor acetyl-transferase activity, and glutamate decarboxylase

activity (R=22.52). Other categories were lactate dehydrogenase activity (R=15.02) and peroxiredoxin activity (R=12.51). The number of differentially abundant proteins varied with condition. IES alone or BOP alone led to abundance changes in 50 and 32 proteins, respectfully. IES followed by BOP led to abundance changes of 14 proteins. Interestingly, each condition influenced specific biological processes pathways. Small molecule metabolism (16%) dominated IES. Transport (7%) and small molecule metabolic process (6%) were affected by BOP. In IES with BOP, most proteins sorted to platelet activation and blood coagulation as well as axon guidance (3%, each). Studies are in progress to determine which disease pathways are identified by IES, BOP, and IES followed by BOP. Conclusions. A greater understanding of unique changes in the brain proteome as a consequence of stress and blast will pave the way for novel biomarker discovery and therapeutics relevant to PTSD and mTBI.

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Poster

607. Traumatic Brain Injury: Cellular Events and Repair

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Program#/Poster: 607.26/Q3

Topic: C.10. Trauma

Title: MALDI analysis of lysophosphatidic acid levels after experimental traumatic brain injury in the rat

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Abstracts: Many traumatic brain injured (TBI) patients sustain permanent physical, cognitive and emotional disabilities that persist throughout their lives. The natural response of the brain to TBI suggests a specific molecular system may be involved in injury pathogenesis as well as limit functional recovery. Lysophosphatidic acid (LPA) is a bioactive phospholipid that increases in the CSF after injury and alone, can activate initial injury responses like cell death and inflammation. In the brain, there are several LPA species involved in physiological processes but

after injury the specific LPA species that's modulated and the brain regions affected by these molecular changes have not been identified. Determining the specific molecular changes that underlie secondary injuries of trauma is essential to developing targeted therapeutics for TBI that can promote cognitive and physical recovery. To bridge this gap in knowledge we've utilized Matrix-Assisted Laser Desorption Ionization imaging mass spectrometry (MALDI -IMS) to identify the regional- and species-specific changes of LPA in the brain after TBI. Brains were obtained from adult, CD1 male rats at 3 hours after controlled cortical impact (CCI), aged-matched naive rats were used as controls. The brains were harvested, sectioned and MALDI spectra collected for each 50 micron tissue location using a Bruker Daltonic Solarix 7T mass spectrometer. The identities of the analyte molecules were confirmed by on-tissue collision induced fragmentation, and each was co-localized to adjacent H&E stained coronal sections. The results revealed an approximate 1.5-fold increase in LPA 18:1 species in the contralateral white matter and contralateral CA1 and CA3 as compared to control. Significant increases in LPA precursors Lysophosphatidylcholine (LPC) and Lysophosphatidylethanolamine (LPE) within the hippocampus and white matters regions were also identified in the injured brains. This data suggests that within 3 hours after injury molecular changes in LPA production within the hippocampus and white matter tracts may contribute to the long terms cognitive and motor disabilities associated with TBI. This study has provided much needed insight into the early molecular changes that may underlie the longer-term effects of TBI.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.01/Q4

Topic: C.10. Trauma

Support: NSC 102-2321-B-038-004

Title: Glucose-dependent insulinotropic polypeptide (GIP) ameliorates traumatic brain injury-induced memory deficits in rats

Authors: *Y. YU[1], T.-H. HSIEH[2,3], J.-H. LAI[4,3], K.-Y. CHEN[2,3], J.-W. LIN[4], Y.-H. CHIANG[2,3,4];
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Abstracts: Mild traumatic brain injury (mTBI) becomes a major public health issue that represents 75-90 % of all treated TBI cases. mTBI leads to various cognitive, emotional and physical and sleep-related symptoms for a period of time. To date, no clear pharmaceutical-based therapies are used to manage the development of pathological deficits associated with mTBI. Glucose-dependent insulinotropic polypeptide (GIP), an incretin similar to glucagon-like peptide-1(GLP-1), showing potential for providing neurotrophic and neuroprotective effects. In this study, we aim to investigate the impact of GIP on cognitive deficits induced by mTBI *in vivo* animal model. An mTBI rat model generated by mild controlled cortical impact (mCCI) were used to evaluate the therapeutic potential of GIP. Time-course analysis of learning and memory ability following mTBI were tested by Morris water maze (MWM) and novel object recognition (NOR) for identifying the therapeutic effects of GIP in cognitive function. In results, mTBI animals showed that the impaired long-term memory were observed following mTBI lesion under the tests of MWM and NOR. When compared to the saline-treated group, administration of GIP were significantly ameliorated mCCI-induced memory deficits. We thus conclude GIP produces significant beneficial effects in memory recovery after mTBI. With the identities of neuromodulatory role of GIP in the future preclinical studies, our results may provide mechanistic insights on the development of novel strategy of GIP for mTBI treatment in the near future.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.02/Q5

Topic: C.10. Trauma

Support: Alberta Children's Hospital Foundation

Title: TBI-induced memory deficits are fully rescued by cannabinoid receptor agonist administration

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Abstracts: A traumatic brain injury (TBI) is caused by a force applied to the head, resulting in damage to neural tissue. TBIs are among the most common sources of disability in young adults and are often associated with sport-related impacts or motor vehicle accidents. A moderate or severe TBI often leads to long-term cognitive challenges including learning and memory deficits, for which there are no known effective treatments. The endocannabinoid system has diverse physiological functions and is involved in reward pathways, appetite, anxiety, synaptic plasticity, and bioenergetics. In the context of a TBI, cannabinoid 1 receptors (CB1Rs) are a potentially interesting target because the activation of CB1Rs could counteract several of the known pathological processes initiated by a TBI including excitotoxicity, neuroinflammation, and changes in bioenergetics. We tested the hypothesis that administration of a CB1R agonist would prevent TBI-induced deficits in learning and memory in young adult male Sprague-Dawley rats. Using the controlled cortical impact TBI model (5mm diameter impact head, 4.0 m/s velocity, 2.5mm depth, 100ms duration), we replicated previously reported results showing that rats with a TBI (n=8) show deficits in short-term memory (Novel Object Discrimination Test) and spatial learning and memory (Morris Water Task). We administered a CB1R agonist (ACEA, 2mg/kg) daily for one week after the injury in a second group of animals with TBI (n=8), and conducted the same behavioral tests 2 weeks post-surgery. We found that the TBI + CB1R agonist-treated animals spent significantly more time exploring a new object in their environment than the TBI-only animals in the Novel Object Discrimination Test (One-way ANOVA, $p<0.01$, with Tukey post hoc tests) and that the performance of TBI + CB1R agonist-treated animals was indistinguishable from the sham surgery-drug (n=6) or sham surgery-vehicle (n=7) groups, and naïve animals (n=4). Similarly, in the Morris Water Task, TBI + CB1R agonist treated animals were significantly better at finding the hidden escape platform after five days of training than TBI-only animals (One-way ANOVA, $p<0.0001$, with Tukey post hoc tests). Once again, the performance of TBI + CB1R agonist-treated animals was indistinguishable from sham surgery-drug (n=6), or sham surgery-vehicle, and naïve animals (n=4). These results demonstrate that CB1R activation rescues learning and memory functions after a moderate-to-severe TBI in young adult rats.

Disclosures: M.B. Arain: None. **L.A. Craig:** None. **S.T. Nakanishi:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

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Topic: C.10. Trauma

Support: UBACYT 00149

PIP Grant 00323

Title: Enriched environment can prevent most behavioral alterations induced by different schedules of noise exposure

Authors: S. J. MOLINA[1], M. SAINT-MARTIN[1], F. CAPANI[2], *L. R. GUELMAN[1]; [1]Dept. Farmacologia, Fac Med, UBA-CEFYBO-CONICET, Buenos Aires, Argentina; [2]Lab. de plasticidad y citoaerquitectura neuronal, ININCA-CONICET, Buenos Aires, Argentina

Abstracts: It is known that acute noise exposure can induce transient or permanent hearing loss. However, few data are available regarding its effects on extra-auditory structures, in particular within developing Central Nervous System (CNS). Previous studies of our laboratory showed that exposure of 15-days-old rats to moderate noise during 2 hours can induce hippocampus (HC)-related behavioral, biochemical and histological alterations, including changes in anxiety-like behaviors. Nevertheless, no data on the behavioral effects induced by noise in other exposure schedules have been obtained yet. Moreover, since the use of potential strategies of neuroprotection has not been explored in our model, rearing noise-exposed animals in an enriched environment (EE) was used as a non-pharmacological tool. Therefore, the aim of the present work was to test if EE can prevent behavioral changes induced by exposure to noise using different exposure schedules. Rats of 15 days were exposed during 2 hours to white noise (95-97 dBA), for one or five consecutive days, using an “ad-hoc” sound camera. After weaning, groups of 3-4 rats were transferred to an enriched cage, consisting of toys, a wheel, tunnels and ramps, while other groups were placed in standard cages. One week later, different behavioral tests were performed, including open field (OF), elevated plus maze (EPM) and inhibitory avoidance (IA) tasks. Results show that whereas no changes in associative or habituation memory were found in rats exposed to noise for 2 h, significant changes were observed when rats were exposed for 5 consecutive days. In addition, a decrease in anxiety-like behaviors was observed in both groups, which could be interpreted as a maladaptive behavior. Rearing rats in EE almost fully prevented these noise-induced behavioral changes. These findings suggest that visual, social and/or physical stimulation during the peri-adolescence period, after exposure to a physical agent such as noise, might contribute to stabilization of normal emotional and behavioral parameters.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.04/Q7

Topic: C.10. Trauma

Support: Phoenix Children's Hospital Mission Support

Title: Novel allosteric inhibitors of TNF-R1 modulate post-traumatic sleep resulting from experimental diffuse TBI in the mouse

Authors: R. K. ROWE[1], J. L. HARRISON[2,1], H. ZHANG[3], D. P. HESSON[3], M. GREENE[3], *J. LIFSHITZ[1,2];
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Abstracts: Clinical studies indicate as many as 70% of traumatic brain injury (TBI) survivors suffer from sleep-wake disturbances. We have previously shown diffuse TBI acutely (1-6 hours post-injury) increases sleep in the mouse_a phenomenon we termed post-traumatic sleep. During this acute window of post-traumatic sleep, cortical levels of inflammatory cytokine tumor necrosis factor-α (TNF-α) were significantly increased, suggesting a relationship between inflammation, sleep regulatory cytokines and sleep. In this study, we administer three novel allosteric inhibitors of TNF receptor 1 (TNF-R1) to test their effect on post-traumatic sleep in the mouse following diffuse TBI. We hypothesize that the administration of TNF-R1 inhibitors immediately following TBI will modulate post-traumatic sleep in the mouse through suppression of neuroinflammatory signaling. In these experiments, adult male C57Bl/6 mice were subjected to moderate midline fluid percussion injury (n=26; 1.3 atm; 6-10 min righting reflex) or sham injury (n=7). Cohorts were divided into groups receiving either high (20 mg/kg) or low (2 mg/kg) doses of novel TNF-R1 inhibitors (Compound 7, SGT11, F002) or vehicle (10% DMSO in PBS). Immediately following TBI or sham injury, mice were given intraperitoneal injections of a TNF-R1 inhibitor or vehicle. Post-traumatic sleep was recorded via non-invasive piezoelectric sleep cages. After 6 hours, mice were euthanatized and cortical biopsies were collected for analysis of cytokine levels by ELISA. From 3 to 6 hours post-injury, vehicle-treated brain-injured mice exhibited increased post-traumatic sleep compared to vehicle-treated uninjured shams ($F(1,11)=11.56$, $p=0.0059$). Brain-injured mice treated with high dose

Compound 7 (F(1,9)=2.115, p=0.1798) low dose Compound 7 (F(1,7)=4.525=0.0710), or low dose F002 (F(1,9)=1.656, p=0.2303) showed no significant difference in post-traumatic sleep compared to vehicle-treated uninjured shams, suggesting a positive drug effect. However, brain-injured mice treated with SGT11 and high dose F002 slept similarly to vehicle-treated brain-injured mice. These data indicate that injury-induced post-traumatic sleep can be decreased by allosteric inhibition of TNF-R1. Compound 7 and low dose F002 decreased post-traumatic sleep to vehicle-treated uninjured sham levels. Further analysis of cortical cytokine levels will confirm TNF inhibition. These preliminary studies will guide future investigation of these novel compounds on functional and pathological outcomes following diffuse TBI in the mouse, potentially identifying a therapeutic intervention for sleep-wake disturbances following TBI.

Disclosures: R.K. Rowe: None. **J.L. Harrison:** None. **H. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. patent on experimental compounds F002, SGT11, and Compound 7. **D.P. Hesson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. patent on experimental compounds F002, SGT11, and Compound 7. **M. Greene:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. patent on experimental compounds F002, SGT11, and Compound 7. (US 8,318,699 B2). **J. Lifshitz:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.05/Q8

Topic: C.10. Trauma

Title: Effects of tamoxifen in hindlimb kinematics after a penetrating injury in CA1 hippocampal region

Authors: *J. R. LOPEZ RUIZ[1], L. P. OSUNA CARRASCO[1], B. DE LA TORRE VALDOVINOS[1], N. E. FRANCO RODRIGUEZ[1], C. R. MOYA GARCÍA[2], I. JIMÉNEZ[4], J. M. DUEÑAS[1], S. DUEÑAS[3];
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Abstracts: Tamoxifen recovers many hindlimb kinemtic parameters after a hippocampal penetrating injury, these effects were clearly observed 30 days after injury1. The present experiments were made in rats to study both, hindlimb kinematic changes and morphologic alterations produced by a penetrating injury in hippocampal CA1 region, and to study tamoxifen effects (1 mg/kg applied for three days) in kinematic changes and neuron survival 30 days after injury. Angular changes in kneee and ankle ocurred in non treated animals 30 days after injury. In trated animal, similar angular changes were observed. Tamoxifen produced an statistical significant recovery in the ipsilateral maximal knee angle. The number of pyramidal neurons in CA1 region is greater in treated vs untreated rats. The better outcome in kinemetics recovery produced by tamoxifen in hippocampal injury seems to be related to a larger number of pyramidal neurons compared to non treated animals. The injured CA1 produced more restricted kinematic changes than when the dentate gyrus was damaged. Experiments are under analysis to value the tamoxifen effects in forelimb kinematics after CA1 hippopcampus penetrating injury.

Disclosures: J.R. Lopez Ruiz: None. **L.P. Osuna Carrasco:** None. **B. De la Torre Valdovinos:** None. **N.E. Franco Rodriguez:** None. **I. Jiménez:** None. **J.M. Dueñas:** None. **S. Dueñas:** None. **C.R. Moya García:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

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Topic: C.10. Trauma

Support: National Science Foundation Grant #2013165053

Penn Medicine Neuroscience Center

Title: A strategy to restore brain circuitry using micro-tissue engineered neural networks

Authors: *J. P. HARRIS[1,2], L. A. STRUZYNA[1], P. L. MURPHY[1], D. K. CULLEN[1,2]; [1]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [2]Philadelphia Veterans Affairs Med. Ctr., Philadelphia, PA

Abstracts: Disruption of the connectome plays a prominent role in neurotrauma and many neurological diseases; however, there is currently no strategy to repair long-distance axonal connections in the brain. Therefore, we have developed micro tissue-engineering techniques to

create miniature tubular constructs containing discrete neuronal populations spanned by long integrated axonal tracts. Our previous work demonstrated that these preformed micro-tissue engineered neural networks (TENNs) stereotaxically delivered via a needle can reconstruct corticothalamic pathways in a rodent. Here, our objective was to minimize insertion trauma by creating an advanced biomaterial encasement strategy for needle-less delivery of micro-TENNs. The micro-TENNs were composed of a small hollow agarose hydrogel shell (≤700μm OD) filled with a laminin-collagen (350μm ID) extracellular matrix. Micro-TENNs were seeded with primary cortical neurons at the tube extremities to form defined populations connected by long bi-directional axonal tracts. Immunocytochemistry revealed that this neuronal and axonal architecture was maintained up to 21 days *in vitro*. Micro-TENNs were coated with low viscosity carboxymethyl cellulose (CMC) to enable needle-less delivery. The CMC becomes rigid with mild dehydration but softens upon brain insertion. Qualitatively, coated and uncoated micro-TENNs show similar architecture while coating had no effect on viability with nearly 100% live cells in both cases. We also assessed the insertion and buckling force of coated micro-TENNs to determine if the coating stiffness was sufficient for brain insertion. Penetration force was measured via an Instron mechanical tester and, based on previous work, 0.6% agarose was used to mimic the brain. We found that coated micro-TENNs were sufficiently strong to be implanted, withstanding ~900 mN before buckling while the insertion force needed was only ~21 mN. Uncoated micro-TENNs required a needle 60% bigger than coated micro-TENNs; therefore, we anticipate that this coated method will minimize insertion damage due to a reduced form factor. We are currently evaluating the host response and micro-TENN survival and integration following needle-less micro-TENN delivery. Micro-TENNs represent the first strategy capable of simultaneously providing neuronal replacement and re-establishing long-distance axon pathways, potentially facilitating nervous system repair following connectome disruption in TBI, Parkinson's disease, stroke, and brain tumor excision.

Disclosures: J.P. Harris: None. **L.A. Struzyna:** None. **P.L. Murphy:** None. **D.K. Cullen:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.07/R1

Topic: C.10. Trauma

Support: NRSA F32 NS079148-02

NIH T32-HL07446-29

Title: Traumatic brain injury and posttraumatic epilepsy: *In vivo* neuroprotective role of M-type K+ channel

Authors: *S. M. BIERBOWER, M. S. SHAPIRO;
Physiol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstracts: Traumatic head injury (TBI) is a leading cause of death and disability among all age groups, occurring from a variety of causes, including car accidents, falls and battlefield events. Among the most common consequences of a TBI are seizures and development of epilepsy. Currently, there are few-to-none preventative treatments to prevent epilepsy after a TBI; a gap that this project seeks to address. In neurons throughout the brain, "M-type" K+ currents, underlied by the KCNQ family of ion channels, play dominant roles in control over excitability, and are thus implicated in myriad neurological and psychiatric disorders. Recently, the use of M-channel "openers" has emerged as novel anti-convulsive and anti-nociceptive compounds, and the FDA-approved drug, retigabine (RTG), is now in the clinic. However, beyond its anti-convulsive efficacy, we have shown in rodent models that RTG is neuroprotective against cell death, deleterious inflammation and motor impairment after a stroke, and we here show such a benefit after a TBI as well. Importantly, cytotoxic and vasogenic edema, neuronal death and morphological changes are all associated with acquired epilepsy after a TBI. Our data indicate that M-channel openers represent a novel and powerful anti-epileptogenic therapy that can prevent acquired seizures and epilepsy after a TBI, not solely by reducing electrical excitability, but by reducing the inflammatory and cell edema consequences of a TBI that are linked to irreversible brain damage. We are testing this hypothesis in mice with a controlled cortical impact "blunt force" TBI that best simulates trauma after vehicular accidents or falls. The effects of RTG on brain damage and pro-epileptic changes were assessed by video monitoring of seizure behavior and EEG recordings, and brain slice staining for fiber "sprouting" and loss of neuronal cell bodies in the hippocampus. Our data so far show strong effects of RTG administered within 30 min of a TBI, with a reduction in vasogenic edema and cell death by nearly half, compared to vehicle-only controls.

Disclosures: S.M. Bierbower: None. **M.S. Shapiro:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.08/R2

Topic: C.10. Trauma

Support: NIH/NINDS T32-NS077889

NIH/NINDS R01-NS048191

NIH/NINDS R01-NS062993

Title: A "NEET" mitochondrial target: The importance of mitoNEET in pioglitazone mediated neuroprotection following TBI

Authors: *H. M. YONUTAS[1], J. D. PANDYA[2], A. H. SEBASTIAN[2], W. J. GELDENHUYS[3], R. T. CARROLL[3], P. G. SULLIVAN[1];
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Abstracts: A major focus has developed to discover neuroprotective therapeutic agents to help the estimated 1.7 million Americans who receive traumatic brain injury (TBI) annually. Due to the complicated nature of TBI, the most promising compounds target multiple mechanisms such as neuroinflammation, ROS production and mitochondrial dysfunction. Previous reports have shown that pioglitazone, a known PPAR agonist, can alter neuroinflammation and decrease ROS production. Additionally, pioglitazone has been found to increase mitochondrial bioenergetics, cortical sparing and functional recovery following TBI, which aligns well with our theory that mitochondrial dysfunction is a pivotal link in the neuropathological sequelae of brain injury. The positive effects seen with pioglitazone seem to be independent of interactions with PPAR and may be attributed to its ability to bind with a novel mitochondrial protein called mitoNEET. Therefore, we hypothesize that pioglitazone's neuroprotective mechanism is dependent on interactions with mitoNEET. To test this hypothesis we have used mitoNEET null mice and a novel mitoNEET ligand called NL-1. *Ex vivo* dose response studies show that pioglitazone can increase mitochondrial bioenergetics in isolated mitochondria with and without Ca2+ insult, which is an effect lost in the mitoNEET null mice. Next, wild-type and mitoNEET null mice (pioglitazone and NL-1 study) and Sprague Dawley rats (NL-1 study) who were subjected to sham or severe controlled cortical impact (CCI) TBI surgery. Preliminary results demonstrate that pioglitazone loses its ability to increase mitochondrial respiration and provide neuroprotection in mitoNEET null mice and that treatment with a specific mitoNEET ligand (NL-1) increases cortical sparing and motor recovery following TBI. Therefore, we believe pioglitazone to be a novel mitochondrial targeting drug which is able to alter mitochondria bioenergetics following TBI through interactions with mitoNEET. Results from these studies will help to shed light on the fundamental processes involved in TBI neuropathology and may pinpoint potential novel interventions and targets for the treatment of TBI.

Disclosures: **H.M. Yonutas:** None. **J.D. Pandya:** None. **A.H. Sebastian:** None. **W.J. Geldenhuys:** None. **R.T. Carroll:** None. **P.G. Sullivan:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.09/R3

Topic: C.10. Trauma

Support: NIH Grant NS065017

Title: The membrane-resealing agent, Poloxamer 188, provides protection from blood brain barrier disruption and neuronal damage following traumatic brain injury

Authors: ***L. L. KRAFJACK**, D. P. FOX, R. RAGHUPATHI;
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Abstracts: Mechanically-induced pore formation (mechanoporation) within cell bodies and axons has been implicated as one of the early events of traumatic brain injury (TBI) that contributes to neurodegeneration and traumatic axonal injury. Previous studies demonstrated that intracerebroventricular (ICV) injection of the membrane-resealing agent, Poloxamer 188 (0.6mg in 5μL), prior to TBI decreased the extent of traumatic axonal injury in white matter tracts below the site of impact and in the thalamus at 4hr post-injury. The present study sought to determine the effect of Poloxamer 188 on neuronal damage, blood brain barrier (BBB) disruption, and membrane permeability immediately (10min) following lateral fluid-percussion brain injury. Neuronal damage was determined using microtubule-associated protein 2 (MAP2) and neuronal nucleus (NeuN) protein immunoreactivities; BBB damage was evaluated using IgG extravasation; and membrane permeability was assessed by uptake of membrane-impermeable dextrans (average mol. wt. = 10kDa). Brain injury resulted in a loss of MAP2 immunoreactivity in the cortex of the injured hemisphere following injury (8% of total area of the cortex), which was not attenuated by pre-injury ICV administration of Poloxamer 188 at any dose (0.6, 1.2, or 1.8mg). Injury also resulted in a decrease in NeuN immunoreactive cells in the cortex and the thalamus of the injured hemisphere by 67% and 27%, respectively, compared to sham-injured animals, which was not affected by pre-injury administration of Poloxamer 188. Approximately 50% of the remaining NeuN(+) cells in the cortex, exhibited intense NeuN immunoreactivity, whereas the rest were weakly immunoreactive. Pre-injury administration of Poloxamer 188 increased the proportion that was intensely immunoreactive for NeuN in a dose-dependent

manner, with the 1.2mg dose being the most effective dose (75% intensely immunoreactive). In addition, diffuse extracellular IgG immunoreactivity was observed within the cortex, white matter and hippocampus covering 23% of total area of the injured hemisphere; pre-injury administration of any of the 3 doses of Poloxamer 188 reduced the area of IgG immunoreactivity by approximately 50% ($p<0.05$ compared to saline-treated animals). Although dextran-flooded profiles were observed in both the cortex and thalamus of the injured hemisphere, indicative of mechanoporation, Poloxamer 188 did not reduce the number of these profiles. Taken together, these data suggest that Poloxamer 188 may provide partial neuroprotection early after injury that may be independent of its ability to reseal cell membranes.

Disclosures: L.L. Krafjack: None. **D.P. Fox:** None. **R. Raghupathi:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.10/R4

Topic: C.10. Trauma

Title: Targeting the p53 pathway to protect against traumatic brain injury

Authors: *L.-Y. YANG[1], K.-H. CHANG[1], Y.-H. CHU[1], N.-H. GREIG[2], J.-Y. WANG[1]; [1]Grad. Inst. of Med. Sci., Taipei Med. Univ., Taipei City, Taiwan; [2]Inst. on Aging, NIH, Baltimore, MD

Abstracts: Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Programmed death of neuronal cells plays a crucial role in acute and chronic neurodegeneration following TBI. p53 tumor suppressor protein, a transcription factor, has been recognized as an important regulator of apoptotic neuronal death. Pifithrin-α (PFT-α) was shown to specifically prevent cell death by inhibiting p53 transcriptional activity.PFT-α has been shown to be neuroprotective against stroke and trauma injury. Previous study indicates that PFT-α oxygen analogue is more stable and active *in vivo* than PFT-α. The aim of our study is to investigate whether inhibition of p53 using pifithrin-α (PFT-α) or PFT-α oxygen analogue (PFT-α(O)) would be a potential neuroprotective strategy for TBI. To investigate whether these drugs protect excitotoxicity *in vitro*, primary rat cortical cultures were treated with glutamate (50mM) in the presence or absence of various concentration of p53 inhibitor PFT-α or PFT-α(O). Cell survival or death was estimated by LDH assays. *In vivo*, adult SD rats were subjected to controlled cortical impact (CCI, with 4m/s velocity, 2mm deformation). Five hours after injury, PFT-α or

PFT-α(O) (2 mg/kg, i.v.) was administered to rats. Sensorimotor and cognitive functions were evaluated by behavioural tests. Apoptotic cells and p53-positive neurons were identified by doubled staining with cell -specific markers. Levels of mRNA encoding for proinflammatory related proteins (IL-6 and IL-1β) and p53-regulated genes (BAX, PUMA, Bcl-2 and p21) were measured by reverse transcription followed by real time-PCR from TBI without or with PFT- α (O) treatment. We found that PFT-α(O) (10uM) enhanced neuronal survival against glutamate-induced cytotoxicity more effectively than PFT-α (10uM). PFT-α (O) treatment enhanced functional recovery and decreased contusion volume at 24 hours post-injury. Neuroprotection by PFT-α (O) treatment also reduced p53-positive neurons in the cortical contusion region and the area CA1 of the rat hippocampus. In addition, Pro-inflammatory cytokines (IL-1β, IL-6) andp53-regulatedPUMA mRNA levels at 8h significantly reduced by PFT-α(O) administration after TBI. Our data suggest that post-trauma administration of PFT-α (O) improves histological and functional outcomes after experimental TBI and reduces inflammatory responses and apoptosis. The inhibition of p53-induced apoptosis by PFT-α (O) may develop into a novel neuroprotective strategy for TBI.

Disclosures: L. Yang: None. **K. Chang:** None. **Y. Chu:** None. **N. Greig:** None. **J. Wang:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.11/R5

Topic: C.10. Trauma

Title: A new treatment method for severe TBI

Authors: *S. LU;
411th Navy Hosp, Shanghai, China

Abstracts: Research Program on the Application of Open Cerebral Decompression in Severe Cerebral Injury **Background** The mortality and disability rate in TBI, especially in severe TBI (Glasgow 3-8), now still remains high. Of the 150,000 patients in the United States who died of trauma each past year one-third (about 50,000) had developed secondary cerebral injury. In China, the death rate in severe craniocerebral injury (GSC 3-5) is as high as 90%. Therefore it can be seen that the current method used for the treatment of severe TBI and its curative effect are unsatisfactory. In this paper, the author suggests a method of open cerebral

decompression for patients of severe craniocerebral injury according to his years of experience, practice and study in neurosurgery so as to make a fundamental change in the treatment of severe craniocerebral injury. **Object** To make a substantial increase in the survival rate and a decrease in the death rate in the treatment of severe TBI patients through a series of systematic study of open cerebral decompression and therefore make a major breakthrough in the treatment of severe TBI patient **Matters Relating to Collaboration** The author is seeking a collaborator who is interested in the application of open decompression in severe craniocerebral injury to make a joint research. It is proposed that: A. The two sides should sign a cooperative research project contract (on the experiment and clinical study of the application of open decompression in severe craniocerebral injury). B. All the funds for research should be offered by the collaborator side or acquired through the brain trauma foundation or other channels. C. The sites for experimental studies and the necessary experimental researchers should be offered by the collaborator side. The author should participate in the joint research. D. The author is intended to apply for the registration of three invention patents in the collaborator's country. If the side of collaborator succeeds in assisting the medium to transfer the possession of the patents, the two sides should share the benefits (with the costs in paying patent application fee, annual fee, taxes, etc. deducted) in the ratio of 5:5. **A Brief Description of the Three Patents** A. Non-bleeding scalp incision nail B. Skull protective device C. Tentorium cerebelli incision knife

Disclosures: S. Lu: None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.12/R6

Topic: C.10. Trauma

Support: Veterans Affairs Rehab R&D, BRRC Center Grant B6793C

BRRC Innovation Award No. 0612BRRC-7

Merit Review # B6570R and B78071

Title: Ischemic lesion in the white matter produces enduring spasticity in a rodent model

Authors: *P. K. BOSE[1,2,3], G. MUSTAFA[2], J. HOU[2], R. NELSON[1], J. DALY[1,3], S. E. NADEAU[1,3], S. DORE[4], F. J. THOMPSON[1,2,5];

[1]North Florida/South Georgia VAMC, Gainesville, FL; [2]Physiological Sci., [3]Neurol., [4]Anesthesiol., [5]Neurosci., Univ. of Florida, Gainesville, FL

Abstracts: The human periventricular white matter (PVWM), a vascular endzone, is particularly vulnerable to injury in middle cerebral artery strokes and ischemic damage and this site is the primary cause of enduring paresis and spasticity. Rehabilitation approaches leveraging use-dependent neuroplasticity have improved recovery but the ultimate potential for recovery is limited by loss of WM substrate. There is a critical lack of knowledge regarding the full neurobiological spectrum of the injury. Little is known, for example, about the functional potential of damaged neurons in overlying motor cortices. A preclinical model is needed that fully recapitulates injury to PVWM in humans, thereby providing a means to fully study the neurobiological consequences of this ischemic lesion and a test platform for the development and refinement of effective rehabilitation therapies. In this study, we used simultaneous stereotaxic microinjection of two potent vasoconstrictors, endothelin-1 (ET-1) and L-NIO, into the internal capsule (IC) of adult anesthetized rats (2.04 mm posterior and 3.6 mm lateral to bregma) to produce the WM injury. During post-operative follow-up, the velocity-dependent ankle torques and time-locked triceps surae EMGs were recorded as a quantitative measure of spasticity across a wide range of velocities (612 - 49 deg/sec) to permit analyses of tonic (low velocity) and dynamic (high velocity) contributions to lower limb spasticity. The ET-1+L-NIO injected animals developed both dynamic and tonic spasticity accompanied by time-locked elevation of triceps surae EMGs. This spasticity persisted at post-injection week 12. Moreover, injected animals exhibited hemiparesis and placing deficits contralateral to the injection side. The 3-D angular Kinematics (Vicon) and the Catwalk gait analyses (Noldus) also revealed significant and detectable deficits in gait kinematics, step sequence, phase dispersion, and impairment of other gait parameters in the limbs contralateral to injection site. However, these deficits in gait parameters were not enduring, and significant spontaneous recovery occurred within 2 weeks following the injections. Fluoro-Jade staining of the post-fixed brain samples revealed significant demyelination in the IC. Our data to date suggest that ET-1+L-NIO injection in the IC can produce an ischemic lesion in the WM sufficient to produce enduring spasticity and detectable deficits in the gait that are relevant to human stroke. This preclinical injury model may enable better understanding of the neurobiology of WM injury and the development and refinement of effective therapies.

Disclosures: **P.K. Bose:** None. **G. Mustafa:** None. **J. Hou:** None. **R. Nelson:** None. **J. Daly:** None. **S.E. Nadeau:** None. **S. Dore:** None. **F.J. Thompson:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.13/R7

Topic: C.10. Trauma

Support: Veterans Affairs RR&D Merit Review Grant B6570R

Veterans Affairs RR&D Merit Review Grant B78071

Title: Mild traumatic brain injury (mTBI) produces alteration in orofacial and peripheral pain in a rodent model

Authors: *G. MUSTAFA[1,2], J. HOU[1,2], S. TSUDA[1,2], R. NELSON[1], A. SINHAROY[1], R. M. CAUDLE[3], J. K. NEUBERT[4], F. J. THOMPSON[1,2], P. BOSE[1,2,5];
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Abstracts: TBI-induced diffuse axonal injury results chronic pain syndromes in patients including neuropathic, central and thalamic pains which are often difficult to manage. TBI-induced pain has not been well studied, although the prevalence of chronic pain is common among TBI patients, and is higher (72%) in mild TBI than severe TBI (32%). It is proposed that TBI-induced neuroplasticity is likely to affect both central and peripheral mechanism of pain processing and may include a wide array of changes in the expression of pain signaling molecules including serotonin (5-HT), norepinephrine (NE), GABA and substance P. Accordingly, a detailed study of the distribution of these molecules in both spinothalamic and spinal trigeminal pathway, could build upon the strengths of this line of inquiry. In particular, new insights could provide a better understanding of chronic pain secondary to TBI such as post-TBI headache pain, neck and shoulder pain, back pain, orofacial pain, and neuropathic pain. Therefore, the primary purpose of this study is to provide a comprehensive and quantitative evaluation of TBI-induced thermal sensitivity and to identify changes in several key signaling molecules that are known to relate to pain modality. We used a rodent model of closed head mTBI (modified Marmarau's model, 450 g X 1.25 m, anesthetized helmeted adult Sprague Dawley rats) and measured thermal sensitivity in hind-paws and orofacial regions using a conventional hot plate assay and an orofacial pain assessment device respectively before and after mTBI. Our data to date indicate that animals with mild TBI exhibit significant increases in thermal sensitivity starting at 4 weeks post-TBI; this increased sensitivity was enduring and was prominent when testing was repeated at post-TBI week 8. A separate cohort of animals showed significant increases in their facial thermal sensitivity compare to that of the pre-TBI state. Immunohistochemical studies of 5-HT, GABA, NE and substance P receptors (NKR1) in post-fixed dorsal root ganglia, and brain samples indicate significant increases in serotonin immunoreactivity in the ventral posteromedial thalamic nucleus, and also in the facial nucleus

after mTBI. Both GABA and DβH expressions are increased in the spinal trigeminal nuclei, Cuneate and Gracile nuclei, while up-regulation of NK1R was found in trigeminal nuclei in the mTBI tissues compared to levels tested in normal tissues. These data indicate a wide array of changes in the pain signaling molecules following mTBI. Accordingly, we propose a working hypothesis that includes an interconnected neuromodulatory role of these key molecules in the development chronic pain over time after mTBI.

Disclosures: G. Mustafa: None. **J. Hou:** None. **S. Tsuda:** None. **R. Nelson:** None. **A. Sinharoy:** None. **R.M. Caudle:** None. **J.K. Neubert:** None. **F.J. Thompson:** None. **P. Bose:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.14/R8

Topic: C.10. Trauma

Support: Medtronic Inc.

Veterans Affairs (VA) Merit Review # B6570R

Title: Acute intrathecal baclofen (ITB) reduces TBI-Induced spasticity in a dose-dependent manner without adversely affecting cognitive performance

Authors: *F. J. THOMPSON[1,3,4], J. HOU[3], R. NELSON[2], G. MUSTAFA[3], A. SINHAROY[2], R. PANDEY[5], Z. WILKIE[2], S. TSUDA[3], L. PAGE[7], P. BOSE[2,3,6];
[1]Brain Rehabil. Res. Ctr., North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; [2]Brain Rehabil. Res. Ctr., North Florida/South Georgia Veterans Hlth. Syst., GAINESVILLE, FL; [3]Physiological Sci., [4]Neurosci., [5]Pediatrics, [6]Neurol., Univ. of Florida, Gainesville, FL; [7]Medtronic Neuromodulation,Targeted Drug Delivery R & D, Medtronic, Minneapolis, MN

Abstracts: Spasticity is a major health problem for patients with moderate to severe traumatic brain injury (TBI). Although the use of anti-spastic medications, particularly ITB, can decrease the severity of TBI-spasticity, the current federal guidelines preclude the use of ITB therapy during the first year following TBI due to insufficient data to determine potential risk associated with early therapies on cognitive function, balance, and motor recovery. Therefore, an aim of the present study was to provide a comprehensive evaluation of the safety, feasibility, and efficacy

of early intervention treatments (initiated at one week after TBI and continued for 4 weeks) on the long-term outcome of spasticity, cognition function, and balance recovery. These studies tested three different doses: low (0.4μg/hr), medium (0.8μg/hr), and high (1.6μg/hr) of ITB (Lioresal®) treatments using Alzet osmotic mini-pumps in a TBI-induced spastic rodent laboratory model that we recently reported. These studies utilized tests of lower limb spasticity, balance, and serial learning to compare data obtained from normal, TBI-untreated, and TBI-ITB treated animals. Velocity dependent ankle torque & ankle extensor muscle EMGs (as measures of spasticity), rotorod balance performance, and the Morris water maze for spatial learning were used to investigate the dose-response effects of these early ITB treatment doses. Our data to date indicate that compared with time-matched data obtained from untreated TBI animals, ITB treatment significantly reduced spasticity in a dose-dependent manner. The higher dose (6.4 μg/μl) blocked the early (post-ITB treatment week 1) and late onset (post-ITB treatment week 3) spasticity with no negative impact on cognitive performances. However, animals receiving this dose, exhibited balance test deficiencies. In contrast, the medium dose blocked the early onset spasticity, significantly attenuated the late onset spasticity, and produced no negative impact on balance and cognitive performances. The lowest dose only attenuated the spasticity level (i.e. did not block) in both early and late stages of spasticity development with no adverse impact on balance and cognitive performances. These observations indicated that initiating ITB at one week post-TBI and continuing for one month of treatment using a medium dose (0.8μg/hr) was safe, feasible, and effective without detectable impact on cognitive, balance, and motor recovery. These studies also indicate that progressively, the data will reinforce confidence in the safety, feasibility, and efficacy of early intervention treatments for TBI-spasticity.

Disclosures: **F.J. Thompson:** None. **J. Hou:** None. **R. Nelson:** None. **G. Mustafa:** None. **A. Sinharoy:** None. **R. Pandey:** None. **Z. Wilkie:** None. **S. Tsuda:** None. **L. Page:** None. **P. Bose:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.15/R9

Topic: C.10. Trauma

Support: Veterans Affairs RR&D Merit Review Grants B6570R and B78071

Title: Transcranial magnetic stimulation (TMS) improves TBI-induced spasticity, balance and anxiety disorders

Authors: *J. HOU[1,2], R. NELSON[1], Z. WILKIE[1], J. JOHN[1], G. MUSTAFA[2], S. TSUDA[2], A. SINHAROY[1], R. PANDEY[3], P. BOSE[1,2,4], F. J. THOMPSON[1,2,5];
[1]North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; [2]Physiological Sci., [3]Pediatrics, [4]Neurol., [5]Neurosci., Univ. of Florida, Gainesville, FL

Abstracts: Mild traumatic brain injury (mTBI) is a common cause of military combat, sports and car accidents which can produce life-long disabilities including motor, anxiety and cognitive deficits. Currently, no clear understanding exists regarding the neurobiology of these long-term disabilities and thus the effective therapy still remained limited. Therefore, development of a safe and effective therapy for these long-term disabilities is urgently needed. Accordingly, the present study was conducted to test the therapeutic effects of TMS on our recently developed rodent model of mTBI which produce enduring spasticity, balance, anxiety and cognitive deficits. Mild TBI was produced by dropping a 450 g impactor onto the helmeted skull from 1.25 m height (modified Marmarou model) in anesthetized adult Sprague Dawley rats. The TMS was delivered three times per week for one month. The TMS treatments consisted of 75 single pulses TMS delivered to the surface of the cranium through a 25mm figure 8 coil (Magstim Rapid, UK) using an intensity ladder protocol that we recently reported (Hou et al., 2014). The velocity-dependent ankle torques (AT) and time-locked triceps surae EMGs were recorded as a measure of spasticity at pre-injury and PO week 5. At the completion of treatment (PO week 5), the untreated TBI animals showed significant increases in AT and EMGs (44.4% and 84.9%, respectively, compared to pre-injury level) at the highest test velocity. In contrast, the TMS treated animals revealed a significant reduction in spasticity (86.8% reduction AT). Balance performance was also measured using Rotorod. The TMS treated group showed 14% improvement in rotorod balance performance compared to TBI-untreated group. Anxiety behavior was measured using an elevated plus maze (EPM) and automated tracking software (EthoVision XT, Noldus Information Technology). The TBI animals spent significantly less time in the open arms compared to intact animals (1% vs. 36%). The TMS treatment group spent significantly longer time in the open arms, 10% more than the TBI untreated group. The Morris water maze (MWM) was used to assess cognitive function. Both TBI untreated and TMS treated groups showed significant increases in the latency and total distances to locate the platform compared to intact animals. However, no changes in serial learning were detected between TBI TMS treated and TBI-untreated animals. Our data to date indicate that TMS treatment significantly reduced the tested TBI-induced spasticity, balance, and anxiety disorders. Studies are on progress to further investigate the long-term residual therapeutic effects of TMS treatment on these disabilities utilizing chronic time points.

Disclosures: **J. Hou:** None. **R. Nelson:** None. **Z. Wilkie:** None. **J. John:** None. **G. Mustafa:** None. **S. Tsuda:** None. **A. Sinharoy:** None. **R. Pandey:** None. **P. Bose:** None. **F.J. Thompson:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

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Program#/Poster: 608.16/R10

Topic: C.10. Trauma

Support: Veterans Affairs RR&D Merit Review Grant B6570R

Veterans Affairs RR&D Merit Review Grant B78071

Title: Closed-head traumatic brain injury (cTBI) disrupts the integrity of central noradrenergic system in rat

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Abstracts: cTBI has been a major component of combat, sport, and other accident-related injuries which lead to life-long multiple morbidities in survived individuals, including cognitive, anxiety, and pain disorders. However, currently, no clear understanding exists regarding the eurobiology of long-term disabilities induced by cTBI, and no established herapeutic treatment has been available for these morbidities. We have eported that TBI dsrupts noradrenergic (NA) cells in locus coeruleus (LC, the principal site for norepinephrine [NE] biosynthesis) and their projections to spinal cord. However, it is not known to what extent NA cells in LC and NA fiber projections to specific neuronal substrate centers known to be involved in these morbidities are affected by cTBI. Considering the wide distribution of NE-releasing axons throughout the central nervous system (CNS) and well-known physiological roles of NE in related behavioral functions, NE potentially plays a prominent role in the pathophysiology of these morbidities. Accordingly, the purpose of this study is to provide a more comprehensive and quantitative evaluation of cTBI-induced alterations in LC cells and NA fiber projections to the neuronal substrate centers related to these morbidities. In the present study, we used post-fixed brain tissue samples taken from animals 4 months after cTBI (Marmarou TBI Model, 450g x 1.25 m) in which these multiple morbidities (cognitive, anxiety and pain disorders) were detected. Coronal sections of the brain (40μm) were stained for dopamine β-hydroxylase (DβH, a surrogate marker for NA-cells/fibers) and NE using standard fluorescent immunohistochemical techniques**.** Stained sections were then quantitatively analyzed by counting the total number of DβH-positive LC

cells and the DβH-positive fibers. Our results to date revealed that cTBI resulted in significant loss of DβH-positive neuronal cells in both dorsal and ventral aspects of the LC. However, the exact topographic positions of LC cell loss varied among individual cTBIs. There was significant reduction of DβH-positive fibers in the central nucleus of amygdala. Double immunohistochemistry revealed colocalization of DβH-positive neuronal fibers with NE. These results suggest that cTBI disrupts the neurobiological integrity of central noradrenergic system which provides significant inputs to the substrate centers related to anxiety, cognitive and pain disorders. These decreases in the central NA cells and fibers may coherently tie to many seemingly disparate CNS disabilities induced by cTBI.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.17/R11

Topic: C.10. Trauma

Support: NIH Intramural

Title: Moderate docosahexaenoic acid deficiency impairs recovery from traumatic brain injury

Authors: *J. BARNES, A. DESAI, K. KEVALA, M. RASHID, H.-Y. KIM;
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Abstracts: Recent studies have shown that omega-3 (n-3) fatty acids, particularly docosahexaenoic acid (DHA 22:6 n-3), are important for brain development and function and improves outcome of traumatic brain injury (TBI). In the current study, we have examined the effects of DHA on the behavioral and histological outcome of brain injury in a mouse model of TBI inflicted by controlled cortical impact (CCI). Brain DHA status was manipulated by dietary means from pregnancy, and the offspring animals were tested at around 5 months age for motor function using rotarod/beam walk tests and cognitive behavior such as learning and memory using fear conditioning. Protein expression changes were evaluated by western blotting and immunohistochemistry. The mice with higher brain DHA status (by approximately 30%) exhibited significantly faster motor deficit recovery as assessed by beam walk and rotarod tests. Also, the group with higher brain DHA status showed significantly more freezing behavior

during contextual fear conditioning tests indicating greater fear memory. Preliminary results indicated that DHA modulated the expression of proteins implicated in inflammation, such as IkBa, as well as proinflammatory metabolites such as PGE2 in TBI mice. The phosphoTau level after TBI was also modulated according to the brain DHA status, indicating that DHA has a role in axonal pathology after brain injury. Moreover, the TBI-induced cleavage of the cytoskeletal protein alpha-spectrin was also less in mice that had more brain DHA, indicating lesser activation of calpain. These data suggest that DHA status in the brain is relevant to the outcome of TBI. Higher brain DHA ameliorates pathophysiology associated with TBI and improves functional recovery.

Disclosures: J. Barnes: None. **A. Desai:** None. **K. Kevala:** None. **M. Rashid:** None. **H. Kim:** None.

Poster

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NIH Grant R21NS075338

AHA Award-0840110N

Title: Preservation of whisker sensation due to Wnt administration following traumatic brain injury in the barrel cortex of mice

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Abstracts: Traumatic brain injury (TBI) is the leading cause of morbidity and mortality in children and young adults in the US. However, there are currently no effective pharmacological therapies. We previously reported on the effectiveness of Wnt therapy for promoting functional

recovery via the augmentation of endogenous neurogenesis. Since then, we have discovered that Wnt may play a neuroprotective role as well, and that this secondary benefit contributes to the functional preservation during the acute stages following TBI. We delivered the injury using the well-established controlled cortical impact (CCI) model of TBI in C57BL/6 mice (male, 11 week old). The injury was directed to the barrel cortex, which is the domain involved in whisker sensation. Three experimental groups included 1) sham surgery, 2) TBI plus intranasal administration of a recombinant Wnt ligand, and 3) TBI plus saline control. After the surgery, two behavior assays, the corner test and a home cage monitoring system, were used to assess behavioral recovery from TBI. The corner test is sensitive for whisker sensation and was performed on post-surgical days 3, 7, and 14, while the home cage monitor measures a variety of native behavior over the course of 24 hours and was performed on post-surgical days 2, 7, and 14. Mice received training and baseline establishment for these two tests before the TBI. Furthermore, mice were sacrificed at either 2 days or 14 days after TBI for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining and Nissl analysis. To assess the extent of cell death, the brains were sectioned (10 μm thickness) and stained for TUNEL and NeuN, which are markers of cell death and mature neurons, respectively. Stereological counting showed that Wnt administration resulted in neuroprotection, with a significant reduction in TUNEL+ cells and TUNEL+/NeuN+ colabeled cells in Wnt-treated animals vs. the saline treated TBI controls. In functional tests, we found that the Wnt-treated mice were more resilient against the whisker sensory deficits following the barrel cortex injury, with significant reductions in aberrant turning behavior for Wnt-treated animals, as scored by the corner test. Furthermore, the home cage test showed that On day 14, saline-treated TBI animals displayed significantly attenuated total sleep times as compared to both sham animals and to Wnt-treated animals. Wnt therapy preserved sleep habits following TBI, which is associated with insomnia and sleep disturbances. In summary, the Wnt pathway is suggested to be an efficacious therapeutic target that can provide both neuronal and functional protection following TBI.

Disclosures: **J.Y. Zhang:** None. **J. Lee:** None. **X. Gu:** None. **S.P. Yu:** None. **L. Wei:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

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Topic: C.10. Trauma

Support: VA Merit Pilot award (PY)

Maryland Stem Cell Research Fund (PY).

Title: Magnetic retention of human neuroprogenitor cells transplanted into a traumatic brain injury model

Authors: W.-B. SHEN[1], C. PLACHEZ[1], D. YARNELL[7], O. TSYMBALYUK[2], S. XU[3], A. C. PUCHE[4], J. SIMARD[2,7], P. S. FISHMAN[5,7], *P. J. YAROWSKY[6,7];
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Abstracts: Stem cell therapy has been suggested as a treatment for traumatic brain injury (TBI). But a non-invasive delivery method and retaining stem cells at the site of TBI has not been addressed. We have devised a method of loading human neuroprogenitor cells (hNPCs) with superparamagnetic iron-oxide nanoparticles (SPION, Molday ION Rhodamine B™; MIRB; BioPAL, Worcester, MA) and assessed the ability of external magnets to retain MIRB-labeled hNPCs at the site of injury following transplantation. MIRB labeling does not affect hNPCs viability or proliferation or differentiation *in vitro* [Shen et al., 2013]. We tested 1) the viability of MIRB-hNPCs *in vitro* in a magnetic field; and 2) the survival, of MIRB-hNPCs in host rat brains after graft. First, we optimized the MIRB labeling conditions for dissociated hNPCs and hNPC neurospheres, including the concentration of MIRB, time for effective labeling hNPCs, and the time of labeled hNPCs hold the SPION. We found that hNPC lysosomes (Lamp1+) contained SPION. It remained stable inside the hNPCs over 3 weeks in cell culture (longest time tested). By fixing a magnet on the scalp on the same side as the injury prior to transplantation, hNPCs were retained at the site of injury in the cortex more than two-fold greater as compared to hNPC-injected TBI animals without a magnet. Detection of magnet-enhanced hNPC-MIRB retention was also found using T2-weighted MRI (Magnetic Resonance Imaging) and confirmed by Perl's staining. Post-transplantation, the cortex under the magnet had a greater percentage of live hNPC-MIRB cells than the comparable cortex in the non-magnet animal. Thus the delivery pathway and the method of retaining the cells are appropriate for this preclinical model in order to evaluate the effectiveness of this stem cell therapy.

Disclosures: W. Shen: None. **C. Plachez:** None. **D. Yarnell:** None. **O. Tsymbalyuk:** None. **S. Xu:** None. **A.C. Puche:** None. **J. Simard:** None. **P.S. Fishman:** None. **P.J. Yarowsky:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

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Topic: C.10. Trauma

Support: NIH Intramural

Department of Defense

Title: Behavioral changes in docosahexaenoic acid deficient female mice after traumatic brain injury

Authors: *A. DESAI, J. BARNES, K. KEVALA, H.-Y. KIM;
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Abstracts: Traumatic brain injury (TBI) may not only lead deficits in cognitive and vestibulomotor functions, it may also cause emotional problems. Deficiency of n-3 polyunsaturated fatty acids (PUFA), especially docosahexaenoic acid (DHA) has been linked to cognitive abnormalities as well as anxiety and depression. Most published studies that link DHA levels to recovery from TBI have used male mice, considering the influences of hormonal fluctuation in female mice. The objective of this study is to evaluate whether the recovery from TBI in female mice is similarly affected by DHA deficiency. Pregnant C57BL/6N mice (E14) were placed on n-3 deficient or n-3 adequate control diet. Female pups were weaned on the same diet and controlled cortical impact was performed on these mice at 5-6 months of age. The mice were then subjected to a battery of tests that consisted of beam walk test, open field test, light dark transition test, tail suspension test, forced swim test and fear conditioning to compare motor recovery, anxiety-like and depression-like behaviors and fear learning. The n-3 deficient diet significantly lowered DHA level in the brain, which was compensated by higher docosapentenoic acid (DPA) levels. The DHA deficient mice had impaired motor recovery and relatively poor memory as compared to the controls, implicating DHA deficiency as an important factor that may predict spontaneous recovery after TBI. However, significant differences were not found in anxiety-like and depression-like behavior. This study indicates that despite hormonal differences, DHA depletion can impair recovery in female mice.

Disclosures: **A. Desai:** None. **J. Barnes:** None. **K. Kevala:** None. **H. Kim:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

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Program#/Poster: 608.21/S3

Topic: C.10. Trauma

Support: Ontario Neurotrauma Foundation

Title: Classifying pediatric traumatic brain injury using an automated digital measurement algorithm to detect cerebral edema

Authors: *E. TA[1], S. LAUGHLIN[4], C. S. PARSHURAM[2], J. HUTCHISON[3], A. M. GUERGUERIAN[3], Y. INVESTIGATORS[5];
[2]Child Hlth. Evaluative Sci., [3]Neurosci. and Mental Hlth. Program, [1]The Hosp. For Sick Children - Res. Inst., Toronto, ON, Canada; [4]Radiology, The Hosp. For Sick Children, Toronto, ON, Canada; [5]Canadian Critical Care Trials Group, Toronto, ON, Canada

Abstracts: Introduction/Rationale: Traumatic brain injury is the leading cause for long-term disability and death in North American children. In the absence of a reliable and objective method of classifying injury severity, there is a growing need for early identification and classification tools. Computed tomography (CT) is the most accessible imaging modality for front-line clinical care in children with TBI and new software applications may be used to detect cerebral edema, a risk factor for poor outcome. While magnetic resonance imaging is more sensitive, it is far less accessible than CTs immediately after injury. This project will assess the ability of an automated digital measurement algorithm (ADMA) that can predict the need for medical interventions and outcome at hospital discharge by quantifying cerebral edema detectable on initial CT scan. The diagnostic accuracy of ADMA is hypothesized to be better than usual radiological interpretation and is expected to be associated with outcomes as measured by the Pediatric Cerebral Performance Category score. Experimental Approach: We propose to apply ADMA to CT scans of two epidemiological cohorts of children hospitalized with TBI (120 children; age: 0 -18 years) using MIPAV (Medical Image Processing, Analysis, and Visualization) software, using an algorithm developed with special considerations to the developmental brain maturity of the subjects. The first step of this project was to measure the capacity of ADMA to predict overall functional outcome. Results: Preliminary results in 77 subjects suggest the capacity to classify children with and without new cerebral dysfunction on hospital discharge using the Pediatric Cerebral Performance Categorical scale has an area under the receiver operating characteristic curve of 0.8083 (95% CI: 0.6756 - 0.8976). Impact of Research: We report promising findings for quantifying visible cerebral edema on standard clinical CT scans in a selected sample of children following TBI. Our future goal is to cross validate our findings in a separate sample, to evaluate the relationship to late brain MRI findings, all with a vision of producing a rapid objective method for to classify TBI in children early after injury.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.22/S4

Topic: C.10. Trauma

Support: Israel Ministry of Science, Technology and Space

Title: Neurotherapeutic effect in mice after traumatic brain injury by CD45+ hematopoietic cells from human cord blood

Authors: *P. LAZAROVICI[1], H. ARIEN-ZAKAY[1], G. GINCBERG[1], A. NAGLER[2], G. COHEN[1], S. LIRAZ-ZALTSMAN[1], V. TREMBOVLER[1], A. G. ALEXANDROVICH[1], I. MATOK[1], H. GALSKI[2], U. ELCHALAL[3], P. LELKES[4], E. SHOHAMI[1];
[1]Hebrew Univ. Jerusalem, Jerusalem, Israel; [2]Chaim Sheba Med. Ctr., Tel-Hashomer, Israel; [3]Hadassah Univ. Hosp., Jerusalem, Israel; [4]Temple Univ., Philadelphia, PA

Abstracts: Treatment of traumatic brain injury (TBI) is still an unmet need. Cell therapy by human umbilical cord blood (HUCB) has shown promising results in animal models of TBI and is under evaluation in clinical trials. HUCB contains different cell populations, but to date only mesenchymal stem cells have been evaluated for therapy of TBI. Here we present the neurotherapeutic effect, as evaluated by neurological score, using a single dose of HUCB-derived mononuclear cells (MNCs) upon intravenous (iv) administration one day post-trauma in a mouse model of closed head injury (CHI). Delayed (8 days post-trauma) intracerebroventricular administration of MNCs showed improved neurobehavioral deficits thereby extending the therapeutic window for treating TBI. Furthermore, we demonstrated for the first time, that HUCB-derived pan-hematopoietic CD45 positive (CD45+) cells, isolated by magnetic sorting and characterized by expression of CD45 and CD11b markers (96-99%), improved the neurobehavioral deficits upon iv administration, which persisted for 35 days. The therapeutic effect was in a direct correlation to a reduction in the lesion volume and decreased by pre-treatment of the cells with anti-human-CD45 antibody. At the site of brain injury, 1.5-2 h after transplantation, HUCB-derived cells were identified by near infrared scanning and immunohistochemistry using anti-human-CD45 and anti-human-nuclei antibodies. NGF and VEGF levels were differentially expressed in both ipsilateral and contralateral brain hemispheres, thirty-five days after CHI, measured by ELISA. These findings indicate the

neurotherapeutic potential of HUCB-derived CD45+ cell population in a mouse model of TBI and propose their use in the clinical setting of human TBI.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

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Topic: C.10. Trauma

Support: AHA 13GRNT15730001

NIH K01AG031926

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NIH R01NS078026

Title: Flavanol (-)-epicatechin is neuroprotective after intracerebral hemorrhage

Authors: ***J. WANG**, C.-F. CHANG, S. CHO;
Anesthesiology/Critical Care Med., Johns Hopkins Univ., Sch. of Med., BALTIMORE, MD

Abstracts: In the wake of intracerebral hemorrhage (ICH), a devastating stroke with no effective treatment, hemoglobin/iron-induced oxidative injury leads to neuronal loss and poor neurologic outcomes. (-)-Epicatechin (EC), a brain-permeable flavanol that modulates redox/oxidative stress via the NF-E2-related factor (Nrf) 2 pathway, has been shown to be beneficial for vascular and cognitive function in humans. Here, we examined whether EC can reduce early brain injury in ICH mouse models and investigated the underlying mechanisms. ICH was induced by injecting collagenase, autologous blood, or thrombin into mouse striatum. EC was administered orally at 3 h after ICH and then every 24 h for 3 days. Lesion volume, neurologic deficits, brain edema, reactive oxygen species, and protein expression and activity were evaluated. EC significantly reduced lesion volume and ameliorated neurologic deficits in both male and female ICH mice. Cell death and neuronal degeneration were decreased in the perihematomal area and were

associated with reductions in caspase-3 activity and HMGB-1 level. These changes were accompanied by attenuation of oxidative insults, increased phase II enzyme expression, and increased Nrf2 nuclear accumulation. Interestingly, in addition to providing neuroprotection via Nrf2 signaling, EC diminished heme oxygenase-1 induction and brain iron deposition via an Nrf2-independent pathway that downregulated ICH-induced activating protein-1 activation and decreased matrix metalloproteinase 9 activity, lipocalin-2 levels, iron-dependent cell death, and ferroptosis-related gene expression. Collectively, our data show that EC protects against ICH by activation of Nrf2-dependent and Nrf2-independent pathways and may serve as a potential intervention for patients with ICH.

Disclosures: J. Wang: None. **C. Chang:** None. **S. Cho:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

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Program#/Poster: 608.24/S6

Topic: C.10. Trauma

Support: CBIR14FEL005

Title: Digitally controlling the biomechanics of fluid percussion injury to better understand the range of mild tbi

Authors: *M. LONG[1,2], S. P. SINHA[3,2], N. NADPARA[4], K. PANG[5,2], B. PFISTER[1]; [1]Dept. of Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [2]Grad. Sch. of Biomed. Sci., Rutgers Biomed. and Hlth. Sci., Newark, NJ; [3]Dept. of Neurol. and Neurosci., New Jersey Med. Sch., Newark, NJ; [4]Dept. of Biol., The Col. of New Jersey, Ewing Township, NJ; [5]Veterans Affairs Med. Ctr., East Orange, NJ

Abstracts: Awareness, diagnosis and management of mild traumatic brain injury (mTBI) are often difficult and not well defined. Furthermore, understanding the neural mechanisms involved in mTBI in the clinical setting remains elusive. However animal models of mTBI provide the ability to investigate pathologies - not otherwise available in humans. Many studies involving animal models of mTBI use magnitude levels of 1.0 - 1.5 atm and average 20 ms duration of impact. However, the classification of mild TBI is a wide range that should encompass, not only the high end, but also include magnitudes that are lower than 1.0 atm or have shorter temporal duration then previously reported. Here, we introduce a novel voice-coil fluid percussion injury

(vFPI) device that digitally delivers a reproducible and well-controlled percussion with control over the magnitude, rise time and duration of the pressure wave that can target the wide range of mTBI. First, We aim to compare our vFPI to the traditional pendulum fluid percussion injury (pFPI) device. We will assess the capabilities of the vFPI to that of the pFPI to produce injury duration comparable to current head impact studies. Adolescent male Sprague-Dawley rats (approx. 1 month) will be randomly assigned to SHAM or injury group. We will manipulate duration parameters available by each device to mimic head impact studies. The vFPI device efficiently produced ≤ 10 ms injury durations. We assessed acoustic sensory reactivity utilizing the acoustic startle reflex (ASR) test. The neural mechanisms of ASR are well documented and conserved between rodent and humans, thus providing a translational study on functional outcome. Sprague-Dawley rats were matched and randomly assigned into either SHAM or mTBI groups based on pre-injury baseline of ASR (acoustic stimuli: 100 ms white noise bursts at 82, 92 and 102 db). At 24 hrs post-injury, ASR showed persistent attenuation in mTBI subjects compared to SHAMS lasting up to 4 weeks post injury. Finally we targeted a wide-range of mTBI injury magnitude levels of 0.37 - 2.19 atm and observed the apnea levels of 0.0 - 56.5 s, latency of righting reflex levels of 23 - 1147 s. We aimed to create a device that aids in exploring the lower range of mTBI, and have demonstrated that the vFPI device facilitates this. We demonstrated that acute deficits are still present when the levels of pressure fall below the range suggested in the literature, which supports that mild TBI encompasses a range of parameters that should be studied.

Disclosures: **M. Long:** None. **S.P. Sinha:** None. **K. Pang:** None. **B. Pfister:** None. **N. Nadpara:** None.

Poster

WITHDRAWN

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.25/S7

Topic: C.10. Trauma

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.26/S8

Topic: C.10. Trauma

Support: John D. Dingell VA Medical Center

Wayne State University Department of Psychiatry and Behavioral Neurosciences

Wayne State University Department of Neurosurgery

Title: Enhanced fear learning and increased cortical GABA following mild traumatic brain injury in mice

Authors: *B. SCHNEIDER[1,2], F. GHODDOUSSI[3], J. CHARLTON[1], R. KOHLER[2], S. A. PERRINE[2], A. C. CONTI[1,4,2];
[1]John D. Dingell VA Med. Ctr., Detroit, MI; [2]Psychiatry and Behavioral Neurosci., [3]Anesthesiol., [4]Neurosurg., Wayne State Univ., Detroit, MI

Abstracts: Background Individuals with mild traumatic brain injury (mTBI) often develop symptoms that resemble anxiety, depression, and posttraumatic stress disorder (PTSD). It is unclear how mTBI results in PTSD-like symptoms, although studies suggest decreased prefrontal cortex (PFC) activation alters responses in downstream regions associated with fear learning, such as the amygdala and hippocampus. To investigate this, we used a mouse model of mTBI and examined the effects of mild injury on conditioned fear behavior and neurochemical alterations in the PFC. Methods Anesthetized male C57BL/6 mice (10-12 wks) were impacted over the sagittal suture of the intact skull or exposed to surgery alone (sham controls). PFC was harvested for proton magnetic resonance spectroscopy analysis *ex vivo* at 11.7 T at 8 d post-injury to assess neurochemical levels, including glutamate and gamma-amino-butyric acid (GABA). A second cohort was used to assess fear response (freezing) to contextual fear conditioning at 14 d post-injury. Fear conditioning consisted of 5 phases: habituation, acquisition, extinction, reinstatement, and extinction recall. Results Mice with mTBI demonstrated significantly increased freezing during acquisition and extinction compared to controls. There were no differences in baseline freezing or freezing during reinstatement or extinction recall. GABA levels were significantly increased in the PFC of mTBI mice compared to controls, but levels of glutamate were not different. Discussion The increased acquisition and slower extinction of conditioned fear observed in mTBI mice resemble features of fear conditioning reported in PTSD and mTBI cases. Increased GABA in the PFC may reflect an increase in inhibitory activity and support the hypothesis that mTBI-induced PFC hypoactivity limits top-down control over subcortical areas involved in FC; thereby increasing susceptibility to affective disorders. Therefore, this model of mTBI-induced changes in PFC may give an

improved understanding of mechanisms involved in developing affective alterations following mTBI.

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Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.27/S9

Topic: C.10. Trauma

Support: Seed Funding from the School of Behavioral Health at Loma Linda University

Title: The emergence of a depressive phenotype after mild concussions in the adult mouse

Authors: *N. MISTRY[1], S. HALAVI[1], M. HAMER[2], A. OBENAUS[2,3,4], R. E. HARTMAN[1]; [1]Dept. of Psychology, [2]Dept. of Pediatrics, [3]Div. of Interdisciplinary Studies, Loma Linda Univ., Loma Linda, CA; [4]Cell, Mol. and Developmental Biol. Program, Univ. of California Riverside, Riverside, CA

Abstracts: Mild traumatic brain injuries (mTBI), including concussions, can lead to long-lasting cognitive and motor deficits, increasing the risk of behavioral problems and the development of future neurological disorders. Few studies have comprehensively examined both acute and long-term neurological and behavioral effects from mTBI. Our study focused on long-term behavioral deficits after single or repeated mild closed head injuries (mCHI). Mice received either a single mCHI, a repeated mCHI consisting of a single concussion to each hemisphere separated by 3 days. A moderate controlled cortical impact injury (CCI) along with sham-only anesthesia served as controls. We hypothesized that mice with repeated mCHI would have persistent long-term neurological and behavioral deficits. Neurological and behavioral tests were administered at 1, 3, 5, 7, and 90 days post-injury (dpi). Balance beam impairments began to emerge at 7 dpi in CCI mice and remained at 90 dpi. Impaired performance on the balance beam was only observed at 90 dpi in repeated mCHI mice. Perhaps most interestingly, depression-like behaviors and social passiveness were observed in repeated mCHI animals at 90 dpi. These data suggest that mCHI leads to delayed motor deficits and the emergence of affective disorders that are not observed after CCI.

Disclosures: **N. Mistry:** None. **S. Halavi:** None. **M. Hamer:** None. **A. Obenaus:** None. **R.E. Hartman:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

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Topic: C.10. Trauma

Support: Seed Funding from the School of Behavioral Health at Loma Linda University

Loma Linda University School of Behavioral Health Intramural Grant

Title: The effects of closed-head injury on scent marking behaviors in mice

Authors: ***S. HALAVI**[1], N. MISTRY[1], M. HAMER[2], M. EVANS[2], B. SEMPLE[4], A. OBENAUS[2,3,5], R. HARTMAN[1];
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Abstracts: Depression has been reported as the most common neuropsychiatric consequence of TBI. We have shown that closed head injury (CHI) in mice leads to social passiveness and learned-helplessness/depression-like behaviors on the tail suspension test (TST; see Mistry, et al. poster). In the current study, we assessed the acute and long-term effects of CHI on scent marking behavior in male mice exposed to novel females. Urine and fecal boli were collected on a piece of paper during exploration of an open field (30.5 cm x 30.5 cm) for 20 min. Baseline measurements were collected in an empty open field, and a novel female was placed in a small cage (6.3 cm wide x 10.3 cm tall) in the center of the open field for stimulus trials. Fecal boli were counted and the paper was sprayed with ninhydrin to visualize and quantify urine markings. In the acute experiment, 12 mice underwent baseline scent marking testing (SMT) followed by the presentation of a novel female stimulus 7 days later. Two days later, the mice were randomly assigned to control (n=6) and anesthesia/CHI (n=6) groups. Animals in the anesthesia/CHI group underwent 20 minutes of 3% isoflurane anesthesia. Five days later, both the groups underwent a second novel female stimulus trial. One day after the second stimulus, animals in the anesthesia/CHI group were anesthetized again and given a CHI over the right temporal-parietal cortex. Two more novel female stimuli were presented 3 and 7 days after injury, followed by the

TST 8 days after injury. In the long-term experiment, the mice in the control group (n=6) were anesthetized, and the mice in the treatment group (n=6) were anesthetized and received a CHI over the right temporal-parietal cortex. SMT (baseline and 1 novel female) and the TST were administered 3 months after injury. All mice, at both time points, engaged in significantly more scent-marking behaviors when exposed to a novel stimulus compared to the baseline. In general, CHI mice scent-marked slightly more than controls 7 days after injury, but CHI mice marked less than controls 3 months after injury. Additionally, the groups' TST performance did not differ after 8 days, but CHI mice gave up significantly more quickly on the TST after 3 months, suggesting that a depression-like phenotype may take some time to emerge after CHI. *Funding came from Loma Linda University School of Behavioral Health Seed grants to AO and RH.*

Disclosures: S. Halavi: None. **N. Mistry:** None. **M. Hamer:** None. **M. Evans:** None. **B. Semple:** None. **A. Obenaus:** None. **R. Hartman:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.29/S11

Topic: C.10. Trauma

Support: Indiana CTSI CBR/CTR Pilot Program Grant- UL1TR001108

Title: Impairments in social familiarity-induced anxiolysis (SoFiA) after mild blast-induced traumatic brain injury (mbTBI)

Authors: *S. M. VEGA ALVAREZ[1,2], E. LUNGWITZ[4,5], N. RACE[3], T. R. WARNER[4,5], W. TRUITT[6,5,7], R. SHI[1,3];
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Abstracts: Traumatic brain injury (TBI) recently took center stage as the signature wound of recent war conflicts. As the most common form of TBI, blast-induced traumatic brain injuries (bTBI) occur when the blast waves produce inertial forces leading to strain, shearing and compression of the brain. Due to the multifocal nature of TBI, a complex cascade of distinct but interconnected cellular events is initiated and leads to the development of secondary injuries.

Furthermore, bTBIs have been linked to a variety of pathologies and neurobehavioral deficits, including increased susceptibility to psychiatric, somatic and cognitive symptoms, which have the potential to become permanent. Consequently, the prevalence of mental health disorders observed in injured veterans continues to rapidly increase, which only adds to both direct and indirect economic burden on US economy from TBI. Therefore, understanding, preventing and treating TBIs must be a national priority. While extreme cases of bTBI result in early diagnosis, immediate treatment and monitoring of injury progression, symptoms of mild bTBI (mbTBI) can go unnoticed for quite some time and result in delayed therapeutic intervention. mbTBI are considered primary injuries caused by the overpressure component of the explosive wave and are characterized by absence in visible wounds, loss of consciousness or acute symptoms. Emotional and social deficits have been widely reported in TBI patients and these transient or permanent deficits have also been shown to significantly affect normal daily function. Consequently, by employing a model of mbTBI that closely resembles the human condition, we expect to elucidate subclinical cognitive deficits and behavioral abnormalities involved in altering coping mechanisms during social stress. Thus far, the mbTBI-causing pressure intensity wave has been correlated with a lack of motor deficits, evident by a lack of statistical significance between mbTBI and sham rats in terms of the length of time on a rotarod test. After mbTBI, the animal subjects do not perform different than uninjured rats when tested for cognitive impairment or basal anxiety. Interestingly, an assessment of Social Familiarity-induced Anxiolysis (SoFiA) revealed that, when subjected to a mbTBI, rats present social processing impairments. Hence, our model replicates subclinical cognitive and behavioral effects observed in humans after mbTBI. Currently, we are evaluating the role of oxidative agents, the oxidative stress-triggered inflammatory response and the benefits of multiple therapeutics in mbTBI cognitive and behavioral deficits.

Disclosures: **S.M. Vega Alvarez:** None. **E. Lungwitz:** None. **N. Race:** None. **T.R. Warner:** None. **W. Truitt:** None. **R. Shi:** None.

Poster

608. Traumatic Brain Injury: Mechanisms and Therapeutics II

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 608.30/S12

Topic: C.10. Trauma

Support: Department of Anesthesiology and Critical Care Medicine, and Brain Science Institute, Johns Hopkins University School of Medicine

Title: Neuroinflammation and cognitive deficits in a rabbit pediatric traumatic brain injury model

Authors: *Z. ZHANG[1,2], M. SARASWATI[2,1], R. C. KOEHLER[2,1], C. ROBERTSON[1,2,3], S. KANNAN[2,1,3];
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Abstracts: Traumatic brain injury (TBI) is a common cause of disability in childhood, yet the mechanisms responsible for its complex spectrum of pathologies remain largely unknown. Pediatric rodent models of TBI often do not demonstrate the spectrum of motor and cognitive deficits as seen in pediatric TBI patients. To address these, we developed a model of pediatric TBI in New Zealand rabbits that mimics pediatric brain development better in the case of white matter maturation, microglial presence and response to injury. On postnatal day 5-7 (P5-7), the rabbits were injured with a standardized controlled cortical impact at a velocity of 5.5 m/s and a depth of 2 mm. Rabbits from the same litter were served as control (no intervention) and sham (craniotomy alone). Functional abilities (cranial nerve, motor and sensory functions) and activity levels (open field) were measured before TBI, 1-d and 5-day after TBI. Maturation level was monitored daily. The cognition was tested with spontaneous alternation in T-maze (spatial learning and memory) and novel object recognition at P14-24. Animals were sacrificed 1, 3, 7 and 21 days after TBI surgery for the evaluation of lesion volume (crystal violet staining) and microglia activation [ionized calcium-binding adapter molecule 1 (IBA1) and translocator protein (TSPO) staining]. Significant decrease in the overall motor functions, such as suck and swallow, head and hind leg movements, and a significant increase of muscle tone of the limbs, was noted 1d after TBI (13-23 kits/group, $p<0.05$), which well represent the acute phase of the TBI injury in patients; while these functional changes returned to normal 5 days after injury. In addition, TBI kits showed delayed achievement of normal developmental milestones, and 5/19 kits lost cliff-avoidance. Moreover, TBI kits showed significant cognitive deficits, including less percentage of correct alternation rates in T-maze (9-16 kits/group, $p<0.001$) and less discrimination between novel and old objects (8-12 kits/group, $p<0.001$). Lesion volume increased from 15% at 3-d to 30% at 7-d after injury, indicating ongoing secondary injury. In addition, intense microglial activation and TSPO expression were seen not only at the injury site but also in white matter regions of both the ipsilateral and contralateral hemispheres, representing diffused injury seen in patients. In conclusion, this pediatric TBI model produces short-term and long-term impairments comparable to those reported clinically, providing key insights into human pediatric TBI that may ultimately provide unique opportunities for therapeutic interventions.

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Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.01/T1

Topic: C.10. Trauma

Support: NIH Grant NS082095

Neilsen Foundation Grant 260853

Title: α-Synuclein increases in the injured spinal cord and is linked to axon loss

Authors: *A. D. SAUERBECK, D. M. MCTIGUE;
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Abstracts: Accumulation of the protein α-synuclein is linked to development of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and multiple system atrophy. Recent work suggests that α-synuclein also plays a role after spinal cord injury (SCI). Even though its function in the normal CNS is still under investigation, numerous studies have shown that α-synuclein is linked to neuropathology. Because of chronic inflammation and the presence of toxic molecules like iron in the injured spinal cord, both of which increase the expression of α-synuclein, we hypothesize that SCI induces accumulation of α-synuclein and this contributes to post-injury processes. To test this hypothesis, adult rats received a mid-thoracic spinal contusion. Animals survived for up to 42 days post-injury and expression of α-synuclein was examined by western blot and immunohistochemistry to determine its spatial and temporal distribution. Following moderate SCI, α-synuclein accumulation was present in a subset of axons as early as 12 hours post-injury. Expression of α-synuclein continued to rise over time, reaching significant levels by 1 day post-injury (dpi) and remaining elevated for at least 42 days after SCI. During the first 3 dpi, the number of axons expressing excess α-synuclein rose. The axons with the highest level of α-synuclein expression were eventually lost, linking accumulation of α-synuclein to tissue loss after SCI. Concomitant with axonal α-synuclein accumulation, neighboring astrocytes also upregulated α-synuclein. Subsequent to loss of α-synuclein+ axons, astrocytes maintained robust expression of α-synuclein chronically. Beyond one week post-injury, most of the α-synuclein in the injured spinal cord was present in astrocytes in the spared white matter, with the majority bordering the lesion cavity. Our study shows for the first time that α-synuclein increases after traumatic SCI, localizes in damaged axons and astrocytes, and correlates with progressive tissue loss. α-synuclein accumulation begins by 12 hours post-injury suggesting it is an early pathological event after spinal trauma. These results position α-synuclein

as a possible contributor to acute and progressive cell death and degeneration after SCI. Given the well-established link between excess α-synuclein accumulation and cell death, and that α-synuclein is known to induce a toxic inflammatory response in astrocytes, these studies indicate the targeting α-synuclein may be a novel and exciting therapeutic target for attenuating deficits after SCI.

Disclosures: A.D. Sauerbeck: None. **D.M. McTigue:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.02/T2

Topic: C.10. Trauma

Support: NINDS Grant NS059776

Title: Chronic oligodendrogenesis and remyelination after spinal cord injury in mice and rats

Authors: *Z. C. HESP[1], E. Z. GOLDSTEIN[1], C. J. MIRANDA[3], B. K. KASPAR[3], D. M. MCTIGUE[2];
[2]Neurosci., [1]The Ohio State Univ., Columbus, OH; [3]Nationwide Children's Hosp., Columbus, OH

Abstracts: Spinal cord injury (SCI) induces extensive cell death as well as significant cell proliferation. For example, while oligodendrocyte (OL) apoptosis continues for 2-3 weeks post-SCI, oligodendrocyte precursor cells (OPCs) concurrently proliferate around the lesion area. It is known that a least a portion of these proliferating OPCs differentiate into new OLs in the first two weeks post-injury (wpi). However, for how long OPCs proliferate or differentiate into OLs after SCI is unknown. Our work and that of others show that oligogenic factors are expressed in the spinal cord beyond 2wpi. Therefore, we hypothesized that OPCs continue to proliferate and differentiate beyond 2wpi. To test this hypothesis, mouse and rat SCI models were used. Sprague-Dawley rats and two OPC reporter mouse lines (PDGFαRCreER:ROSA and PDGFαR-CreER:mT/mG) received a moderate spinal contusion. At various times after injury, rats received a 1μl intraspinal injection of a GFP-expressing retrovirus to label proliferating cells, and mice received a 4-day oral regimen of tamoxifen (200-300mg/kg) to induce GFP expression in OPCs. GFP+ OPCs in both rat and mouse models retain GFP expression upon differentiation into OLs. To allow time for differentiation and myelination, animals were perfused 3-4 weeks after

virus/tamoxifen administration. OPC fate was assessed between 0-7wpi in rats and 0-12wpi in mice. Overall, we found evidence that oligodendrogenesis occurred at every time examined. New OLs were generated as late as 8-12wpi in mice and 4-7wpi in rats (the latest time available). New OLs expressed mature myelin proteins and wrapped axons in spared and lesioned tissue. The new OL-derived myelin segments displayed shortened internodes - a sign of remyelination. Functional remyelination was further verified by detecting the paranodal protein Caspr adjacent to new myelin segments. In addition, the expression of the pro-OL survival proteins CNTF and FGF-2 were increased chronically in both models. We conclude that oligodendrogenesis and remyelination are ongoing for 2-3 months after SCI in mice and rats. Additionally, the chronic injury environment is dynamic and amenable to OPC differentiation and functional remyelination. Understanding this endogenous response and chronic tissue milieu will be crucial for planning remyelination-based studies and may also provide insight into environments in which spontaneous OL differentiation occurs in the injured adult nervous system.

Disclosures: Z.C. Hesp: None. **E.Z. Goldstein:** None. **C.J. Miranda:** None. **B.K. Kaspar:** None. **D.M. McTigue:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.03/T3

Topic: C.10. Trauma

Support: NS082095

Title: Toll-like receptor 4 (TLR4) deficiency impairs oligodendrocyte lineage cell responses after spinal cord injury

Authors: *J. S. CHURCH, P. G. POPOVICH, D. M. MCTIGUE;
Neurosci., The Ohio State Univ., Columbus, OH

Abstracts: Prominent oligodendrocyte (OL) loss occurs after spinal cord injury (SCI), however the numbers rebound in the first 7-14 days post-injury (dpi) due to proliferation and differentiation of surviving OL progenitor cells (OPCs) into mature OLs. The mechanisms controlling this endogenous response, however, are not understood. Previous work from our group showed activation of toll-like receptor 4 (TLR4) on microglia induced OPC proliferation and oligodendrogenesis in the intact spinal cord. Thus, the current work tested the hypothesis

that TLR4 signaling contributes to oligodendrogenesis after SCI. Wild-type (WT, HeOuJ) and TLR4-deficient (TLR4d, HeJ) mice were given a midthoracic moderate contusion SCI and sacrificed for spinal cord histology and polymerase chain reaction (PCR) at 1, 7, 14, or 21dpi. Adjacent tissue sections were stained with antibodies recognizing nerve/glial antigen 2 (NG2) expressed by OPCs and glutathione S-transferase pi (GSTpi), a marker of mature OLs. NG2 expression increased over the first week post-injury in epicenter sections of WT and TLR4d mice. NG2 levels were reduced, however, in TLR4d mice rostral to epicenter at 21dpi and caudal to the epicenter from 7 - 21 dpi. The greatest accumulation of NG2 both rostral and caudal was seen in regions of motor tract dieback or degeneration. In both genotypes, significant OL loss occurred by 1dpi but returned to baseline numbers by 14dpi. OL numbers continued to rise above baseline values in WT mice distal to the epicenter between 14 - 21dpi. In contrast, OLs did not increase in TLR4d mice during this time and were significantly lower than in WT caudal to the epicenter. This indicates that intraspinal TLR4 signaling is essential for the delayed rise in OLs after SCI. Surprisingly, the oligogenic factors ciliary neurotrophic factor (CNTF) and insulin-like growth factor (IGF) were significantly elevated in TLR4 deficient spinal cords, revealing that their absence was not the cause of reduced OL numbers. TLR4d and WT spinal cords had similar levels of integrin alpha M (CD11b) and macrosialin (CD68) expression, but TLR4d cords had elevated Fc gamma RII (CD32) and reduced hemoglobin scavenger receptor (CD163) mRNA. Thus, an altered inflammatory environment may have changed the response of OL lineage cells after SCI. Collectively, this work shows that post-SCI OL replacement is influenced by the inflammatory environment, and highlights the importance of TLR4 signaling for normal post-SCI cellular repair mechanisms, growth factor expression, and inflammatory gene expression.

Disclosures: **J.S. Church:** None. **P.G. Popovich:** None. **D.M. McTigue:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

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Program#/Poster: 609.04/T4

Topic: C.10. Trauma

Support: Craig H. Neilsen 164246

Ray W. Poppleton Endowment

Title: Disruption of the gut microbiome enhances inflammation and impairs recovery after spinal cord injury

Authors: *K. A. KIGERL, J. C. HALL, P. G. POPOVICH;
Dept. of Neurosci., The Ohio State Univ., Columbus, OH

Abstracts: The intestinal microbiome contains approximately 10-fold more genes than the human genome. This vast genome can regulate numerous functions in the host, including metabolism, digestion and absorption of nutrients, and the development of the adaptive immune system. The gut microbiome also can influence disease development and CNS function. Intestinal "dysbiosis" is a condition in which an imbalance between beneficial, non-pathogenic and pathogenic or inflammatory bacteria develops in the gut. Common causes of gut dysbiosis include antibiotics, stress, and altered or impaired gastrointestinal tract peristalsis; all are associated with or caused by traumatic spinal cord injury (SCI). Although gut dysbiosis has been implicated in autoimmune diseases (e.g. multiple sclerosis, type I diabetes, rheumatoid arthritis), allergy and metabolic disorders, the consequences of dysbiosis have not been explored after SCI. Here, data are presented that show time-dependent changes in immune cell number and phenotype in gut-associated lymphoid tissues (GALT), i.e., the Peyer's patches and mesenteric lymph nodes (MLNs), after SCI. Specifically, the number of B- & T-cells in the GALT increases after injury and is associated with enhanced expression of inflammatory cytokines (i.e. TNFα, IL-1β). Changes in immune cell subsets and cytokine profiles occur coincident with SCI-induced changes in the composition of the intestinal microbiota. To determine whether changes in the intestinal microbiome affect intraspinal pathology and recovery of function after SCI, we intentionally induced dysbiosis in gut microbiota prior to injury. New data will show that disrupting the gut microbiome exacerbates intraspinal inflammation and lesion pathology, alters the composition of leukocyte subsets in GALT and impairs spontaneous locomotor recovery. Specifically, in mice receiving a combination regimen of broad-spectrum antibiotics prior to SCI to induce gut dysbiosis, axon and myelin pathology is exacerbated and recovery of locomotor function is impaired. These novel data illustrate the fundamental importance of the gut microbiome in regulating immune cell function and recovery after SCI.

Disclosures: K.A. Kigerl: None. **J.C. Hall:** None. **P.G. Popovich:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.05/T5

Topic: C.10. Trauma

Support: NS082095

Title: TLR4 signaling promotes iron storage but enhances iron-mediated oligodendrocyte death in the CNS

Authors: *E. GOLDSTEIN, J. S. CHURCH, P. G. POPOVICH, D. M. MCTIGUE;
The Ohio State Univ., Columbus, OH

Abstracts: Traumatic spinal cord injury (SCI) induces concomitant secondary cascades that exacerbate tissue loss. One prominent feature of secondary injury is elevated intraspinal iron from intraparenchymal hemorrhage that accumulates and persists in the lesion, leading to oxidative damage and cell loss. Neurons and oligodendrocytes (OLs) are especially vulnerable to iron-induced death. A potential strategy to combat iron-mediated damage is to stimulate iron sequestration by macrophages through toll-like receptor 4 (TLR4) activation. TLR4 stimulation promotes iron uptake and storage by macrophages and prior work from our group showed intraspinal TLR4 activation in uninjured spinal cords also promotes OL progenitor proliferation and new OL formation. Therefore, TLR4 is an attractive target for promoting tissue repair after SCI. To test the hypothesis that intraspinal TLR4 activation would be protective from iron-induced injury in the spinal cord, we microinjected a TLR4 agonist (lipopolysaccharide; LPS), iron or LPS + iron into the intact spinal cord and examined the tissue 24 hours later to determine if iron sequestration was enhanced and tissue damage reduced by concomitant TLR4 activation. LPS treatment concomitant with iron did promote iron storage as detected by a significant increase in ferritin expression and increased ferritin+ macrophages in the injection site. However, the combined treatment exacerbated OL loss. LPS or iron microinjected alone each caused ~50% loss of OLs, while co-injection of LPS + iron caused almost complete loss of OLs (95% reduction). Surprisingly, the combination did not enhance neuron loss, with all groups exhibiting a 25-30% reduction in neurons. Thus, concomitant TLR4 activation in the presence of excess iron does promote iron uptake and storage by microglia, however it also increases oligodendrocyte loss. These data demonstrate a deleterious role for microglia attempting to regulate excess iron in an injury environment. Ongoing studies are investigating mechanisms of enhanced microglial toxicity when exposed to concomitant TLR4 activation and excess iron.

Disclosures: E. Goldstein: None. **J.S. Church:** None. **P.G. Popovich:** None. **D.M. McTigue:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.06/T6

Topic: C.10. Trauma

Support: Ray W. Poppleton Endowment

Title: Activation of dectin-1, an anti-fungal immune receptor, by endogenous CNS proteins causes destructive inflammation after traumatic spinal cord injury

Authors: *Y. WANG[1], J. HALL[2], Z. GUAN[2], P. POPOVICH[2];
[1]Wexner Med. Ctr. At Ohio State Univ., Columbus, OH; [2]Ctr. fro Brain and Spinal Cord Repair, Waxner Med. Ctr. at the Ohio State Univ., Columbus, OH

Abstracts: Dectin-1 is a pattern recognition receptor (PRR) found on the surface of myeloid cells including neutrophils, dendritic cells, monocytes and macrophages. Pathogen-associated molecular patterns (PAMPs) are small pathogen-specific molecular motifs that are recognized by PRRs. Dectin-1 recognizes beta glucans on fungi. Many PRRs can also be activated by endogenous proteins that are released by necrotic cells at sites of injury. These so-called “danger associated molecular patterns” (DAMPs) may activate myeloid cells via dectin-1. Here, using co-immunoprecipitation techniques followed by mass spectrometry, we identified several proteins, many of which are derived from resident glia, that bind dectin-1 and may function as DAMPs after traumatic spinal cord injury (SCI). Moreover, SCI increases expression of dectin-1 mRNA and protein, predominantly in monocyte-derived macrophages, which migrate to the injury site and initiate an inflammatory response with adverse effects on neural repair. Indeed, dectin-1 knock out mice exhibit improved locomotor recovery and reduced lesion volume after SCI. Ongoing experiments will seek to further characterize endogenous non-pathogenic dectin-1 ligands and block their effects on activating intraspinal macrophages.

Disclosures: Y. Wang: None. **J. Hall:** None. **Z. Guan:** None. **P. Popovich:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.07/T7

Topic: C.10. Trauma

Support: FRS-FNRS grant

Title: Is placental growth factor involved in spinal cord repair?

Authors: *R. /. FRANZEN, L. CHABALLE, P. ROWART, F. SCHOLTES, J. SCHOENEN; GIGA Neurosciences, Univ. of Leige, Leige, Belgium

Abstracts: Following axon injury, regeneration outcome depends on the central or peripheral localization of the lesion, thus on the environment through which the axon regrows. In the peripheral nervous system (PNS), the post-injury Wallerian degeneration (WD) inflammatory process occurs rapidly and involves mainly Schwann cells and monocyte-derived macrophages, leading to successful regeneration. In the central nervous system (CNS), WD that involves resident microglia and monocyte-derived macrophages is much slower, leading to a poor clearance of myelin debris and a hostile environment for the axon, whose regeneration is aborted. In this context, the cellular and molecular mechanisms regulating WD in the PNS and the CNS have been extensively studied. In particular, infiltration of monocyte-derived macrophages has been shown to be essential for recovery after spinal cord injury (SCI), but this recruitment needs to be well orchestrated. Among the molecules likely involved in this control, the Placental Growth Factor (PlGF) possesses various properties that could make it an interesting actor in the repair process. Indeed, PlGF is an angiogenic factor with neuroprotective and neurotrophic properties. Moreover, PlGF is able to attract and activate monocytes, and has recently been shown to be involved in macrophage M1/M2 polarization process. We thus decided to assess PlGF role in the molecular and cellular mechanisms that follow SCI. Using Pgf null mice (Pgf -/-), we compared their behavioural recovery to the one of their wild-type (WT) littermates after a thoracic spinal cord contusion injury. Quantification of lesion extension and immunohistological studies were performed on their respective spinal cord tissues. Surprisingly, behavioural data show that Pgf -/- mice recover their motor (BMS and BMS subscores) and sensory (Von Frey test) functions significantly better than wt mice. This is correlated to a significant decrease of the lesion volume in Pgf -/- mice (Luxol Fast Blue/Eosin staining), 28 days after SCI. However, no difference in axon regrowth was observed when assessed with Neurofilaments and Gap-43 immunostainings. We then examined the injured tissues 5 days after SCI, to compare the inflammatory response, and more particularly the M1/M2 macrophage polarity. We found that in the lesioned site of Pgf -/- mice, there is a significantly higher number of M2 - polarized macrophages than in wt mice. These original data, which will be further confirmed by RT-qPCR assays for pro- and anti-inflammatory cytokines, strongly suggest that in absence of PlGF, the lesion environment is an anti-inflammatory milieu, allowing tissue preservation and supporting recovery.

Disclosures: R./. Franzen: None. **L. Chaballe:** None. **P. Rowart:** None. **F. Scholtes:** None. **J. Schoenen:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.08/T8

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Support: Manitoba Spinal Cord Injury Research Committee (Canadian Paraplegic Association (Manitoba) Inc., Rick Hansen Institute and Government of Manitoba)

Manitoba Medical Service Foundation

Title: Transforming growth factor beta-induced expression of chondroitin sulphate proteoglycans in reactive astrocytes: roles of non-Smad signaling pathways and autophagy

Authors: *S. S. HANNILA, N. JAHAN, S. GHAVAMI;
Human Anat. and Cell Sci., Univ. of Manitoba, Winnipeg, MB, Canada

Abstracts: Following spinal cord injury, reactive astrocytes express high levels of chondroitin sulphate proteoglycans (CSPGs), which have been shown to inhibit axonal regeneration both *in vitro* and *in vivo*. The factors responsible for inducing CSPG expression have not been fully defined, but there is strong evidence that transforming growth factor β (TGFβ) plays an important role in this process. Administration of anti-TGFβ antibodies reduces glial scarring following spinal cord injury, and expression of CSPGs is significantly increased when astrocytes are treated with TGFβ. Inhibiting TGFβ signaling may therefore be an effective way to reduce CSPG levels in the injured spinal cord. Canonical TGFβ signaling is initiated by binding to the TGFβ receptor, which in turn leads to activation of the Smad2/3 signaling pathway. We have recently shown that levels of Smad2 are significantly reduced in neurons in response to elevation of intracellular cyclic AMP (cAMP), and so, we initially hypothesized that expression of the CSPG neurocan would be reduced following treatment with dibutyryl cAMP (dbcAMP). Surprisingly, while Smad2 levels were significantly reduced in astrocytes treated with dbcAMP and TGFβ, neurocan expression remained elevated, suggesting that Smad signaling is not required for CSPG expression. Subsequent experiments confirmed that activation of the TGFβ receptor is necessary for expression of neurocan, brevican, and aggrecan, but when astrocytes were incubated with either Smad2 or Smad4 siRNA prior to TGFβ treatment, CSPG levels were still significantly increased, which further suggests that TGFβ-induced CSPG expression is Smad-independent. In addition to the Smad signaling pathway, TGFβ also activates several non-Smad signaling pathways, and to identify which of these pathways is involved in CSPG expression, astrocytes were treated with inhibitors of the PI3K-Akt-mTOR or Ras-Erk pathways. No significant reductions in CSPG levels were observed when astrocytes were treated with the Erk inhibitor U0126, but levels of neurocan, brevican, and aggrecan were all significantly

reduced following treatment with two inhibitors of the PI3K-Akt-mTOR pathway: LY294002 and rapamycin, which suggests that CSPG expression is mediated primarily through this pathway. In addition, TGFβ induces several markers of autophagy, and CSPG expression was reduced in response to treatment with choloroquine, which indicates that autophagy may also play a role in this process. Targeting these pathways could potentially prevent CSPG deposition within the spinal cord and thereby create a more favorable environment for axonal regeneration.

Disclosures: **S.S. Hannila:** None. **N. Jahan:** None. **S. Ghavami:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.09/T9

Topic: C.10. Trauma

Support: Shriners Research Foundation SHC-85310

Title: Epigenetic regulation of axonal regeneration

Authors: ***M. I. SHIFMAN**, J. CHEN, C. LARAMORE, A. CORNICK, J. SHAHOUD;
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Abstracts: Spinal cord injury (SCI) causes permanent disability because interrupted axons fail to regenerate. Even with regeneration-inducing interventions, some neurons are much worse than others at regenerating their axons. The reasons for this are not known, and the complexity of the mammalian spinal cord makes it difficult to distinguish true regeneration of injured axons from collateral sprouting of spared axons. By contrast, in lampreys, identified reticulospinal neurons (RS) have large axons whose regeneration can be followed in the living animal or verified unequivocally in histological preparations. Moreover, it is possible to correlate molecular expression patterns with regenerative abilities in individual neurons. Only about 50% of all injured axons regenerate through the scar. Therefore, factors intrinsic to the neurons could be involved in determining their regenerative abilities. Previous work has shown that successful axon regeneration dependent upon the transcription of a large number of regeneration-associated genes (RAGs). CNS axons fail to regenerate because they do not initiate intrinsic regenerative gene programs and they remain sensitive to multiple extrinsic inhibitory influences present within the adult CNS. However, the mechanisms underlying inability of CNS neurons to reactivate transcription of RAGs remain poorly understood. In eukaryotes, genomic DNA is

organized into chromatin and it structure regulated gene expression by preventing the transcriptional machinery from interacting directly with promoters and other DNA regulatory elements. Epigenetic mechanisms - DNA methylation and histone modifications - result in changes in the chromatin structure, which in turn influence gene transcription. We hypothesize that epigenetic modifications function as "master switches" that activate or suppress gene expression of RAGs after SCI. The existence of such a "master switch" could explain the major differences in regenerative potential between the good-regenerating and bad-regenerating neurons. Using unique ability of lamprey CNS to retrograde labeled regenerated and non-regenerated RS neurons we showed that histones H3 and H4 acetylation (indicator of transcriptional activation) is detected only in regenerating RS neurons and not in non-regenerating ones. Using *in situ* hybridization we are testing ***in vivo*** that bad-regenerating neurons have higher levels of histone deacetylases (HDACs) expression and as result, have lower level of histone acetylation that contribute to regeneration failure after SCI.

Disclosures: M.I. Shifman: None. **J. Chen:** None. **C. Laramore:** None. **A. Cornick:** None. **J. Shahoud:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.10/T10

Topic: C.10. Trauma

Support: NIH NS079631

Shriners Hospitals for the children

Title: Nogo, MAG, OMgp and CSPGs are not critical barriers preventing intraspinal regeneration of dorsal root axons

Authors: J. ZHAI[1], H. KIM[1], S. HAN[1], J. K. LEE[2], B. ZHENG[3], G. M. SMITH[1], *Y.-J. SON[1]; [1]Shriners Hosp. Pediatric Res. Ctr., Temple Univ. Sch. of Med., Philadelphia, PA; [2]The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL; [3]Dept. of Neurosciences, Univ. of California, San Diego, CA

Abstracts: Dorsal root (DR) axons fail to regenerate through the dorsal root entry zone (DREZ), the CNS/PNS border. This regeneration failure has been attributed largely to a number of

extrinsic factors abundantly present at the DREZ and within the spinal cord, which include myelin-associated inhibitors such as Nogo, oligodendrocyte-myelin glycoprotein (OMgp), myelin-associated glycoprotein (MAG) and various chondroitin sulfate proteoglycans (CSPGs). To test whether these factors indeed play a critical role in preventing axonal regeneration at the DREZ, we are evaluating regeneration of dorsal root axons in triple knockout mice lacking Nogo, OMgp and MAG. Two weeks after cervical or lumbar root crush, regenerating axons selectively labeled with AAV-eGFP are analyzed in sections or wholemounts. We have found that AAV-GFP extensively labels all subclasses of DRG axons and more importantly, helps us to easily distinguish regenerating from spared axons, which has been a major challenge in many analyses of dorsal root injury and regeneration. We have found that most axons fail to penetrate the DREZ in the tKO mice. Moreover, regeneration of dorsal root axons in the tKO was not improved even after additional removal of CSPGs with lentiviruses expressing chondroitinase. We are currently examining additional mice and extending our analysis to later time points. We also plan to test whether enhancing intrinsic growth ability by a conditioning lesion will promote intraspinal regeneration of axons in the tKO mice. Our results thus far suggest that myelin inhibitors and CSPGs are unlikely to play a critical role in preventing regeneration across the DREZ.

Disclosures: J. Zhai: None. **H. Kim:** None. **S. Han:** None. **J.K. Lee:** None. **B. Zheng:** None. **G.M. Smith:** None. **Y. Son:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Topic: C.10. Trauma

Support: NIH P30 HD018655

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Regeneration occurs in the CNS of CAST/Ei mice as a consequence of enhanced Activin signaling

Authors: *T. OMURA[1,2], K. OMURA[2], P. RIVA[2], A. TEDESCHI[2], L. ROJAS[2], J. MARTIN[2], H. A. HUEBNER[2], M. PAINTER[2], A. LATREMOLIERE[2], Y. YIN[2], L. BARRETT[2], B. SINGH[2], S. LEE[2], T. CRISMAN[3], F. GAO[3], S. LI[4], D. GESCHWIND[3], G. COPPOLA[3], Z. HE[2],

S. T. CARMICHAEL[4], L. BENOWITZ[2], M. COSTIGAN[2], C. WOOLF[2];
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Abstracts: We screened nine genetically diverse inbred mouse strains for differences in axonal growth of adult dorsal root ganglion (DRG) neurons on CNS myelin. Naïve DRG neurite outgrowth on myelin was very limited, but preconditioning the neurons by a prior sciatic nerve crush increased axonal growth substantially across all strains, with by far the greatest change in neurons from CAST/Ei mice, which was 5.5 fold greater than the average of the eight other strains. Three independent *in vivo* CNS injury models consisting of dorsal column injury, optic nerve injury and stroke model revealed greater capacity for CNS axonal regeneration in CAST/Ei than C57BL/6 mice. Full-genome expression profiling of naïve and preconditioned DRGs across all strains revealed Activin-βA (Inhba) as the transcript whose expression most closely correlated with axonal growth on myelin. *In vitro* and *in vivo* gain- and loss-of-function experiments confirmed that Activin promotes axonal growth in the CNS. Substantial regeneration is possible, therefore, in the injured mammalian CNS when Activin signaling is intrinsically high, as in CAST/Ei, or when extrinsically modulated in other strains.

Disclosures: T. Omura: None. **K. Omura:** None. **P. Riva:** None. **A. Tedeschi:** None. **L. Rojas:** None. **J. Martin:** None. **H.A. Huebner:** None. **M. Painter:** None. **A. Latremoliere:** None. **Y. Yin:** None. **L. Barrett:** None. **B. Singh:** None. **S. Lee:** None. **T. Crisman:** None. **F. Gao:** None. **S. Li:** None. **D. Geschwind:** None. **G. Coppola:** None. **Z. He:** None. **S.T. Carmichael:** None. **M. Costigan:** None. **C. Woolf:** None. **L. Benowitz:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.12/T12

Topic: C.10. Trauma

Support: DFG

Title: The role of epigenetics in axonal regeneration and functional recovery following spinal cord injury

Authors: A. HERVERA[1], L. ZHOU[3], I. PALMISANO[1], G. KONG[3], R. PUTTAGUNTA[3], R. LINDNER[3], *S. DI GIOVANNI[2];
[1]Imperial Col. London, London, United Kingdom; [2]Imperial Col. London, London,, United Kingdom; [3]HIH, Tuebingen, Germany

Abstracts: Lack of a regenerative glial environment and of a neuronal intrinsic regenerative gene expression program compromise axonal regeneration after injury in the central nervous system. However, regenerative gene expression, mainly characterized by the induction of regeneration-associated genes (RAGs), is activated after regenerative PNS injury, including in dorsal root ganglia (DRG) neurons. Pseudounipolar sensory DRG neurons have a central and a peripheral axonal branch belonging to the same cell body, with opposite regenerative capacity. Lack of regeneration of the injured DRG central axon in the spinal cord can be reversed by an injury to the corresponding peripheral branch (conditioning lesion) of those DRG neurons. Interestingly, this conditioning lesion induces the expression of regeneration associated genes (RAGs) and axonal regeneration in DRG central axons. In search for molecular mechanisms that may rule this shift in injury induced-expression programs, we hypothesized that epigenetic modifications may play a role as master regulators. In fact, we recently found that the neuronal regenerative programme is supported by selected epigenetic modifications, including histone acetylation mediated by p300 and PCAF on regeneration-associated genes in DRG neurons and retinal ganglia cells. Additionally, p300 and PCAF, which induce gene expression via acetylation of histones and key transcription factors, can promote axonal regeneration in the injured optic nerve and in the injured spinal cord respectively. Recently, we systematically investigated the expression of histone modifying enzymes and of DNA methylation contributing to epigenetic regulation of the axonal regeneration programme. DNA methylation seemed to play a role in mechanisms other than axonal regeneration. However, we found that specific histone modifying enzymes are selectively down regulated in DRG neurons following injury in the peripheral (regenerative) but not central axonal branch. We also found that axonal retrograde signalling pathways are responsible to modulate their expression. Indeed, we determined that inhibition of specific enzymes results in increased outgrowth of DRG neurons in culture and *ex vivo* after the spinal delivery of specific inhibitors. Last, but not least, mice treated with novel specific inhibitors displayed improved locomotion as compared to vehicle treated littermates following SCI. This supports histone modifying enzymes as novel epigenetic modulators of the axonal regeneration programme, whose inhibition may lead to regeneration and locomotor recovery after spinal injury.

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Poster

609. Spinal Cord Signaling in Trauma

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Topic: C.10. Trauma

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NIH Grant NS050243

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Hulman George Endowment Funds

Indiana Spinal Cord and Brain Injury Research Foundation

ISDH Grant A70-2-079609

Title: Blocking cPLA2 ameliorated motor deficits and reduced tissue damage after spinal cord injury

Authors: *N.-K. LIU[1], L.-X. DENG[1], Y.-P. ZHANG[2], Q.-B. LU[1], X.-F. WANG[1], J.-G. HU[1], C. L. WALKER[1], J. V. BONVENTRE[3], C. B. SHIELDS[2], X.-M. XU[1];
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Abstracts: Several lines of evidence suggest that phospholipase A2 (PLA2) may play a key role in mediating secondary spinal cord injury (SCI). PLA2 are a diverse family of lipolytic enzymes which hydrolyze phospholipids to produce free fatty acids and lysophospholipids. Cytosolic PLA2 (cPLA2) is one of the most important PLA2 isoforms. However, the role of cPLA2 in the pathogenesis of SCI is not fully understood, and is even controversial. In this study, we investigated whether cPLA2 plays a role in the pathogenesis of SCI using multiple approaches including molecular, pharmacological, genetic, and behavior assessments. Our results showed that SCI significantly induced cPLA2 expression and activation. Treatment with AACOCF3, a cPLA2 inhibitor, significantly reduced cPLA2 activity and PGE2 production in C57BL/6 mice after SCI as well as restored Na+-K+-ATPase activity (a marker for membrane integrity or damage) and reduced SCI-induced MPO activity (a marker for neutrophil infiltration). Remarkably, blocking cPLA2 with AACOCF3 reduced tissue damage accompanied by a

corresponding increase in white matter sparing in C57BL/6 mice after SCI. In addition, Luxol fast blue staining showed that the AACOCF3 treatment resulted in a corresponding increase in myelin sparing. Behavioral recovery was also improved after AACOCF3 treatment as shown in BMS, beam walking and foot print analyses. An important finding of the present study was that genetic deletion of cPLA2 resulted in neuroprotection and behavioral recovery following SCI. Genetic deletion of cPLA2 also inhibited the expression of active caspase-3 after SCI, suggesting that cPLA2 activation mediates neural apoptosis. These findings collectively suggest that cPLA2 may play a key role in the pathogenesis of SCI, and this molecule could be an attractive therapeutic target for ameliorating secondary tissue damage and promoting recovery of function after SCI.

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Poster

609. Spinal Cord Signaling in Trauma

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VA CDA 1 IK2 RX001123-01A2

Department of Veterans Affairs (VA) Medical Research Service and Rehabilitation Research Service

Paralyzed Veterans of America

Title: Targeting sci induced dendritic spine dysgenesis to attenuate neuropathic pain

Authors: *A. M. TAN[1], S. LIU[2], B. VOHRA[2], S. G. WAXMAN[2];
[1]Neurol., Yale University/VA Connecticut Healthcare Syst., West Haven, CT; [2]Yale University/VA Connecticut, West Haven, CT

Abstracts: Over fifty percent of patients with spinal cord injury (SCI) live a severely diminished quality of life due to neuropathic pain and spasticity that are refractory to current medical

treatment. A variety of factors contribute to these chronic complications after SCI. The primary goal of our research is to extend our mechanistic understanding of dendritic spine behavior in the spinal cord after injury and identify a clinically applicable strategy for addressing intractable pain and spasticity. Dendritic spines, micron-sized postsynaptic structures, have been of great interest within the learning and memory field. Because dendritic spines directly contribute to circuit function and represent modifiable sites of synaptic contact, dendritic spine morphology provides crucial insight into how neural networks form and retain function. Knowledge of dendritic spine behavior in the spinal cord would therefore elucidate mechanisms of spinal sensory-motor circuit dysfunction after SCI. We have previously shown that acute pharmacological inhibition of Rac1-mediated dendritic spine remodeling on nociceptive sensory neurons in the dorsal horn one-month after SCI significantly reduces the presence of neuropathic pain. These findings demonstrate that dendritic spine remodeling contribute to the maintenance of hyperexcitability in the spinal nociceptive system. Given this information, we address the following questions: 1) is acute Rac1-inhibitor treatment efficacious in the long-term? 2) If not, will a gene therapy approach be more effective and durable in SCI induced circuit remodeling and neuropathic pain? 3) Are there alternative molecular targets, downstream of Rac1-GTPase signaling, that are involved in regulating dendritic spine remodeling and neuropathic pain after SCI? Our recent preliminary studies demonstrate that acute pharmacological Rac1 inhibition after SCI reduces evidence of neuropathic pain. However, follow-up assessment demonstrates a partial loss of analgesic effect upon drug cessation, suggesting the return of abnormal dendritic spines and the need for a more durable therapeutic strategy. We are currently utilizing pharmacological and gene therapy approaches to investigate the mechanistic role of the Rac1 signaling pathway in dendritic spine remodeling in pain and other complications after SCI.

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Poster

609. Spinal Cord Signaling in Trauma

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.15/U3

Topic: C.10. Trauma

Title: Disrupted autophagy after spinal cord injury is associated with neuronal cell death

Authors: *S. S. LIU[1], C. SARKAR[2], E. Y. KOH[1], J. WU[2], M. LIPINSKI[2];
[1]Orthopaedics, [2]Anesthesia, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstracts: Autophagy is a lysosome-dependent intracellular degradation pathway, which plays a neuroprotective function in several neurodegenerative diseases. Although elevated autophagic markers have been reported after SCI, its mechanism, cell type specificity, and relationship with cell death remain unknown. In a rat model of moderate contusive SCI, we found increased levels of autophagy marker, LC3-II, by western blot, and increased numbers of cells accumulating LC3-positive autophagosomes by immunohistochemistry (IHC), starting at 1 day after injury and continuing for up to 5 weeks (Fig. 1). Initial accumulation of LC3 was accompanied by pronounced elevation in the levels of the autophagy substrate, p62 (Fig. 1). This indicates that the initial increase in markers of autophagy was due to defective lysosomal clearance of autophagosomes and their cargo. Accumulation of p62 was resolved by day 7 after SCI (Fig. 1), and was associated with elevated levels of the lysosomal enzyme cathepsin D. Therefore, increase in the size and activity of the lysosomal compartment may help restore autophagy flux at that time. Furthermore, we used IHC to study cell type specificity of autophagy after SCI. LC3 preferentially accumulated in oligodendrocytes and microglia in the white matter, and co-localized with the neuronal cell marker NeuN in the gray matter. LC3 was especially pronounced at day 1 after SCI in motor neurons in the ventral horn. Since our data indicate that at that time autophagic clearance is blocked, we hypothesize that it may contribute to neuronal cell death. Consistently, we found that p62 labeled cells were also positive for apoptotic cell death markers, cleaved caspase 3 and caspase 12, indicating association between disrupted autophagy and cell death. Together, our data indicate that autophagic degradation is temporarily blocked and may contribute to neuronal cell death after SCI.

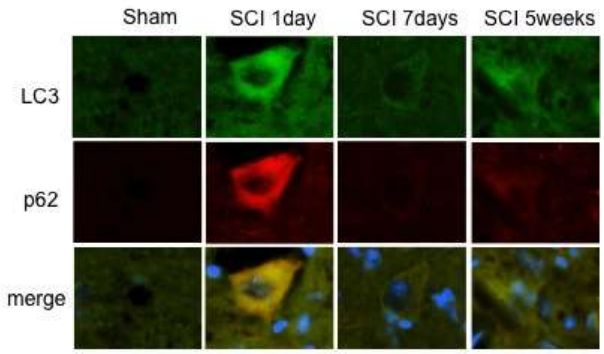

Figure 1: Immunohistochemistry of spinal cord cross-sections from ventral horn of gray matter: expression of LC3 (top row) and p62 (middle row) increased after SCI, and peaked at day 1, with a strong co-localization.

Disclosures: **S.S. Liu:** None. **C. Sarkar:** None. **E.Y. Koh:** None. **J. Wu:** None. **M. Lipinski:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Topic: C.10. Trauma

Support: CIHR Grant 6218

MFE-104430

Title: Factors influencing macrophage polarization in spinal cord injury

Authors: *A. KRONER-MILSCH, A. D. GREENHALGH, J. G. ZARRUK, S. DAVID; Neurol., Ctr. For Res. In Neuroscience, Res. Inst. of the McGill Univ., Montreal, QC, Canada

Abstracts: Macrophages/microglia are rapidly activated after CNS injury and appear to have both detrimental and beneficial effects. Recent work suggests that the tissue environment influences macrophage polarization towards different phenotypes, referred to as M1 and M2. M1 polarization includes the production of nitric oxide and pro-inflammatory cytokines, while M2 macrophages are viewed as anti-inflammatory which contribute to wound healing and tissue repair. We and others have previously reported that M1 markers dominate the macrophage/microglial response after spinal cord injury (SCI). Phagocytosis *in vitro* by macrophages was also shown to reduce secretion of pro-inflammatory cytokines and we demonstrated that myelin phagocytosis skews M1 activated bone marrow derived macrophages (BMDMs) and microglia towards an M2 phenotype. We now show that TNF, which is rapidly upregulated after SCI in various cell types, is a crucial factor in preventing the myelin induced switch from M1 to M2. *In vitro*, rTNF completely abrogates the switch. TNF null mice show a better functional recovery after SCI and have more M2 macrophages at the injury site. Interestingly, BMDMs from TNF null mice show a strong reduction in the expression of M1 markers after LPS stimulation, which can be further reduced by myelin phagocytosis, suggesting a TNF dependent and a TNF independent mechanism. Furthermore, the reduction in neurite growth from DRG neurons treated with conditioned medium from LPS treated BMDMs is completely prevented when BMDMs from TNF null mice are used. We have also previously shown that the presence of iron in macrophages, mainly derived from phagocytosis of red blood cells (RBCs), is correlated with the expression of TNF. We now provide evidence that systemic iron loading in mice with SCI exacerbates TNF expression, increases production of reactive oxygen species and cell death in the injured cord, and also reduces locomotor recovery. We also show *in vitro* that BMDMs from TNF null mice loaded with RBCs show a significant reduction of M1 markers and increase in M2 markers as compared to BMDMs from wildtype control mice. These finding suggest that iron and iron induced TNF production play a crucial role in

maintaining the pro-inflammatory M1 phenotype of macrophages after SCI. Funded by a grant from the Canadian Institutes of Health Research (CIHR).

Disclosures: A. Kroner-Milsch: None. **S. David:** None. **A.D. Greenhalgh:** None. **J.G. Zarruk:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.17/U5

Topic: C.10. Trauma

Support: JSPS DC1 25-5321

Title: Genetic modifications of Crmp enhance axonal regrowth after spinal cord injury by reducing cytoskeletal destabilization and inflammatory responses

Authors: *J. NAGAI[1], Y. KITAMURA[1], K. OWADA[1], Y. GOSHIMA[2], T. OHSHIMA[1]; [1]Wakamatsu-cho, Waseda Univ., Tokyo, Japan; [2]Dept. Mol. Pharmacol. Neurobiol., Grad. Sch. Med., Yokohama City Univ., Yokohama, Japan

Abstracts: The recovery after central nervous system (CNS) injury in the adult mammal by targeting suggested molecules were substantially limited perhaps because they contribute to either axonal elongation or glial scar formation. One prevailing theory is the presence of inhibitory molecules that prevent injured axons from regenerating beyond the injury site. Myelin-associated inhibitors (MAIs), which are expressed in myelin and mature oligodendrocytes and bind to Nogo-66 receptor on axonal membranes, are non-permissive substrates for neurite outgrowth. Extracellular matrix molecules, such as Semaphorin3A (Sema3A), secreted from astroglial scar tissue at the CNS injury site are likely to be important for the inhibition of axonal regrowth by acting on the axonal cytoskeleton. However, loss-of-function studies of MAIs still produce conflicting results on their role in axon regeneration after CNS trauma such as spinal cord injury (SCI) and the high lethality of Sema3AKO mice raises concerns over possible strong side effects of targeting Sema3A. To develop more effective therapies for CNS injuries, the focus should be on common downstream molecules of these signals that control axonal growth. Cytoskeletal dynamics is a key factor limiting regenerative capacity in terms of axonal formation, glial inflammation and scarring. Collapsin response mediator protein (CRMP) has been demonstrated to regulate the cytoskeletal responses to the

axonal outgrowth inhibitory signals *in vitro*, but the role of CRMP axonal regrowth and inflammatory responses *in vivo* after CNS trauma remains to be elucidated. Here, we found that inhibitory and toxic forms of CRMP were significantly increased in injured wild-type spinal cords. We generated mutant mice of CRMP genes and found remarkable locomotor recovery with robust axonal regrowth through microtubule polymerization and cell survival in CRMP4KO mice. Moreover, we also observed CRMP4 upregulation in inflammatory cells; activated microglia/macrophages and reactive astrocytes. Mice lacking CRMP4 exhibited suppression of non-traumatic and post-traumatic inflammation and dramatic reduction of scarring after SCI, providing permissive environment for the regenerative growth of injured spinal cord axons. These results provide us with new insights into CRMP as a novel factor that bridges two major inhibitory factors after SCI.

Disclosures: J. Nagai: None. **Y. Kitamura:** None. **K. Owada:** None. **Y. Goshima:** None. **T. Ohshima:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Support: NIH Grant RO1N5083983

Bryon Riesch Paralysis Foundation

Title: Virally delivered ADAMTS-5 as a strategy to promote axon growth in inhibitory CSPG environments

Authors: *K. WINSOR, Z. WANG, D. COLEY, C. NIENHAUS, M. BLACKMORE; Marquette Univ., Milwaukee, WI

Abstracts: Chondroitin Sulfate Proteoglycans (CSPGs), a diverse family of proteins adorned by glycosaminoglycan side chains, are deposited at sites of injury to the central nervous system (CNS) and present a major barrier to axon growth. Thus to improve axon growth and recovery from CNS injury it will likely be necessary to enzymatically degrade CSPGs, or to render injured axons insensitive to CSPG-mediated inhibition. ADAMTS enzymes are expressed in nervous system tissue and can act to cleave the protein core of CSPGs. In particular, infusion of

ADAMTS-4 enzyme to sites of spinal injury has been reported to degrade the CSPG aggrecan and to improve functional outcomes. Here we test whether ADAMTS-5, which is structurally related and reportedly more enzymatically active than ADAMTS-4, can also improve axon growth in CSPG-rich environments. We tested full length ADAMTS-5 as well as two truncated forms that mimic endogenous degradation products with enhanced activity. Lentiviral constructs encoding ADAMTS-5 or its derivatives were applied to cell lines or to primary astrocytes in order drive production of ADAMTS enzymes, and conditioned media from transduced cells was applied to neurons cultured on CSPG substrates. Western blotting confirmed expression of exogenous ADAMTS-5 in transduced cells, but showed minimal secretion. Consistent with this, media from virally-transduced cells produced neither significant degradation of CSPGs, nor enhanced axon growth. Unexpectedly, however, media from early postnatal astrocytes dramatically enhanced axon growth on CSPG substrates, regardless of viral treatment. The astrocyte media improved axon growth only when neurons were exposed to the media, and not when the CSPG substrates were pre-exposed, suggesting that rather than degrading CSPGs the astrocytes produce a signal that acts directly on neurons. Moreover, although a secreted protein called periostin has recently been proposed to explain astrocyte-mediated improvements in axon growth on CSPG substrates, we detected no periostin expression in the astrocyte cultures, suggesting an alternative mechanism. Combined, these data illustrate challenges in the use of virally-expressed ADAMTS-5 enzyme as a potential therapeutic approach to CNS injury, but also illustrate an intriguing ability of early postnatal astrocytes to provide an unknown signal to neurons that renders them insensitive to CSPG-mediated inhibition.

Disclosures: K. Winsor: None. **Z. Wang:** None. **D. Coley:** None. **M. Blackmore:** None. **C. Nienhaus:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Support: by International Foundation for Research in Paraplegia (IFP) (M.T.V.)

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Italian Ministry of Health (Ricerca Corrente - M.M.)

Title: Cannabinoid CB2 receptor (CB2R) stimulation delays rubrospinal mitochondrial-dependent degeneration and improves functional recovery after spinal cord hemisection

Authors: *M. VISCOMI, L. LATINI, E. BISICCHIA, V. SASSO, V. CAVALLUCCI, M. MOLINARI;
Fondazione Santa Lucia, Rome, Italy

Abstracts: Spinal cord injury (SCI) is a devastating neurological disease that results in severe functional impairments for which there are no restorative therapies. In addition to the primary injury, functional impairments following a SCI are attributed to degenerative events in regions that are remote but functionally connected to the primary lesion site_i.e., supraspinal structures. These events include cell death and structural changes and are important predictors of outcome. However, few studies have examined the molecular and biochemical changes in remote neurons after SCIs as targets for therapeutic interventions. This study examines the effects of pharmacological modulation of type-2 cannabinoid receptor (CB2R) on the fate of axotomized rubrospinal neurons and functional recovery in a model of spinal cord dorsal hemisection (SCH) at the cervical level in rats. Beginning 7 days after damage, cross-sectional area and stereological analyses demonstrate that SCH induces severe atrophy and cell loss of contralateral rubrospinal neurons. At the same time, morphological and biochemical analyses demonstrate that SCH causes a significant de novo synthesis of CB2R in the axotomized rubrospinal neurons. Notably, in rubrospinal neurons morphological changes proceed concomitantly with molecular changes in the apoptotic cascade resulting in cytochrome c (cyt-c) release from damaged mitochondria, apoptosome formation, and caspase-3 activity. Pharmacological stimulation of CB2R, by its selective agonist JWH-015, by increasing the bcl-2/bax ratio and decreasing cytochrome c release, delays SCH-induced atrophy and cell loss in rubrospinal neurons as demonstrated by both morphological and biochemical approaches. Furthermore, JWH-015 treatment significantly improves the locomotor performance and the forelimb-hindlimb coordination as demonstrated by beam walking and CatWalk behavioral tests. These findings implicate the Endocannabinoid system (ECS), particularly CB2R, as part of the endogenous neuroprotective response that is triggered after an SCI. Thus, CB2R modulation might represent a promising therapeutic target that lacks psychotropic effects and can be used to exploit ECS-based approaches to counteract the remote degeneration of supraspinal regions after SCI.

Disclosures: M. Viscomi: None. **L. Latini:** None. **E. Bisicchia:** None. **V. Sasso:** None. **V. Cavallucci:** None. **M. Molinari:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.20/U8

Topic: C.10. Trauma

Title: FasL modulates disruption of the blood-spinal cord barrier, glial scarring and inflammation after spinal cord injury: Evidence from human tissue and mouse model

Authors: *W.-R. YU, M. G. FEHLINGS;
Genet and Develop, U of Toronto, Divisions of Genet. & Develop. and Neurosurgery, Toronto Western Res., Toronto, ON, Canada

Abstracts: Accumulating evidence indicates that the death receptor Fas and its specific ligand (FasL) have a wide range of physiological non-apoptotic functions. We sought to study the role of Fas ligand-mediated blood spinal cord barrier (BSCB) disruption, glial scarring and inflammation following spinal cord injury (SCI). We examined FasL-mediated BSCB disruption, glial scar formation and inflammatory response in human SCI and in an *in vivo* Fejota™ clip compression model of SCI in FasL-deficient B6Smn.C3-*Tnfsf6*^{gld}/J and wild-type mice using immunohistochemistry, Western blotting, and ELISA with Mouse 32-plex cytokine/chemokine panel bead immunoassay. We report novel evidence that shows leakage of plasma protein (Fibronectin, Fibrinogen and Homeglobin) into the extravascular space and parenchyma of the spinal cord, increased expression of CX3CR1, IL-1β and reduction of anti-inflammatory cytokine IL-10 expression in the injury epicenter after acute and sub-acute human SCI. We also found significantly reduced expression of Fibronectin and Evans blue leakage at 3 days, and reduced glial scarring and inflammatory response (Iba1 and galectin-3 expression). Increased levels of MAP2 and CNPase, expression and improved neurological functional recovery were observed in FasL-deficient mice relative to wild-type mice after SCI. In conclusion, we report multiple lines of evidence that indicate that FasL activation plays a pivotal role in mediating BSCB disruption, glial scarring formation, the inflammatory response and neurological functional recovery after SCI. These data provided a compelling rationale for therapeutically targeting Fas/FasL in human SCI.

Disclosures: W. Yu: None. **M.G. Fehlings:** None.

Poster

609. Spinal Cord Signaling in Trauma

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 609.21/U9

Topic: C.10. Trauma

Support: NJCSCR grant CSCR12IRG007

Reynolds Family Spine Laboratory funds

Foundation of UMDNJ Society of Research Scholars

Title: Role of toll-like receptor 9 activation in the intact spinal cord: Effects on neurons and glia

Authors: *A. PALLOTTIE[1,2], L. NI[2], W. DONG[2], R. F. HEARY[1,2], S. ELKABES[1,2]; [1]Neurolog. Surgery, Grad. Sch. of Biomed. Sci., Rutgers Univ., Newark, NJ; [2]Neurolog. Surgery, Reynolds Family Spine Laboratory, New Jersey Med. School, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstracts: Toll like receptor 9 (TLR9), best known for its role in the induction of innate immune responses, is expressed in the spinal cord and modulates sterile inflammation following spinal cord injury. To better define the effects of TLR9 activation in the spinal cord, naïve mice received a single intrathecal injection of a TLR9 agonist, CpG ODN 1826. Subsequently, the time course of the inflammatory reaction and the effects on neurons, astrocytes and myelin proteins were delineated in the lumbar region. As early as 6 hours post-injection (p.i.), there was a significant increase in CD45 (1.7-fold), CD11b (1.7-fold), and GR1 (4.1-fold)-positive cells in the spinal cord of CpG ODN 1826-treated mice as compared to vehicle-treated controls. At 24 hours p.i. the inflammatory response reached a peak with a 4.2-fold increase in CD45, 4.3-fold in CD11b, and 6.4-fold in GR-1-positive cells in the CpG ODN 1826-treated mice. This was followed by a marked reduction in inflammatory cell counts at 48 hours p.i. and a return to baseline levels by 96 hours. Despite the decline in inflammatory cells, a delayed decrease in myelin basic protein levels was observed at 96 hrs p.i. In contrast, the levels of the astroglial marker glial fibrillary acidic protein and neuronal marker NeuN remained unchanged. These results suggest that activation of TLR9 in the spinal cord can affect expression of myelin proteins or myelin integrity. Ongoing studies are determining the underlying mechanisms and whether this is a direct or indirect effect on oligodendrocytes.

Disclosures: A. Pallottie: None. **L. Ni:** None. **W. Dong:** None. **R.F. Heary:** None. **S. Elkabes:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.22/U10

Topic: C.10. Trauma

Support: UAB TJ Atchison SCI Research Program

Title: Depression-like behaviors and altered serotonergic signaling resulting from spinal cord injury in rats

Authors: *D. STEWART, C. FLOYD;
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Abstracts: Spinal cord injury (SCI) is a devastating condition that affects approximately 12,000 persons annually in the U.S. The initial injury is followed by secondary pathophysiological sequelae, including reduced modulation of serotonin (5-HT) circuits controlling locomotion in the spinal cord. Additionally, psychological conditions such as clinical depression manifest in up to 60% of persons with SCI. Selective serotonin reuptake inhibitors (SSRIs) have been proposed as a therapy to enhance locomotor ability by restoring 5-HT signaling in the spinal cord. However, studies of SSRIs have not evaluated a reduction in depression symptoms in SCI models, a relevant topic due to the potential for globally altered 5-HT signaling in supraspinal circuits. The current study seeks to evaluate the effect of SCI on depression-associated behaviors and changes in 5-HT signaling after SCI in a rodent model. Our overarching hypothesis is that SCI will lead to changes in serotonin transporter (SERT) and 5-HT1a receptor (5HT1aR) expression in brain and spinal cord. In addition, an increase in depression-associated behavior will be evident in SCI animals. Adult male Sprague-Dawley rats were randomly assigned to receive either a moderate contusion SCI at thoracic level 10 or sham surgery (uninjured), recovering for either 14 or 28 days. Depression-associated behavior (sucrose preference test) and corticosterone (CORT) levels were measured before injury and at 14 or 28 days after SCI. Animals were euthanized and brain and spinal cord samples collected for Western blot analysis of 5-HT1aR and SERT levels. We found that sucrose preference decreased at 28, but not 14, days after surgery in SCI animals. In contrast, CORT levels were significantly increased at 28 days post-SCI in the injured group versus the uninjured group. Similarly, SERT expression increased in the raphe nuclei at both 14 and 28 days after SCI and decreased in the spinal cord rostral to the lesion epicenter, comparing SCI to uninjured group. Levels of 5-HT1aR were increased in the spinal cord rostral and caudal to the lesion and in the raphe nuclei at 28 days post-SCI in the injured group versus uninjured controls. These results indicate that SCI induces physiological and psychological changes in male Sprague-Dawley rats that are accompanied by region-specific 5-HT signaling changes. Animals receiving SCI exhibited signs of depression, including anhedonia and increased CORT. Future studies evaluating the effects of SSRIs will further elucidate the mechanism of 5-HT changes after SCI. Supported by the UAB TJ Atchison SCI Research Program.

Disclosures: **D. Stewart:** None. **C. Floyd:** None.

Poster

609. Spinal Cord Signaling in Trauma

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Program#/Poster: 609.23/U11

Topic: C.10. Trauma

Support: Ro1 NS 054221

Ro1 NR 013601

R21 NR 014053

Title: The severity-dependent negative effects of isolated thoracic spinal cord contusion in mice on cognitive and affective behaviors

Authors: ***Z. ZHAO**[1], B. A. STOICA[2], A. I. FADEN[2], J. WU[2];
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Abstracts: Clinical studies have reported cognitive and affective deficits after spinal cord injury (SCI). Roth et al. demonstrated that 40-60% of SCI patients show impairment in attention, concentration, memory, learning, and/or problem-solving ability. The cause of the cognitive deficits in SCI patients has been debated, because of potentially confounding factors such as concurrent traumatic brain injury (TBI). Some have tried to address this issue by focusing on SCI patients without signs of TBI, and confirmed that these patients also show impairments in cognitive function. Yet few clinicians or experimentalists in SCI recognize that such injuries can cause chronic neurodegeneration in the brain, or that such progressive changes can negatively impact long-term cognitive outcomes or affective state. It is known that cognitive/affective impairments are detrimental to SCI patients not only in their own right but because they can compromise rehabilitation. Here we examined effects of isolated thoracic SCI in mice on cognition, depression, and brain neurodegeneration. To determine whether cognitive and affective impairments increase as a function of injury severity we exposed mice to sham, mild, moderate, or severe SCI using the Infinite Horizon Spinal Cord Impactor and evaluated performance on a variety of neurobehavioral tests that are less dependent on locomotion. We showed that locomotor function measured by BMS scores was reduced in an injury severity

manner. Cognitive impairments in the tests of Y-maze, novel objective recognition, and step-down fear conditioning increased with injury severity at two months post-injury. SCI also caused deficits in affective behavior as quantified in the sucrose preference, tail suspension, and forced swim test, dependently on injury severity. Stereological analysis demonstrated SCI severity-dependent neuronal loss chronically in the cerebral cortex, thalamus, and hippocampus. Our data suggest that spinal cord contusion in mice induces a chronic neurodegeneration in important brain regions associated with cognitive decline and physiological depression. Thus, these findings provide the experimental confirmation of clinical evidence suggesting SCI-related cognitive/affective deficits considerably revising concepts about the nature of SCI as a focal acute neurodegenerative disorder.

Disclosures: **Z. Zhao:** None. **B.A. Stoica:** None. **A.I. Faden:** None. **J. Wu:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 610.01/U12

Topic: C.08. Ischemia

Title: Neuroprotective effect of sodium nitroprusside depends of cellular differentiation stage and damage conditions in different cell lineages

Authors: *R. MACIAS-VÉLEZ[1], L. SAUCEDO ARELLANO[1], M. RIVERA CERVANTES[1], R. SCHLIEBS[2];
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Abstracts: Under hypoxic conditions the formation of free radicals exceeds the antioxidant mechanisms, triggering multiple alterations to cellular components.Oxidative stress has been defined as a disturbance in pro-oxidant and antioxidant balance. If pro-oxidant stress goes beyond, it can cause cell damage and death.Sodium nitroprusside (SNP) is a nitric oxide (NO) donor, which has been used for testing the oxidative stress, particularly in in-vitro experimental models; as well to value the neuroprotective properties of NO, by the modulation of specific intracellular signaling pathways.The aim of this study was to value differentially incubation of two different cell lines and primary-neurons culture with addition SNP in culture media, favoring cell viability and determining differential susceptibility to hypoxia and oxidative stress caused by H2O2. And let to propose a potential explanation about how cell susceptibility depends of damage nature. Also this study valued the dual effect of NO released from SNP in culture media. This study used two cellular lines (5151, GT1-7) and primary culture neurons. Both were incubated for 2 and 6 hours in culture media containing different concentrations of SNP (1,10,100 y 1000µM) in order to obtain a dose-response curve, by a cellular viability test. Later, the cultures was submitted to an oxidative stress model with H2O2 (using two concentrations, 250 and 500µM) and a hypoxia model (by perfusion of 95% N2 and 5% CO2 in culture media). Subsequently under these damage conditions, we valued the SNP neuroprotective effect (1 and 10µM) which was added to culture media and tested by MTT analysis.Our results shown that SNP protect non-differentiated GT1-7 cells from damage cause by H2O2. But not in the other cell line and primary culture neurons.Also SNP plays a protective roll in differentiated GT1-7 cells damaged by hypoxia. By the same way SNP exert a protective effect in primary culture

neurons under hypoxic damage. So, the neuroprotective roll of SNP over cells subjected to pro-oxidant stress depends of stage of differentiation and nature of damage.

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Poster

610. Neuroprotection in Ischemia, Stress, and Injury

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 610.02/U13

Topic: C.08. Ischemia

Support: CalciGenix

Title: The neurotherapeutic effects of the calcium binding protein apoaequorin

Authors: *V. L. EHLERS[1], E. L. ADAMS[1], N. B. FETTINGER[1], S. C. MICHELS[1], J. R. MOYER, Jr.[1,2];
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Abstracts: Stroke is a devastating condition that accounts for 1 out of every 19 deaths in the United States alone (Go et al., 2013). During ischemic stroke, deprivation of oxygen and glucose leads to intracellular calcium accumulation, triggering molecular cascades and activation of proteases, caspases, and calpains that lead to cell death (Choi, 1992). Several studies demonstrate that buffering excess intracellular calcium using endogenous calcium binding proteins (CaBPs) provides neuroprotection from ischemia (e.g., Fan et al., 2007). Apoaequorin (AQ), a CaBP isolated from the jellyfish Aequorea victoria, previously demonstrated neuroprotection using an *in vitro* model of ischemia. Specifically, an intrahippocampal infusion of AQ administered prior to ischemia resulted in reduced cell death, in addition to elevation of cytokine and chemokine mRNA (Detert et al., 2013). The anti-inflammatory cytokine IL-10 was significantly increased 1 h following AQ infusion, and remained elevated for up to two days post-infusion, suggesting the potential involvement of a neuroimmunomodulatory response in neuroprotection. Oral administration of AQ for 7 days prior to an ischemic insult also resulted in significantly reduced cell death (Hochstetter et al., SfN 2013). In the present studies, we investigated the neuroprotective effects of AQ oral administration using additional doses (0, 3.6, 48, 240, 480 mg/kg AQ mixed with peanut butter for 7 d) and lengths of administration (48 mg/kg AQ for 0, 1, 2, or 7 d). Using an *in vitro* model of ischemia, the present study found that oral administration

of AQ is neuroprotective in a dose- and time-dependent manner. Specifically, a dose of 48 mg/kg resulted in significantly reduced cell death when administered for 7 days prior to ischemia ($p < .05$). The results of these studies support our previous findings indicating hippocampal infusion of AQ is neuroprotective, and demonstrate that oral administration of AQ may serve as an additional route for neuroprotection. Ongoing studies are investigating whether oral administration of AQ leads to measurable levels of the protein in the brain, and if not, how AQ oral administration is providing its neuroprotective effects.

Disclosures: V.L. Ehlers: None. **E.L. Adams:** A. Employment/Salary (full or part-time):; Quincy Bioscience. **N.B. Fettinger:** None. **S.C. Michels:** None. **J.R. Moyer:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Program#/Poster: 610.03/U14

Topic: C.08. Ischemia

Support: Junta de Castilla y León reference LE 184A12-2

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Title: GABA treatment induces significant differences in the unfolded protein response (UPR) in cerebral cortex and hippocampus in an oxygen and glucose deprivation (OGD) model

Authors: *M. SANTOS GALDIANO[1], D. PEREZ-RODRIGUEZ[2], B. ANUNCIBAY-SOTO[2], E. FONT[2], I. FERNANDEZ-UGIDOS[2], P. GONZALEZ-RODRIGUEZ[2], C. C. PEREZ-GARCIA[3], A. FERNANDEZ-LOPEZ[2];
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Abstracts: GRP78/BIP is a molecular chaperone located in the RE that regulates the protein folding and elicits the unfolded protein response (UPR) following ER stress. GRP78/BIP is widely used as a measurement of the UPR a condition elicited by stroke. We here report the UPR response to oxygen and glucose deprivation (OGD) and glutamate excitotoxicity in the external and internal pyramidal cells of the cerebral cortex and CA1 and CA3 hippocampal pyramidal cells. The study has been performed in a *in vitro* slice model. While the presence of glutamate

elicited a strong UPR in both cerebral cortex and hippocampus, the OGD-dependent UPR was only detected in the hippocampus. The presence of GABA in the incubation media increased significantly the URP in the cerebral cortex to levels similar to those observed in the presence of glutamate. GABA did not modified the UPR elicited by glutamate. Thus, UPR seems to play a crucial neuroprotective role in the excitotoxic stress in both cerebral cortex and hippocampus. In contrast the neuroprotective role of UPR following OGD appears in the hippocampus but not in the cerebral cortex. Our results support that UPR in the cerebral cortex would be related with GABA receptors and would fit with previous studies indicating a relevant role of chloride channels.

Disclosures: **M. Santos Galdiano:** None. **D. Perez-Rodriguez:** None. **B. Anuncibay-Soto:** None. **E. Font:** None. **I. Fernandez-Ugidos:** None. **P. Gonzalez-Rodriguez:** None. **A. Fernandez-Lopez:** None. **C.C. Perez-Garcia:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.08. Ischemia

Support: RO1#NS073832 (AZ)

U54 #NS083932 (MSM NI)

Title: Distinct roles of polycomb group proteins and their associated proteins in neuronal cells and endocrinal cells

Authors: *F. BIAN[1], J. RICE[3,2], L. CAO[1], Y. LI[1], A. ZHOU[1];
[1]Neurosci. Inst., [2]Morehouse Sch. of Med., Atlanta, GA; [3]Spelman college, Atlanta, GA

Abstracts: Background: Polycomb group (PcG) proteins are transcriptional repressors expressed in various cell types. A protective role against ischemic injury has been shown for a few PcG proteins in neuronal cells. Differential changes in PcG proteins and some of their target proteins have been reported for different neuronal ischemic conditions. Little is known for the roles of PcG in different cell types. The objective of this study was to determine (1) whether PcG proteins may have different interacting partners in different cell types, and (2) how cellular proteomes may be regulated differently by changes in PcG protein levels. **Methods:** Mouse

brain-derived neuroblastoma NS20Y cells or pituitary adenoma AtT20 cells were transfected with cDNAs encoding Myc-tagged PcG protein Bmi1 or Ezh2, respectively. PcG-interacting proteins (direct or indirect) were isolated by immunoprecipitation (IP) using an anti-Myc antibody and determined by quantitative mass spectrometry (MS). Cellular proteomes were analyzed with a quantitative MS-based proteomic approach and characterized with the assistance of bioinformatic tools (MetaCore and Proxeon). **Results:** IP-MS analysis showed 12 Bmi1-interacting proteins in both NS20Y and AtT20 cells (common), 59 only in NS20Y cells and 44 only in AtT20 cells. Examples of common proteins include Csnk2a1, Hdac2, and Ywhab, which are the known Bmi1-interacting proteins. Bmi1-interacting proteins that were identified only in NS20Y cells or AtT20 cells also included those previously known and those reported here for the first time. Examples of the latter include Chd4, Ybx3, Ebna1bp2, Ubtf, Uchl1, and Upf1 in NS20Y cells, and Ercc6l2, HMX3, and Tardbp in AtT20 cells. Results of proteomic analysis of total cellular proteins showed enriched presences of different biological processes in WT NS20Y cells and AtT20 cells. Interestingly, overexpression of Ezh2 also induced differential proteomic changes in NS20Y cells and AtT20 cells. Notable examples are decreased levels of proteins associated with DNA damage/Role of SUMO in p53 regulation in NS20Y cells, and glycolysis, gluconeogenesis and glucocorticoid receptor signaling in AtT20 cells. Taken together, these results suggest that individual PcG proteins may play different regulatory roles in different cell types, and they do so by interacting with different partner proteins.

Disclosures: **F. Bian:** None. **J. Rice:** None. **L. Cao:** None. **Y. Li:** None. **A. Zhou:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.08. Ischemia

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Title: Inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) synthesis decreases apoptotic and necrotic neurodegeneration produced by oxygen-glucose deprivation

Authors: L. DU[1], S. M. POLOYAC[1], T. C. JACKSON[1], H. BAYIR[1], P. M. KOCHANEK[1], R. S. B. CLARK[1], *M. D. MANOLE[2];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Univ. Pittsburgh, PITTSBURGH, PA

Abstracts: Neuronal hypoxia-ischemia induces membrane depolarization, phospholipase activation, and arachidonic acid (AA) release. Metabolism of AA by CYP 4A ω- hydrolase generates 20-HETE, a potent vasoconstrictor. 20-HETE increases in brain homogenates after resuscitation from cardiac arrest. Blocking CYP4A with the irreversible inhibitor HET0016 improves cortical perfusion and neurological outcome. The beneficial effect of CYP4A/20-HETE inhibition could be due to improved perfusion, direct neuroprotective effects, or both. The detrimental effect of 20-HETE in neurons is not completely understood. We hypothesize that inhibition of 20-HETE production after hypoxia in primary cortical neuronal rat cultures improves neuronal survival by preserving antioxidant reserves and decreasing apoptosis. Cortical neurons were exposed to the following conditions: oxidative stress using H_2O_2, excitotoxic stress using glutamate and glycine, and oxygen glucose deprivation (OGD) using 95% argon, 5% CO_2 and 0.5 mM Glucose. Cell death was assessed using the MTT assay, lactate dehydrogenate release, and flow cytometry. Incubation with 20-HETE produced a dose-dependent decrease in neuronal survival (73.5±6, 55±7, and 41±4% of control for 1, 10, and 100μM 20-HETE, respectively, $p<0.05$). Inhibition of 20-HETE formation using the CYP4A inhibitor HET0016 increased survival in neurons subjected to oxidative stress induced by H_2O_2 (65±7% vs. 89±15%, $p<0.05$, vehicle vs. HET0016). It also increased neuronal survival after excitotoxic injury induced by glutamate/glycine (69±7% vs. 81±10%, $p<0.05$, vehicle vs. HET0016). OGD impaired the cellular respiration as assessed by the MTT assay. Inhibiting 20-HETE formation with HET0016 increased neuronal survival after OGD (74.3±7.7 vs. 87.4±4.5%, $p<0.05$, OGD vs. HET0016 treated OGD at 2h, and 57.3±2.5 vs. 71.6±6.1%, OGD vs. HET0016/OGD at 24h, $p<0.05$). Treatment with HET0016 reduced pro-apoptotic caspase 3/7 activity (225±6 vs. 147±6, OGD vs. HET0016/OGD, $p<0.05$) and restored intracellular GSH levels (47±0.6 vs. 58±0.1, OGD vs. HET0016/OGD, $p<0.05$). These data suggest that the vasoconstrictor eicosanoid 20-HETE has direct neurotoxic effects, and contributes to excitotoxic and oxidative neuronal death. After neuronal hypoxia induced by OGD, 20-HETE inhibition increased neuronal survival, restored intracellular GSH levels, and reduced caspase 3/7 activation. In conclusion, inhibiting 20-HETE synthesis reduces neuronal death and apoptosis in a model of *in vitro* OGD. Preventing 20-HETE formation is a neurotherapeutic strategy that merits further exploration in models of ischemia-reperfusion.

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Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.08. Ischemia

Support: NIH Grant NS056313

Title: The tri-block co-polymer F-68 (Poloxamer 188) blocks oxygen-glucose deprivation-induced increases in reactive oxygen species and prevents lipid peroxidation

Authors: P. B. SHELAT, J. C. WANG, *J. D. MARKS;
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Abstracts: Inhibition of reactive oxygen species (ROS) production reduces neuronal death following oxygen-glucose deprivation (OGD) *in vitro* and hypoxia-ischemia (HI) *in vivo*, showing that ROS contribute to this death. We have shown that F-68 (also called Poloxamer 188), a tri-block co-polymer of poly[ethylene oxides] and poly[propylene oxides], profoundly rescues hippocampal neurons from severe OGD, preventing mitochondrial apoptosis. F-68 interacts with cell membranes, and blocks exogenous lipid peroxidation in neurons. We hypothesized that F-68 alters lipid peroxidation following OGD in embryonic rat hippocampal neurons. C11-BODIPY$^{581/591}$-labeled hippocampal neurons were exposed for 45 min to either OGD (1% O_2, 0 glucose, 37° C; an insult that kills about 60% of neurons), or saline containing glucose in ambient O_2 (control). Different coverslips were imaged (490 nm) every 15 min over the subsequent 2 hr in control saline, and fluorescence intensity quantified on a cell-by-cell basis. Compared with control cells, C11-BODIPY$^{581/591}$ fluorescence intensity progressively increased, beginning at 30 minutes after OGD and reaching a plateau 90 min after OGD. In contrast, neurons continuously incubated in F-68 (30 μM) following OGD failed to exhibit significant fluorescence intensity increases over time, suggesting that F-68 blocks OGD-induced membrane lipid peroxidation. Lipid peroxidation following OGD is mediated by peroxynitrite, produced by the diffusion-limited reaction of superoxide and nitric oxide. Therefore, we used CellRox Orange to determine F-68 effects on OGD-induced changes in ROS. Compared with control neurons, CellRox fluorescence was increased 1 hr after OGD ($P<.01$) and further increased at 2 hr after OGD ($P<.0001$). Neurons continuously incubated in F-68 following OGD did not exhibit significant mean intensity increases over the 2 hr period compared with F-68 incubated control neurons, indicating that F-68 blocked OGD-induced increases in ROS. We next used linear regression of the raw intensities over 30 min epochs to determine ROS production rates at each time point. Following OGD, mean CellRox slopes markedly and progressively increased from control slopes over 2 hrs after OGD. In contrast, mean CellRox slopes in F-68 treated neurons

following OGD did not significantly increase over time compared with the mean baseline slope. These data indicate that F-68 treatment blocks OGD-induced membrane lipid peroxidation and inhibits ROS increases following OGD. This F-68-induced block of ROS increases after OGD may occur as a result of the previously demonstrated inhibition by F-68 of OGD-induced mitochondrial dysfunction.

Disclosures: **P.B. Shelat:** None. **J.C. Wang:** None. **J.D. Marks:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Maroon Biotech.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

Location: Halls A-C

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Program#/Poster: 610.07/U18

Topic: C.08. Ischemia

Title: 2-(4-Methoxyphenyl)ethyl-2-acetamido-2-deoxy-β-D-pyranoside confers neuroprotection in cell and animal models of ischemic stroke through calpain1/PKA/CREB-mediated induction of neuronal glucose transporter 3

Authors: S. YU, Q. HE, L. WEI, *F. DING;
Jiangsu Key Lab. of Neuroregeneration, Nantong University, China, Jiangsu, China

Abstracts: Objectives: Salidroside is proven to be a neuroprotective agent of natural origin, and its analogue, 2-(4-Methoxyphenyl)ethyl-2-acetamido-2-deoxy-β-D-pyranoside (named SalA-4g), has been synthesized in our lab. The aim of this study was to investigate whether SalA-4g could be developed into a promising neuroprotective drug for treating neuronal insult during cerebral ischemic stroke. Very importantly, the regulation mechanisms underlying the neuroactivity of SalA-4g were further explored. Methods: The *in vitro* and *in vivo* protective effects of SalA-4g were evaluated in primary hippocampal neurons exposed to oxygen and glucose deprivation (OGD) and the male Sprague-Dawley (SD) rat model of ischemic stroke induced by transient middle cerebral artery occlusion (MCAO), respectively. The cell survival and cell apoptosis were evaluated by MTT, Hoechst, TTC staining and TUNEL assays. To explore the mechanism of SalA-4g neuroprotection, glucose uptake and glucose transporter 3 (GLUT3) expression and recruitment were measured with a fluorescent dye (2-NBDG), immunocytochemistry and Western blot analysis. The calpain1/PKA/CREB signaling analysis was determined by Western blot analysis, immunocytochemistry, MTT, Hoechst and intracellular Ca2+ measurement using

the fluorescent indicator (Fluo-3/AM). Results: SalA-4g promoted neuronal survival and inhibited neuronal apoptosis in primary hippocampal neurons exposed to OGD and in rats subjected to ischemia by MCAO, respectively, and that SalA-4g was more neuroprotective than salidroside. SalA-4g elevated glucose uptake in OGD-injured primary hippocampal neurons and increased the expression and recruitment of GLUT3 in ischemic brain. Signaling analysis revealed that SalA-4g triggered the phosphorylation of CREB, and increased the expression of PKA RII in primary hippocampal neurons exposed to OGD injury, while inhibition of PKA/CREB by H-89 alleviated the elevation in glucose uptake and GLUT3 expression, and blocked the protective effects of SalA-4g. Moreover, SalA-4g was noted to inhibit intracellular Ca2+ influx and calpain1 activation in OGD-injured primary hippocampal neurons. Conclusion: SalA-4g neuroprotection might be mediated by increased glucose uptake and elevated GLUT3 expression through calpain1/PKA/CREB pathway.

Disclosures: **S. Yu:** None. **Q. He:** None. **L. Wei:** None. **F. Ding:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Program#/Poster: 610.08/U19

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The ability of a pomegranate husk extract to modify alzheimer's disease pathology in aged transgenic mice

Authors: ***G. M. SUBAIEA**[1,2], A. H. AHMED[3], N. P. SEERAM[2], A. E. EID[3], N. H. ZAWIA[3]; [1]Col. of Pharm., Univ. of Hail, Hail, Saudi Arabia; [2]Biomed. and Pharmaceut. Sci., [3]Interdisciplinary Neurosci. Program, Univ. of Rhode Island, Kingston, RI

Abstracts: Accumulating research has demonstrated that polyphenolic compounds from natural products may have antioxidant and neuroprotective abilities in different models of Alzheimer's disease (AD). Pomegranate in particular has been shown to have promising effects. The present study explores whether the administration of a pomegranate peel extract could have a rescuing effect on AD pathology in aged transgenic mice. Daily doses of the extract or a control solution were fed to groups of aged transgenic mice (R1.40), ranging in age from 24-30 months. These mice already have abundant AD pathology since amyloid Beta (Aβ) deposition continues with age. The mice were treated for thirty-seven days total with assessment starting the last week of treatment. Mice were tested for improvements in spatial and long term memory function, and for

working memory using behavioral tasks including Morris water maze and the Y-maze. This was followed by measurements of cortical APP and total amyloid Beta levels by Western blot and ELISA, respectively. The resulting data demonstrated a specific, but significant decrease in AD pathology manifested by the lowering of Aβ42/40 ratio. Further experiments revealed that this reversal could be the product of the modification of the gamma-secretase enzyme responsible for generating the more amyloidogenic form. In conclusion, pomegranate peel extract appears to contain ingredients that act as gamma-secretase modulators, which may be identified and developed as compounds for use in future drug therapy.

Disclosures: G.M. Subaiea: None. **A.H. Ahmed:** None. **N.P. Seeram:** None. **A.E. Eid:** None. **N.H. Zawia:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 610.09/U20

Topic: C.08. Ischemia

Support: Indian Council of Medical Research

Title: Activated Protein C exert its neuroprotective activity via FAIM2 against oxygen-glucose deprivation in cultured SH-SY5Y Cells

Authors: *M. K. SRIWASTVA[1], R. KUNJUNNI[1], K. PRASAD[2], R. SAXENA[3], V. SUBBIAH[1];
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Abstracts: Introduction: With the recent interest of Activated Protein C (APC) as possible targets for the cerebrovascular injury, we tested whether Activated Protein C protected via FAIM2 against oxygen-glucose-deprivation (OGD) induced ischemic injury in cultured SH-SY5Y cells. It is proposed that FAIM2 have protective role in cerebrovascular ischemic injury by phosphatidylinositol 3-kinase -Akt/protein kinase B pathway. Methods: Oxygen-glucose deprivation of SH-SY5Y cells were prepared in media deprived of glucose and fetal bovine serum and kept in a air-tight chamber containing 95%N2 and 5% CO2 for 3 hours with 24 hours reoxygenation. The ischemic injury was checked by measuring the release of lactate dehydrogenase (LDH) in to the medium and the cell viability was assessed by WST-8 assay. The expression of EPCR, PAR-1 & PAR-3 was quantified by flow cytometry. The expression of

FAIM2 and Fas was measured by real time PCR after 24 hours of reoxygenation. Results: APC pre-treatment at a dose of 100 nM reduce the cell toxicity upto 35% ($P<0.05$); the expression of all three receptors EPCR, PAR-1 and PAR-3 was up-regulated significantly ($p<005$) in both hypoxia treatment and hypoxia and APC both compared to control normoxic condition. The Quantitative Faim2 and Fas mRNA real-time PCR expression analyses at 24 h of reoxygenation revealed the expressions of FAIM2 upregulated while the expression of Fas downregulated upon APC treatment. Conclusion: We concluded that the effects of neuroprotection of APC in ischemic injury also occur through phosphatidylinositol 3-kinase -Akt/protein kinase B pathway via FAIM2. Acknowledgment: Indian Council of Medical Research, New Delhi, India

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Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Program#/Poster: 610.10/U21

Topic: C.08. Ischemia

Support: NIH Grant RO1NS062967

Title: Activation of the delta opioid receptor attenuates ASIC1a-mediated neuronal death

Authors: *J. VICK, T. SHERWOOD, E. SCHIMMOELLER, C. ASKWITH;
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Abstracts: A decrease in extracellular pH accompanies multiple pathologies within the nervous system including stroke/ischemia, traumatic injury, seizure, and multiple sclerosis. Prolonged extracellular acidosis induces neuronal death through the acid sensing ion channels (ASICs) and is enhanced by several endogenous factors. However, whether any endogenous factors can prevent ASIC-mediated death is unknown. We find that enkephalins can dramatically attenuate acidosis-induced neuronal death in cultured cortical and hippocampal neurons. This action is dependent on activation of the delta opioid receptor (DOR) and is mimicked by non-peptide DOR agonists. Activation of DOR has previously been found to prevent ischemic neuronal death in rodent models of stroke. DOR-mediated protection from acidotoxicity was dependent on G-protein activity and occurred through manipulation of signal transduction cascades. Interestingly, while DOR agonists did not reduce ASIC current density or activation, ion selectivity was

altered. These results indicate that ASIC-mediated neuronal death can be attenuated by DOR activation and suggests that DOR agonists, which have shown neuroprotective properties in animal models, may prevent neuronal death in ischemic conditions. Finally, the connection between ASICs and the opioid system is further strengthened by this work. In the spinal cord, ASIC inhibition limits pain through an enkephalin-dependent mechanism. Our work suggests that enkephalins can affect ASIC-induced signaling as well. This observation could impact our understanding of ASICs role in behavior and pain in addition to neuronal death.

Disclosures: J. Vick: None. **T. Sherwood:** None. **E. Schimmoeller:** None. **C. Askwith:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.08. Ischemia

Support: Grants-in-Aid for Scientific Research (S) 23228004

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Title: Glucose-deprivation attenuates sortilin expression via AMP kinase cascade in PC12 cells

Authors: *K.-I. KAWASHIMA, K. FUJINO, Y. OGURA, N. MIYANISHI, T. NEDACHI; Toyo Univ., Gunma, Japan

Abstracts: PGRN (Progranulin) gene mutations are associated with several dementia, such as frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). PGRN is a secreted growth factor that promotes neuroprotection and cell proliferation. However, it remains largely unknown how these physiological actions of PGRN are regulated. Recently, sortilin, a member of a Vps10p domain receptor family, has been well documented as a receptor for PGRN. The expression levels of sortilin in neuronal cells, therefore, could be a factor to determine PGRN action; however, the regulatory mechanisms of sortilin are not fully understood. We have explored the stimuli that controls the expression of sortilin, and finally found the expression levels of sortilin is regulated by extracellular glucose concentration. We further investigated underlying mechanisms how the changes in glucose availability controlled sortilin expression and impact of this changes on PGRN-dependent bio-action. Initialy, we used PC12 cells exposed to two different DMEM medium (High Glucose-DMEM; 4.5 g/l, Glucose

Free-DMEM; 0.0 g/l glucose) to examine sortilin expression. After 24 hours of glucose deprivation, the gene and protein expression of sortilin was significantly reduced. Next, we focused on the molecular mechanisms how glucose deprivation reduced sortilin expression. AMP kinase (AMPK) is a well known kinase that activity was increased upon glucose deprivation. After confirming AMP kinase activation was observed by glucose deprivation, we tested the effects of AMPK activator (AICAR) on sortilin expression. Sortilin expression was reduced by AICAR treatment even in the presence of high concentration of glucose, suggesting that the reduced sortilin expression by glucose deprivation is mainly mediated by AMPK activation. In conclusion, we found that sortilin expression was significantly reduced by glucose deprivation via AMPK activation in PC12 cells. This mechanism may be important for regulating the bioactivity of PGRN in central nervous system.

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Poster

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Support: Academia Sinica grant

National Science Council grant

Title: PPAR-γ by suppressing NADPH oxidase subunit p22-phox transcription attenuates ischemic brain damage

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Abstracts: Our previously data showed that Peroxisome proliferator-activated receptor-γ (PPAR-γ) protects neurons against hypoxic/ischemic injury by attenuating reactive oxygen species (ROS) production. Nonetheless, the detailed molecular mechanism is not fully understood. Resent studies indicate that NADPH oxidase is one of the major sources for ROS production in brain. In the present studies, we aim to investigate whether and how PPAR-γ interacts with NADPH oxidase, thereby downregulating ROS generation, to ameliorate ischemic

brain injury. (1) *In vitro* studies: we demonstrate PPAR-γ agonist (15-deoxy-Δ12,14-Prostaglandin J2) increased primary cortical neurons (PN) viability after oxygen glucose deprivation and reoxygenation (H/R). With pharmacological (PPAR-γ antagonist GW9662), loss-of-function (PPAR-γ siRNA), and gain-of-function (Ad-PPAR-γ) approaches, we demonstrated that PPAR-γ by inhibiting NADPH oxidase activity attenuated ROS formation and neuronal apoptosis. Western blot analysis further showed that PPAR-γ specifically decreased the p22-phox subunit level of NADPH oxidase. Results of reporter, ChIP, and confocol assays revealed that PPAR-γ blocked H/R-induced NF-κB nuclear translocation, which led to inhibited transcription of p22-phox. (2) *In vivo* studies: Western blot analysis revealed that 15d-PGJ2 reduced the level of protein oxidation and p22-phox in brain subjected to 30-min ischemia and 1-day reperfusion. By contrast protein oxidation and p22-phox level were higher in ischemic brain of heterozygous PPAR-γ (L/+) mice than wild type control. Furthermore, p22-phox siRNA significantly reduces cerebral infarct volume, improves functional recovery, and protects blood-brain barrier (BBB) integrity. In summary, we report a novel transrepression mechanism whereby PPAR-γ downregulates ischemia-induced p22-phox transcription and the subsequent NADPH oxidase activation, ROS formation and neuronal apoptosis. This work is supported by grants form National Science Council and Academia Sinica, Taipei, Taiwan, ROC Authors disclose no conflict of interests. Key words: stroke, ROS, primary cortical neuron, 15d-PGJ2

Disclosures: J. wu: None. **H. Tsai:** None. **W. Cheung:** None. **T. Lin:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.08. Ischemia

Support: NIH Grant NS015589

Title: Demonstration of ischemic preconditioning in white matter: Critical role for toll-like receptor-4

Authors: *M. A. HAMNER, R. V. LEE, Z. YE, D. C. HANSSEN, B. R. RANSOM, J. R. WEINSTEIN;
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Abstracts: Ischemic preconditioning (IPC) is a robust protective phenomenon in which a brief ischemic exposure confers tolerance to a subsequent ischemic challenge. Most experimental paradigms for IPC are carried out in rodents that have very little white matter (~10-15%) compared to man (~55%). Consequently, IPC has been studied predominantly, if not exclusively, in gray matter. In humans, most strokes injure both white matter (WM) and gray matter (GM), and clinical outcome depends on damage to both of these areas. We sought to determine if IPC exists in WM, and if present, did it depend on Toll-like receptor-4 (TLR4), a key receptor in innate immune response, as is the case in GM IPC. We used a standard protocol for inducing IPC in mice *in vivo* consisting of 15 minutes of common carotid artery ligation, producing a pattern of diminished blood flow which included the optic nerve. Seventy-two hours later, the mouse optic nerve (MON), a typical CNS WM tract, was removed and exposed *ex vivo* to oxygen-glucose deprivation (OGD) for 45 minutes followed by 5 hours of normoxia/normoglycemia. MON excitability was monitored quantitatively by measuring the area under the supramaximal compound action potential (CAP) before, during and after the acute insult. CAP data was normalized and recovery is shown as a percentage of the baseline level. MON histopathology was assessed by quantitative immunofluorescent microscopy. In wild type (*WT)* animals, preconditioned (ipsilateral) MON showed improved functional recovery (CAP area = 31±3% of baseline CAP area) compared with either sham-operated (21±4%, *$p<0.05$) or contralateral (14±2%, *$p<0.0001$) control MONs. IPC in GM depends on TLR4 and this also appeared to be the case in WM. *TLR4*$^{-/-}$ preconditioned MONs did not differ from either sham or contralateral controls in terms of CAP recovery after OGD. Compared to control nerves, MONs subjected to IPC lost fewer oligodendrocytes; damage to astrocytes and microglia was unchanged. Ischemic WM injury is mediated in part by excitoxicity. Glutamate levels in MON perfusate and AMPA glutamate receptor mRNA subtypes/isoforms were quantified by high performance liquid chromatography and qRT-PCR, respectively. IPC, however, had no effect on OGD-induced glutamate release or AMPA receptor expression. These findings demonstrate that: (i) IPC induces a robust protective effect in WM and (ii) this effect is dependent on functional TLR4.

Disclosures: **M.A. Hamner:** None. **R.V. Lee:** None. **Z. Ye:** None. **D.C. Hanssen:** None. **B.R. Ransom:** None. **J.R. Weinstein:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH Grant NS015589

Title: Alpha(α)-synuclein increases injury vulnerability due to oxidative stress or ischemia

Authors: *X. YANG[1], H. ZHOU[3], M. A. HAMNER[2], Z. YE[2], B. R. RANSOM[2];
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Abstracts: The protein α-Synuclein (α-Syn) is implicated in the pathophysiology of Parkinson's disease. It is expressed in both neurons and glial cells, but its physiological functions in glia remain unclear. It is also unknown if α-Syn expression might condition susceptibility to brain injury, a question suggested by its association with CNS degeneration. To investigate these questions, normal astrocytes expressing α-Syn (wild type, α-Syn$^{+/+}$) were compared to astrocytes from α-Syn knockout (α-Syn$^{-/-}$) animals with regard to oxidative stress sensitivity. Cultured midbrain astrocytes were subjected to oxidative stress by exposure to various concentrations of hydrogen peroxide (H_2O_2). Astrocytes showed time- and dose-dependent H_2O_2 cytotoxicity. Unexpectedly, wild type (WT) astrocytes showed significantly greater injury compared to astrocytes from α-Syn$^{-/-}$ animals. Cytotoxicity was accompanied by progressive accumulation of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and intracellular $[Na^+]$ ($[Na^+]_i$) in a H_2O_2 concentration-dependent manner. The WT astrocytes showed earlier and greater increases in both ions. Increase in $[Na^+]_i$ preceded change in $[Ca^{2+}]_i$, suggesting that the increase in $[Na^+]_i$ mediated the $[Ca^{2+}]_i$ increase, possibly via reversed Na^+/Ca^{2+} exchange. Enhanced vulnerability to oxidative injury was restored in α-Syn$^{-/-}$ astrocytes by transfecting with human α-Syn, confirming that α-Syn was responsible for the WT phenotype. We then tested if α-Syn$^{-/-}$ animals might be less vulnerable to ischemic brain injury than WT animals, using a validated model of CNS white matter injury, the acutely isolated mouse optic nerve (MON). Indeed, α-Syn$^{-/-}$ MONs suffered significantly less injury compared to WT controls after 60 min of ischemia (recovery = 42 ± 3% vs. 20 ± 2%, $p<0.001$). Finally, we used another injury paradigm, anoxia, which has an entirely different injury mechanism, to further test the impact of α-Syn expression on injury severity. As with ischemia, anoxia caused less damage in the α-Syn$^{-/-}$ animal. In summary, α-Syn expression confers markedly increased injury vulnerability to astrocytes and to intact CNS white matter. These findings suggest that for all the adaptive value of α-Syn, its expression has a dark side manifest by both cellular and tissue enhanced injury vulnerability.

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Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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The University Grants Commission, New Delhi, India

Indian Medical Research Council, New Delhi, India

Title: Functionalized magnetic iron oxide nanoparticles induced Ubiquitin and heat shock protein responses following hyperthermia in the central nervous system is attenuated by nanowired cerebrolysin

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Abstracts: Functionalized magnetic iron oxide nanoparticles (FMIONPs) are used for cancer therapy together with whole body hyperthermia (WBH) or for drug delivery to the target tumor tissues using external magnetic guidance. Our laboratory has previously shown that FMIONPs are innocuous in normal healthy rats up to 24 h, however, when administered following WBH an exacerbation of brain pathology occurs. Interestingly, when TiO2 nanowired Cerebrolsyin is delivered together with FMIONPs in WBH significant neuroprotection is seen. However, the detailed mechanisms of cerebrolysin-induced neuroprotection are still unclear. The present investigation was undertaken to explore the possible mechanisms of cerebrolysin-induced neuroprotection in FMIONPs induced brain pathology in WBH. Cerebrolsyin is a balance composition of several neurotrophic factors and active peptide fragments. Thus, a possibility

exists that TiO2 nanowired Cerebrolsyin could attenuate cellular stress responses following WBH together with FMIONPs administration. We used ubiquitin and heats shock protein (HSP) 72 kD to evaluate stress responses in WBH and FMIONPs combination. Young adult Male Sprague Dawley rats (age 25 to 27 weeks) were administered FMIONPs in a dose of 0.50 mg/mL in100 µl intravenously and then subjected to 4 h WBH at 38°C in a Biological Oxygen Demand (BOD) Incubator (relative humidity 45-47 % and wind velocity 20-22 cm/sec). Twenty-four h after WBH immunohistochemistry of Ubiquitin and HSP 72 kD were examined on paraffin sections from the brain passing thorough parietal cerebral cortex and hippocampus. In a separate group rats, TiO2 nanowired Cerebrolsyin (2.5 ml/kg) was also co-administered and the brains were processed under identical conditions for ubiquitin and HSP-72 kD immunoreactivity. In addition, albumin immunoreactivity was also assessed to test any breakdown of the blood-brain barrier (BBB) to the endogenous serum proteins. Our observation show that FMIONPs following WBH resulted in pronounced albumin leakage and upregulation of ubiquitin and HSP immunoreactivity in the cerebral cortex, hippocampus, hypothalamus and thalamus. Interestingly TiO2 nanowired Cerebrolsyin (2.5 ml) exhibited significant reduction (> 90%) in albumin, ubiquitin and HSP immunoreaction in the brain after FMIONPs treatment following WBH. Taken together our results for the first time show that FMIONPs induces enhanced stress reaction in the brain following WBH and TiO2 cerebrolysin treatment reduces these stress signals significantly indicating a potential role of Cerebrolysin in reducing brain pathology in cancer patients where FMIONPs are used as an adjunct therapy.

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Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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CNCSIS ˆUEFISCSU, project number PNII ˆ IDEI 787/2007, Romania

Title: Superior antioxidant effects of nanowired cerebrolysin in heat stroke following intoxication of engineered Ag and Cu nanoparticles

Authors: *D. F. MURESANU[1], A. SHARMA[2], R. PATNAIK[3], A. BUZOIANU[4], H. MOESSLER[5], H. S. SHARMA[2];
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Abstracts: Heat stroke is prevalent in our armed forces especially during summer seasons in desert environment. Heat stroke results in instant death or permanent disability in victims with profound manifestation of neurological disorders. Since our soldiers are also exposed to a variety of nanoparticles e.g., Ag, Cu, Silica dust during their day-to-day work it appears that nanoparticles exposure may aggravate their mental dysfunction in heat stress. Since brain damage following heat stroke is exacerbated by Ag or Cu nanoparticles there is an urgent need to find out suitable drugs to reduce the pathophysiology of brain injury in heat stroke. In this investigation we examined the effects of nanowired cerebrolysin with regard to its antioxidative effects in heat stroke management as compared to the other contemporary stroke therapies employed in clinics. Thus, the possibility that neuroprotective effects of cerebrolysin, a mixture of several neurotrophic factors and active peptides fragments could exert powerful antioxidant properties as compared other stroke therapies, e.g., levertiracetam (44 mg/kg), pregabalin (200 mg/kg), topiramat (40 mg/kg) and valproate (400 mg/kg) was evaluated in a rat model of heat stroke in normal and in Cu or Ag nanoparticles (50 to 60 nm, 50 mg/kg, i.p. /day for 7 days) treated group. Rats subjected to 4 h heat stress in a biological oxygen demand incubator at 38°C (Rel Humid 45-47 %; Wind vel 22.4 to 25.6 cm/sec) resulted in profound increase in brain Luminol, Malondialdehyde and Myeloperoxidase, and a marked decrease in Glutathione. These changes correlated well with the reduction in the cerebral blood flow (CBF) and development of brain edema formation. These pathophysiological responses were exacerbated in nanoparticles treated heat-stressed rats. Pretreatment with cerebrolysin (2.5 ml/kg) daily for 3 days significantly attenuated the oxidative stress parameters and brain edema formation as well as improved CBF in heat stressed group. The other drugs were not effective in reducing these parameters after heat stroke. Interestingly, in nanoparticles treated animals cerebrolysin 5 ml/kg was required to achieve similar reduction in oxidative stress parameters, brain edema formation and improvement in cerebral circulation. However, the other drugs even in higher doses were not able to alter these parameters in nanoparticles treated heat stressed animals. These observations

are the first to demonstrate that antioxidant capacity of cerebrolysin is far more superior in heat stroke than other therapeutic agents currently used to treat stroke related brain damages.

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Poster

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Vanderbilt Brain Institute Scholars Award

Suzanne and Walter Scott Foundation

Neuroscience Scholars Program Award, provided by SfN & NIMH

Title: Autophagic containment of mitochondria: Role in preconditioning protection

Authors: *B. N. LIZAMA-MANIBUSAN[1], A. M. PALUBINSKY[1], I. S. KHAN[3], R. J. SINGER[3], B. MCLAUGHLIN[2];
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Abstracts: Chronic and acute CNS injuries share similar pathology including increased reactive oxygen species (ROS), oxidized protein accumulation and activation of cell death pathways. Identifying conserved mechanisms that determine cell survival under stress will allow us to develop more effective, well-tolerated therapies for stroke. In order to identify endogenous neuroprotective pathways, we use a model of ischemic preconditioning (PC), in which mild ischemic stress confers protection from subsequent stroke-like episodes. We have shown that the sub-toxic PC stress evokes protection in part by temporally and spatially limiting caspase activation and ROS production. Significant gaps remain however, in our understanding of proteins that play a role in the temporal and spatial constraint of caspases as well as other pro-death signals. Using a transient middle cerebral artery occlusion (tMCAO) model of PC in rats, we observed significant differences in the expression of mitochondrial containment and protein

detoxification machinery at 48h. In order to determine if formation of mitophagic containment of organelles is an adaptive event in PC, we developed a neuron-enriched PC model in which cultures are exposed to 15min of oxygen and glucose deprivation (OGD) followed by 24h of recovery. Preconditioned cultures exhibited 50% less cell death when subsequently challenged with an otherwise lethal OGD stress. Preconditioned cells had high levels of stabilized PTEN-inducible kinase 1 (PINK1), a known regulator of mitophagy, 3h-24h following PC. The autophagy protein LC3, as well as the neuroprotective chaperone heat shock protein 70 (HSP70) and its co-chaperone C-terminus of HSC70-interacting protein (CHIP), are also increased in response to PC. To directly test the role of autophagy in neuroadaptation, we exposed neurons to the small molecule autophagy inhibitor, bafilomycin A1, during sub-lethal OGD and assessed viability 24h later. Neurons in which autophagy was halted exhibited increased cell death when subsequently exposed to OGD. This suggests that PC protection requires removal of damaged organelles as part of neuroadaptation to stressful stimuli. Ongoing experiments are aimed at identifying additional molecules that promote autophagy in preconditioned cells. This work is supported in part by the Neuroscience Scholars Program Award (provided by the NIMH and SfN) and the Suzanne and Walter Scott Foundation.

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Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS065789

NIH R01 AG026389

Title: The possible role of PINK1 in cardiolipin mediated mitophagy

Authors: *M. K. DAIL[1], C. T. CHU[2];
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Abstracts: Mitochondrial dysfunction has been implicated in a central role in the pathogenesis of Parkinson Disease, despite ambiguity of the cause of the dysfunction. Maintaining

mitochondrial quality is important to neurons, and a decline in quality of mitochondria has also been observed in PD. Mitophagy is a major mechanism of regulating mitochondrial quality. Dysregulation of mitophagy has been implicated in multiple neurodegenerative disorders including PD. Our group previously reported a novel mechanism of mitophagy in response to mitochondrial injury by PD-related toxins. Mitophagy occurs via the externalization of cardiolipin (CL) from the inner mitochondrial membrane to the outer by phospholipid scramblase (PLSCR3). There CL interacts with microtubule-associated-protein-1-lightchain-3 (LC3), which mediates autophagosome formation and mitochondrial cargo recognition. CL is synthesized by cardiolipin synthase (CLS1). RNAi knockdown of CLS1 and PLSCR3 decreased CL exposure and mitophagy in SH-SY5Y cells treated with rotenone or 6-OHDA, PD related toxins. The role of this pathway in genetic models of PD remains unknown. PINK1, a mitochondrial kinase, is known to regulate mitophagy. Thus we investigated whether PINK1 regulates mitophagy through effects on cardiolipin. We found that PINK1 may play a role in modulating CL availability.

Disclosures: **M.K. Dail:** None. **C.T. Chu:** None.

Poster

610. Neuroprotection in Ischemia, Stress, and Injury

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-5R01NS060123-04

Title: Haplo-insufficiency of autophagy proteins beclin 1 and VPS34 is linked to age-dependent neuroprotection

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Abstracts: Beclin 1 is an essential autophagy protein and its cellular functions intersect with the pathological pathways of AD and PD but we do not know the specific neuroprotective mechanisms of beclin 1 or how it may promote neural repair. Altered expression of beclin 1 is linked to several major diseases such as cancer, infectious diseases and neurodegenerative disorders and evidence demonstrates beclin 1 mediates removal of disease-related proteins

including APP metabolites, α-synuclein and mutant ataxin. In turn, beclin 1 may be a potential drug target but the physiological function of beclin 1 in neurons is poorly understood and whether the neuroprotective function of beclin 1 is mediated through strictly autophagy-dependent or independent pathways is unclear. Beclin 1 was the first described mammalian autophagy protein and a core component of the class III phosphatidylinositol 3-kinase (PI3K-III) complex, which plays an important role in membrane trafficking in autophagy, endocytosis, cytokinesis and phagocytosis. To date beclin 1 has largely been characterized in the context of autophagy. It modulates the lipid kinase activity of PI3K-III catalytic unit VPS34, which generates phosphatidylinositol 3-phosphate (PI(3)P), recruiting other autophagy proteins for the nucleation of autophagosome. Little is known, however, about how beclin 1 regulates specific functions of VPS34. Our lab has recently shown that beclin 1's autophagy-independent functions contribute to its function in neurons and neuronal viability is greatly reduced in the absence of beclin 1. We also illustrate reduced VPS34-associated PI(3)P levels in beclin 1 deficient MEF cells. It is important to understand the precise molecular mechanism whereby beclin 1 regulates VPS34 functions in autophagy, other membrane trafficking pathways, organelle homeostasis and cellular signaling pathways, the disruption of which may underlie the pathogenesis of multiple diseases. Here we show that haploinsufficiency of beclin 1 in the brain leads to alteration in VPS34 lipid kinase activity. Interestingly, we observe differences in behavior in beclin 1 heterozygous mice. We performed a microarray on beclin 1 heterozygous mice and observed significant differences in genes associated with learning and memory as well as mitochondria dynamics and protein kinase signaling. In addition we find that reduced beclin 1 levels and VPS34 activity is associated with enhanced disease related protein levels and we are currently investigating the mechanism. Together our data show the importance of the autophagy independent functions of beclin 1, which contribute to beclin 1's function in the nervous system.

Disclosures: **N.C. McKnight:** None. **M.S. Wold:** None. **Z. Yue:** None.

Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.01/U31

Topic: C.08. Ischemia

Support: NIH Grant 4R00NR013593-03

Title: Characterization of inflammation in acute and non-acute ischemic infarcts in human brain tissue

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Abstracts: Inflammation in the human brain after stroke is relatively uncharacterized. Therefore, it is unknown whether there is a canonical inflammatory response to stroke in the human brain, or if inflammation in the brain after stroke follows a different trajectory in different individuals. In support of the latter, there is evidence to indicate that some humans develop an adaptive immune response to brain antigens after stroke. For instance, brain antigens have been detected in lymphoid tissue after acute stroke, myelin reactive T lymphocytes have been found in some patients after stroke, and the presence of myelin reactive mononuclear cells in the circulation 90 days after stroke is associated with worse outcome. There are also several reports of oligoclonal bands in the cerebrospinal fluid of stroke patients, which suggests that in some people a B lymphocyte response to stroke may occur. Therefore, the goals of this study were to advance our understanding of inflammation in the human brain after stroke, investigate the extent of variability between individuals, and determine if some individuals develop an autoimmune response to stroke. Using both immunohistochemistry and multiplex immunoassay techniques, we characterized immune cell infiltration and measured cytokine levels in acute and non-acute ischemic infarcts in post-mortem human brain tissue.

Disclosures: K.P. Doyle: None. **J. Beischel Frye:** None. **T.V. Nguyen:** None.

Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.02/U32

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA P01 AG022550

NIA P01 AG027956

Title: Effects of LPS pretreatment on body temperature and lesion volume after stroke

Authors: *S. LEWIS, D. N. DOLL, X. REN, H. HU, J. W. SIMPKINS;
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Abstracts: Stroke is a complex condition with many risk factors such as hypertension, diabetes mellitus, and obesity. Many of these risk factors are known to cause systemic inflammation and studies have shown that an increase in systemic inflammation plays a role in both the pathology and etiology of stroke. Additionally, one third of strokes occur during an active infection, which is also associated with a state of heightened systemic inflammation. To better understand the role of inflammation and stroke, we used lipopolysaccharide (LPS) to mimic a bacterial infection in C57Bl/6 mice prior to a transient middle cerebral artery occlusion (tMCAO). Mice were injected i.p. with 100 ug/ kg - 2 mg LPS/kg, thirty minutes or 6 hours prior to the tMCAO and body temperatures were monitored for 48 hours. LPS when combined with tMCAO produced a significant dose-dependent reduction in body temperature as compared to vehicle treated tMCAO animals. We observed that low dose LPS (100 ug/ kg), which only modestly reduced core body temperature exacerbated stroke volume, while high doses of LPS (200 ug/ kg - 2 mg/ kg), which severely reduced core body temperature, reduced stroke volume. This severe hypothermic response to high-dose LPS may contribute to the observation of preconditioning following LPS administration. Collectively, these data indicate that bacterial mimics have a dose-dependent effect on body temperature and a differential effect on lesion volume.

Disclosures: S. Lewis: None. **D.N. Doll:** None. **X. Ren:** None. **H. Hu:** None. **J.W. Simpkins:** None.

Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

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Program#/Poster: 611.03/U33

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant NIH/NINDS R01NS080844

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grant NSC 102-2320-B-030-011 from National Science Council of Taiwan

Title: Interleukin-1 receptor antagonist attenuates neonatal LPS exposure-enhanced neurotoxicity of dopaminergic system by rotenone challenge in adult rats

Authors: *L.-W. FAN[1], L.-T. TIEN[2], J. SHEN[1], H. ZHU[1], A. J. BHATT[1], Y. PANG[1]; [1]Pediatrics/Newborn Med., Univ. Mississippi Med. Ctr., Jackson, MS; [2]Sch. of Med., Fu Jen Catholic Univ., New Taipei City, Taiwan

Abstracts: We have previously reported that neonatal lipopolysaccharide (LPS) exposure induced a chronic neuroinflammation, which was linked to an enhanced susceptibility of substantia nigra dopaminergic neurons to rotenone in adult rats. The objective of this study was to examine whether co-administration of interleukin-1 receptor antagonist (IL-1ra) with LPS attenuates LPS-induced motor behavioral dysfunction and LPS-enhanced susceptibility to rotenone toxicity in later life of these rats. Intracerebral injection of either LPS (1 mg/kg) alone, or LPS (1 mg/kg) with IL-1ra (0.1 mg/kg), or saline was administered in postnatal day 5 (P5) Sprague-Dawley male rat pups. At P70, rats were exposed to rotenone through subcutaneous mini-pump infusion at a dose of 1.25 mg/kg per day for 14 days. Motor behavioral tests were carried out from P70 to P98. Rats were sacrificed and LPS-induced injury to the dopaminergic system was assessed by losses of tyrosine hydroxylase immunoreactive neurons in the substantia nigra. Our results show that co-administration of IL-1ra significantly protected against rotenone-induced neurobehavioral impairments, including bradykinesia, akinesia, and rigidity in rats with neonatal LPS exposure. IL-1ra treatment also provided protection against LPS-enhanced rotenone-induced brain injury in adult rats, including loss of tyrosine hydroxylase positive neurons, decrease in mitochondrial complex I activity, and increase in the number of activated microglia in the substantia nigra. Our data demonstrate that treatment with IL-1ra has long-lasting neuroprotection against perinatal brain inflammation-enhanced adult susceptibility to environmental toxin, suggesting that IL-1ra is critically involved in this double-hit model of dopaminergic neuronal injury.

Disclosures: L. Fan: None. **L. Tien:** None. **J. Shen:** None. **H. Zhu:** None. **A.J. Bhatt:** None. **Y. Pang:** None.

Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.04/U34

Topic: C.08. Ischemia

Support: NIH Grant NS080098

Title: The acute inflammatory response following a cortical microhemorrhage is dominated by brain-resident microglia and not blood-borne macrophages

Authors: *S. AHN[1], J. C. CRUZ HERNANDEZ[1], J. ANRATHER[2], N. NISHIMURA[1], C. B. SCHAFFER[1];
[1]Biomed. Engin., Cornell Univ., Ithaca, NY; [2]Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstracts: The presence of brain microhemorrhages has been linked to accelerated cognitive decline and to increased risk of dementia in patients. Progress on understanding the mechanisms by which these small brain bleeds lead to dysfunction in nearby neurons and other brain cells, however, has been hampered by a lack of good animal models of these lesions. To address this issue, our lab used tightly-focused femtosecond laser pulses to rupture targeted brain arterioles and produced a reliable and reproducible model of microhemorrhage that enables the study of mechanisms of brain injury. In previous experiments with this model, we showed that microhemorrhages do not cause neuronal death nor large-scale degeneration of axons and dendrites near the lesion. We also observed that inflammatory cells are recruited within minutes of the microhemorrhage and that this inflammation persisted near the lesion for weeks. This long-term inflammation could play a role in the cognitive dysfunction linked to microhemorrhages. In these previous experiments, however, we were unable to distinguish between brain-resident microglia and blood-derived macrophages in this inflammatory process. Here, we used bone marrow transplantation to create chimeric animals where we can distinguish between hematogenous and brain-resident inflammatory cells. Lethally irradiated C57Bl/6 mice were retro-orbitally injected with cells isolated from the bone marrow of donor animals. Animals recovered at least 6 weeks before experiments. We used mice that express GFP under the control of the promoter for the CX3CR1 fractalkine receptor, which show labeling in microglia and circulating monocytes. These animals were used as donors into wildtype mice (only monocytes labeled) and, in separate experiments, as recipients of wildtype bone marrow (only microglia labeled). A glass covered cranial window was implanted and animals were allowed to recover for 10 days. We then used two-photon excited fluorescence imaging to visualize brain vasculature (intravenous injection of Texas Red-dextran) and inflammatory cells (GFP+). Femtosecond laser pulses of ~ 0.5-μJ energy were tightly focused on the wall of the descending segment of penetrating arterioles, causing a rupture of the vessel wall and the formation of a small hemorrhage. We repeated imaging of these mice immediately, a few hours, 1 day, 3 days, and 1 week after the lesion. We found that microglia are responsible for nearly all of the inflammatory cells that surround the hematoma up to one week after the lesion.

Disclosures: S. Ahn: None. **J.C. Cruz Hernandez:** None. **J. Anrather:** None. **N. Nishimura:** None. **C.B. Schaffer:** None.

Poster

611. Ischemia Inflammation: White Cells and Cytokines

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Program#/Poster: 611.05/U35

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS038079

NIH NS069537

Title: TNF-alpha inhibition modulates chronic inflammation following cervical spinal cord injury in rats

Authors: *J. R. HUIE[1], R. SAIGAL[1], A. LIN[1], J. SACRAMENTO[1], D. SZYMKOWSKI[2], A. FERGUSON[1], J. BRESNAHAN[1], M. BEATTIE[1];
[1]Brain and Spinal Injury Ctr., UCSF, San Francisco, CA; [2]Xencor, Inc, Monrovia, CA

Abstracts: Spinal cord injury (SCI) is a complex syndrome characterized by an initial mechanical disruption of tissue, followed by a secondary wave of cell death and inflammation. While a number of inflammatory processes are believed to peak and resolve acutely after injury, a growing body of evidence has indicated that inflammation is sustained in the chronic spinal cord, and may contribute to undermining recovery of function. Here we have used a unilateral cervical contusion model of spinal cord injury in the rat to detail the inflammatory profile of injury both acutely (3 hours post-injury) and chronically (100 days post-injury), and to test whether treatment with a dominant negative soluble TNF inhibitor (XPro1595, Xencor, Inc.) can mitigate the expression of inflammatory biomarkers at these timepoints. Inflammation was assessed using commercially-available gene microarrays (Qiagen/SABiosciences) that test the mRNA expression of 84 chemokines and cytokines associated with the inflammatory immune response. As expected, we found that SCI produced a marked upregulation of a broad range of inflammatory markers at the spinal lesion site at 3 hours post injury. This inflammatory response was reduced by a single intraperitoneal (i.p.) injection of XPro1595 (5 mg/kg), given 90 minutes after injury. Interestingly, at 100 days after injury, SCI subjects continued to exhibit robust expression of inflammatory markers when compared to age-matched sham controls. To test whether TNF inhibitor treatment in these animals could reduce chronic inflammation, rats were given i.p. injections of XPro1595 every three days for 10 days. We found that Xpro1595 treatment was able to modulate the inflammatory response compared to vehicle-treated SCI controls. XPro1595 reduced the expression of many inflammatory markers, with the strongest effect on interleukins, toll-like receptors, and C reactive protein, as well as chemokines that are

chemoattractant for neutrophils and monocytes. These findings suggest that in the injured cervical spinal cord, inflammation is a persistent phenomenon that can be observed during the chronic phase of injury. These findings also suggest that anti-inflammatory treatment with a TNF inhibitor may alleviate this inflammation, even if given at chronic timepoints.

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Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.06/U36

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Dept of Veterans Affairs BLR&D BX001686

Dept of Veterans Affairs RR&D

Title: Role of orexin A signaling in dietary saturated fatty acid activated microglial cells

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Abstracts: Excess dietary saturated fatty acids such as palmitic acid (PA) induce inflammation in peripheral and central nervous system tissues, including the hypothalamus. Hypothalamic inflammation, thought to contribute to metabolic dysregulation, is mediated in part by microglial activation. In rodents, high fat diet-induced obesity activates microglia, resulting in nuclear translocation of nuclear factor-κB (NFκB) and increased central and peripheral pro-inflammatory cytokine tumor necrosis factor alpha (TNFα). The hypothalamic neuropeptide orexin A (OXA, hypocretin) is neuroprotective through reducing lipid peroxidation and decreasing caspase-3/7 activity. In cortex, OXA acts through a microglial-mediated pathway to reduce inflammation and prevents neurodegeneration following ischemic injury. Whether endogenous orexin signaling is

altered in activated microglia in the hypothalamus is unknown. We hypothesize that PA induces changes in gene expression for the orexin receptors in microglial cultures. To test this, immortalized murine microglial cells (designated BV2) were treated with or without 0.1 mM PA for 4 h. Microglial cells were collected for gene expression analysis via qRT-PCR. Exposure to PA increased gene expression of orexin-1 receptor (OX1R; $p<0.05$ vs. control) but not orexin-2 receptor (OX2R; $p>.05$ vs. control). To our knowledge, this is the first report demonstrating that PA increases microglial OX1R and the presence of OX2R in a microglial cell line. These data support the idea that OX1R in microglia are responsive to PA. We are currently evaluating OXA's role in microglial activation following exposure to PA in the presence or absence of OXA by profiling inflammatory cytokines using both qRT-PCR and ELISA. The results from these experiments will provide novel insight into the mechanisms by which excess dietary fatty acids provoke inflammatory responses, and how endogenous hypothalamic peptides such as OXA may function as central immunomodulators.

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Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

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Program#/Poster: 611.07/V1

Topic: C.08. Ischemia

Support: NIH NS42617

AHA 12SDG11780023

Title: Excessive dietary α-tocopherol increases post-stroke microglial activation and contributes to brain injury

Authors: *C. L. RINK, S. GNYAWALI, M. HEIGEL, S. ROY, C. K. SEN, S. KHANNA; Surgery, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstracts: Clinical trials testing the α-tocopherol form of vitamin E (αTOC) against a range of diseases, from cancer to heart disease, have largely failed or reported negative outcomes when a "more is better" approach to supplementation was employed. A 2005 meta-analysis of clinical trials testing high-dose αTOC (≥400 IU/d) reported significantly increased risk for all-cause

mortality in 9 of 11 clinical trials reviewed (Miller et al, Ann Intern Med., 2005). In the context of stroke, the Alpha-Tocopherol, Beta Carotene (ATBC) trial reported significantly increased risk for hemorrhagic stroke among participants assigned to αTOC supplementation. While the antioxidant properties of αTOC at physiological levels are widely regarded to attenuate stroke-induced injury, pathological effects of excessive αTOC have yet to be addressed. In this light, the current work was focused to determine the effects of high-dose αTOC on ischemic stroke outcomes in a pre-clinical setting using the intraluminal thread model of middle cerebral artery occlusion. In this work we demonstrate that excessive αTOC supplementation exacerbates stroke-induced lesion volume via increased microglial activation. Elevated brain αTOC levels are associated with increased S100B-mediated microglial activation and iNOS activity in the stroke-affected brain. *In vivo* outcomes are validated in microglial cell culture systems. Taken together, this work identifies new mechanisms by which excessive αTOC supplementation contributes to pathological brain injury.

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Poster

611. Ischemia Inflammation: White Cells and Cytokines

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Topic: C.08. Ischemia

Support: AHA Grant 12SDG11780023

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CCTS Grant UL1RR025755

Title: Tocotrienol vitamin E induces arteriogenesis and protects against ischemic stroke brain injury

Authors: *S. TEPLITSKY, S. KHANNA, M. HEIGEL, K. OLICKAL, C. K. SEN, C. RINK; The Ohio State Univ., Columbus, OH

Abstracts: Cerebrovascular collaterals refer to an interconnected group of blood vessels that anastomose and are capable of retrogradely perfusing the stroke-affected brain. Importantly, cerebrovascular collaterals have been clinically documented to protect the brain from ischemic

stroke brain injury. While there is interest in identifying therapies to improve collateral blood flow, mechanisms and a means to do so remain poorly studied. The current work rests on a key *in vivo* observation that supplementation of lesser-characterized vitamin E family members, tocotrienols (TCT), successfully improves cerebrovascular collateral blood flow (Rink et al., JCBFM 2011). Here, we employ TCT as a tool to study mechanisms of vascular remodeling, termed arteriogenesis, for improved collateral blood flow during acute ischemic stroke. C57/BL6 mice (N=24, 5 wks old) were orally gavaged 5d/week with TCT (50 mg/kg) or a volume-matched vehicle control placebo (PBO). After 4 and 10 weeks of supplementation, mice were subjected to ischemic stroke using the intraluminal thread method of middle cerebral artery occlusion (MCAO). Successful MCAO was validated by laser Doppler flowmetry. While ischemia persisted (30min after onset of MCAO), intracardiac injection of a FITC-conjugated Lycopersicon esculentum lectin (FITC-lectin, 0.5 mg/ml) enabled selective staining of patent vessels in stroke-affected and contralateral control brain. On the basis of staining, TCT supplementation increased collateral perfusion in stroke-affected S1 cortex. FITC-tagged collaterals were then cut (mean = 1.8x10^5 μm^2) and collected using laser capture microdissection. From laser-captured samples, RNA isolation was performed followed by cDNA synthesis and real-time PCR. Compared to placebo controls, TCT supplementation significantly affected expression arteriogenic targets associated with vascular proteolysis (MMP2 and TIMP1), and shear stress (DLL4). No significant difference in arteriogenic targets related to tubulogenesis (CLIC1 and CLIC4) were observed. Taken together, outcomes identify novel TCT-sensitive mechanisms that enable arteriogenesis for protection against acute ischemic stroke.

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Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.09/V3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Impact of chronic neuroinflammation on microcircuit activity in mouse visual cortex *in vivo* using two photon calcium imaging

Authors: *G. K. PRAMANIK[1], E. ELLWARDT[2], E. ROSALES JUBAL[1], Z. BARGER[1,3], E. WITSCH[2], D. LUCHTMANN[2], F. ZIPP[2], A. STROH[1];
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Abstracts: Multiple Sclerosis (MS) is driven by the infiltration of CNS specific lymphocyte cells that are peripherally activated by antigen presenting cells and transmigrate into the CNS resulting in chronic inflammation and demyelination. Here, we explore the association of disease stage identified by the behavioral phenotype with cortical neuronal microcircuit activity in the mouse brain *in vivo*. We utilized *in vivo* two-photon Calcium imaging in the visual cortex of experimental autoimmune encephalomyelitis (EAE) animals, the well-established animal model of MS. Upon a cranial window preparation, the fluorescent Ca2+ indicator Oregon Green Bapta1 (OGB-1) and Sulforhodamine 101 (SR101), a specific astrocyte marker, were injected using the multi-cell bolus loading technique. By a dedicated 2-photon microscope equipped with a resonant scanner, we simultaneously recorded up to 100 neurons at 30-Hz temporal resolution in layer II/III of the primary visual cortex. Since spontaneous activity reflects the basic architecture of cortical circuitry, whereas stimulus driven activity may predominantly be affected by subcortical inputs, we analyzed both spontaneous and evoked activity. Comparing healthy controls with EAE animals in remission phase, in which the animals do not exhibit an obvious behavioral phenotype, we find a differential pattern of spiking activity in the visual cortex.

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Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.10/V4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH AG037320

HHMI Med-into-Grad

Title: Blockade of L-VDCCs or RyRs during chronic neuroinflammation improves spatial memory, normalizes synaptic function, and reduces expression of inflammatory markers

Authors: *S. C. HOPP[1], H. M. D'ANGELO[1,2], S. E. ROYER[1], R. M. KAERCHER[2], L. ADZOVIC[2], A. M. CROCKETT[1,2], G. L. WENK[2,1];
[1]Neurosci., [2]Psychology, Ohio State Univ., Columbus, OH

Abstracts: Chronic neuroinflammation and calcium dysregulation are shared components of Alzheimer's disease and other neurodegenerative processes. Prolonged neuroinflammation produces elevation of pro-inflammatory cytokines and reactive oxygen species which are capable of altering neuronal calcium homeostasis via L-type voltage dependent calcium channels (L-VDCCs) and ryanodine receptors (RyRs). Chronic neuroinflammation also leads to deficits in spatial memory, which may be related to calcium dysregulation. The studies herein use an *in vivo* model of chronic neuroinflammation: rats were treated with intraventricular infusion of lipopolysaccharide (LPS) for 28 days. Synaptosomes from LPS-infused rats had increased calcium uptake, which was decreased by pharmacological blockade of the L-VDCC either *in vivo* or *ex vivo*. LPS-infused rats had significant memory deficits in the Morris water maze; this deficit was ameliorated by treatment with an L-VDCC antagonist. Taken together, these data indicate that calcium dysregulation during chronic neuroinflammation is at least partially dependent on increases in L-VDCC function. However, blockade of the RyRs also slightly improved spatial memory of LPS-infused rats, demonstrating that other calcium channels are dysregulated during chronic neuroinflammation. Calcium-dependent immediate early gene expression was reduced to control levels in LPS-infused rats treated with L-VDCC or RyR antagonists, indicating normalized synaptic function that may underlie improvements in spatial memory. Pro-inflammatory markers are also reduced in LPS-infused rats treated with either drug. Overall, these data suggest that calcium dysregulation via L-VDCCs and RyRs plays a crucial role in neuroinflammation-induced memory deficits.

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Poster

611. Ischemia Inflammation: White Cells and Cytokines

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 611.11/V5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: The role of TNF in pain induction and secondary consequences in the hippocampus following peripheral nerve injury

Authors: *T. DEL RIVERO[1], A. DELLAROLE[3], J. BETHEA[2];
[1]Drexel Univ., Philadelphia, PA; [2]Drexel Univ., philadelphia, PA; [3]Univ. of Miami, Miami, FL

Abstracts: Tumor Necrosis Factor (TNF) is a proinflammatory cytokine which is involved in physiological and pathological processes, systematically and within the central nervous system (CNS). There are two biologically active forms of TNF, soluble TNF (solTNF) and transmembrane TNF (tmTNF) that preferentially bind to TNFR1 and TNFR2 respectively. Recent studies have focused on targeting TNF for neuropathic pain treatment due to the involvement of the immune response in pain propagation. Following peripheral nerve injury, an increase in TNF levels has been observed in the sciatic nerve, spinal cord, and different brain regions. Although anti-TNF drugs such as etanercept and infliximab are currently used to treat pain, they are not completely effective and have some deleterious side effects. Recent studies have shown that certain types of TNF signaling (TNF receptor 1 signaling) are directly associated with an increase in pain following injury while other distinct types of TNF signaling (TNF receptor 2 signaling) help to protect cells. The current anti-TNF drugs inhibit overall TNF activity, so although patients experience a slight alleviation of pain, they may also be deprived of beneficial effects of TNFR2 signaling. This is why in the current study we are interested in the effects of specifically blocking TNF receptor 1 (TNFR1) signaling on neuropathic pain following peripheral nerve injury in a mouse model with the use of both transgenic mice and drug therapy. After undergoing chronic constriction injury (CCI) in the sciatic nerve, knockout mice lacking TNFR1 (TNFR1 -/-) fail to develop a normal pain response in the injured paw compared to wildtype mice as measured by the Von Frey test. Furthermore, following CCI we observe a decrease in hippocampal neurogenesis which corresponds to the time course for pain sensation, but this decrease in neurogenesis is not observed in TNFR1-/- mice. To investigate the therapeutic effects of inhibiting TNFR1 signaling after injury, we delivered XPro1595, a novel drug which creates a biologically inactive form of solTNF, to mice following CCI. Inhibition of TNFR1 signaling via XPro1595 resulted in an accelerated recovery from neuropathic pain which began at 4 weeks following injury. At the 5 week time we see an increase in XPro1595 levels in the CNS as well as in the plasma, therefore the delay in recovery could be due to a requirement for sufficient levels of XPro1595 to accumulate in the CNS. Together, these results suggest that TNFR1 signaling in both the peripheral and central nervous system is necessary for pain induction following CCI, and that TNFR1 signaling additionally plays a role in hippocampal alterations due to the injury.

Disclosures: **T. Del Rivero:** None. **A. Dellarole:** None. **J. Bethea:** None.

Poster

611. Ischemia Inflammation: White Cells and Cytokines

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Program#/Poster: 611.12/V6

Topic: C.08. Ischemia

Support: NINR 1F32NR013611

Title: Sex differences in immediate microglia and astrocyte responses in models of brain injury

Authors: *H. MORRISON[1], J. FILOSA[2];
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Abstracts: Microglia cells possess the title of immune cell; however, the brain houses two inflammatory cell responders, astrocytes and microglia. Due to a parallel function, it is conceivable that these cells coordinate surveillance and inflammatory responses during health and disease via modalities of integrated microglia-astrocyte communication. Sex differences have been well described related to stroke prevalence and stroke outcomes, however, the combination of mechanisms that underlie these sex differences, to include microglia-astrocyte communication, are poorly understood. We tested the hypothesis that lipopolysaccharide (LPS) induced microglia activation would differentially stimulate astrocyte activation in adult male and female mice. Using calcium indicator Rhod-2AM and live imaging techniques, we show that 15-min LPS incubation of female acute brain slices significantly reduced intrinsic astrocyte activity by 36% (vs. aCSF, $p<0.05$) and the Ca2+peak frequency of these active cells was also reduced by 33% (vs. aCSF, $p < 0.05$). While male astrocyte intrinsic activity and peak frequency was unchanged vs. aCSF incubation, the Ca2+ peak amplitude of active cells was increased by 30% after 15-min LPS incubation ($p<0.05$ vs. aCSF). In an additional study, intrinsic Ca2+depended astrocyte activity was significantly increased by 20% in female ($p <0.05$, vs sham), but not male, contralateral and distal ipsilateral brain regions after 60-min focal ischemic stroke. Although the Ca2+ peak frequency of active cells in contralateral and ipsilateral brain regions were similarly unchanged (vs. sham) in both sexes after 60-min of focal ischemia, the coefficient of variability in female data was significantly increased vs. males ($p<0.01$). Together, these data illustrate immediate and diverse astrocyte Ca2+ dependent responses to LPS induced microglia activation. We suggest that LPS induced microglia activation initiates differing microglia-astrocyte signaling modalities between males and females. In addition, and independent of sex, we illustrate contrasting Ca2+ dependent astrocyte responses between LPS incubation and ischemic injury. These early sex differences will influence current and future events that define stroke outcomes.

Disclosures: H. Morrison: None. **J. Filosa:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.01/V7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS F31 NS064872-01

NIMH R01 MH085607

State of Florida, Executive Office of the Governor's Department of Economic Opportunity

Title: Exposure to HIV-1 Tat in brain impairs sensorimotor gating and activates microglia in medial prefrontal cortex of male mice

Authors: *J. J. PARIS[1], H. D. SINGH[2], A. N. CAREY[4], J. P. MCLAUGHLIN[3];
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Abstracts: Human immunodeficiency virus (HIV) infection is associated with a greater incidence of mood disorder and behavioral disinhibition. Recently, impairments in sensorimotor gating have also been described among HIV-infected individuals with associated neurocognitive disorders, but the identity of the HIV-proteins and the mechanisms involved are not known. The regulatory HIV-1 protein, Tat, is neurotoxic and its expression in animal models increases anxiety-like behavior concurrent with neuroinflammation and structural changes in limbic and extra-limbic brain regions. We hypothesized that conditional expression of Tat(1-86) in the GT-tg bigenic mouse model would impair sensorimotor gating and increase microglial reactivity in subregions of the medial prefrontal cortex. Conditional, astroglial-derived Tat induction via doxycycline (Dox) treatment (0-125 mg/kg, i.p., for 1-14 days) significantly potentiated the acoustic startle reflex of GT-tg mice and impaired prepulse inhibition of this response in a bimodal, dose-dependent manner. Effects were observed to peak at brief (Dox 100 mg/kg, for 1 day) or prolonged (Dox 100 mg/kg, for 7 days) conditions of Tat expression. A greater proportion of active/reactive Iba1-labeled microglia was also observed in the anterior cingulate cortex of GT-tg mice when Tat protein was induced under low expression conditions, and in the agranular insular, anterior cingulate, and prelimbic cortices when Tat was induced under high expression conditions. Pretreatment with indomethacin attenuated behavioral effects of brief (but

not prolonged) Tat-exposure. Differences were not observed on any measure among control mice lacking the Tat transgene. Overall, exposure to Tat protein induced sensorimotor deficits associated with HIV, perhaps involving acute neuroinflammatory responses in forebrain regions.

Disclosures: J.J. Paris: None. **H.D. Singh:** None. **A.N. Carey:** None. **J.P. McLaughlin:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

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Program#/Poster: 612.02/V8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIMH R01-MH087332

Title: IFNβ mediated neuroprotection in HIV-1 associated brain injury

Authors: *V. E. THANEY[1], M. M. HOEFER[1], M. KAUL[1,2];
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Abstracts: HIV-1 is known to invade the CNS early following peripheral infection, however, neurologic symptoms are delayed until later in the progression of the disease. This delay has been explained by the ability of the innate immune system to effectively suppress viral replication during the initial stages of CNS infection. Type I interferons (IFNs), with both pro-inflammatory and anti-inflammatory roles, are critical mediators of this response in the brain, and are a major first line of host defense against viral infections. We hypothesized, that the differences in IFNβ production and signaling in the CNS might provide an explanation for progression of the neurological symptoms. Based on preliminary evidence we investigated the neuroprotective effects of IFNβ in the brain against toxicity of HIV/gp120 using *in vivo* and *in vitro* models. We used mixed rodent cerebrocortical cultures (RCC), containing neurons, astrocytes, and microglia, to show that concentration dependent treatment with IFNβ can provide sufficient neuroprotection against gp120-induced toxicity. Additionally, treatment with IFNβ of RCC increased levels of natural ligands of the HIV co-receptor CCR5 and up-regulated expression of anti-viral IFN-stimulated genes. In contrast, treatment with HIV viral envelope protein gp120 alone at neurotoxic concentration did not cause any comparable changes in gene expression. These ligands, MIP-1β and RANTES, are known to suppress HIV-1 infection;

disease progression and all can provide significant *in vitro* protection against gp120-induced injury. We further found that introduction of neutralizing antibodies against MIP-1β, but not RANTES completely abrogated the neuroprotective effect of IFNβ. These results suggest that MIP-1β is a critical part of IFNβ mediated neuroprotective mechanism. To investigate the neuroprotective effects of IFNβ against HIV/gp120-induced neurotoxicity *in vivo*, we performed intranasal IFNβ administration to transgenic mice expressing the viral envelope protein in the brain. HIV/gp120-tg animals manifest several neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density, increased numbers of activated microglia, and pronounced astrocytosis. After one month of once a week treatment, brains were analyzed by quantitative RT-PCR for specific changes in gene expression and by immunohistology for structural neuronal injury. We found that intranasal IFNβ application triggered a biological response that was detectable as IFN-induced gene expression and ameliorated neuronal damage in HIV/gp120-tg mice.

Disclosures: V.E. Thaney: None. **M.M. Hoefer:** None. **M. Kaul:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.03/V9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Strain of LCM virus influences tropism and pathology in the developing brain

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Abstracts: Lymphocytic Choriomeningitis Virus (LCMV) is a common congenital infection that damages the fetal human brain. However, the nature and severity of the injuries differ from case to case. This variability may be due, in part, to differences among LCMV strains. Here, we compare and contrast the patterns of infection and pathological effects of three previously unexplored LCMV strains. Lewis rat pups were injected on PD4 with 1,000 infectious viral particles of one of three LCMV strains (E350, Clone 13, or WE2.2) and monitored over the next two weeks. Brains were harvested at multiple time points for immunological, histological, and molecular studies. The neurotropic strain, E350, behaves similarly to the Armstrong strain, with early widespread infection of astrocytes, followed by restricted infection of mitotically active neuroblasts in the cerebellum, olfactory bulb, hippocampus, and ventricular zone. These animals

live to adulthood, but suffer permanent cerebellar destruction. The Clone 13 strain, which was previously believed to infect only lymphatic tissue, infects many cells of the cerebellum, but has no other neuron or astrocytic targets. These animals do not exhibit overt neurological disease, but die ~10 days after infection. In contrast, the WE2.2 strain does not infect the cerebellum, but shows widespread infection of cortical and hippocampal neurons. The WE2.2 strain produces fatal disease without obvious neurological symptoms. Thus, different strains of LCMV have markedly different cellular targets of infection and pathologic effects within the developing brain.

Disclosures: **J.M. Plume:** None. **B. Karacay:** None. **J. Mahoney:** None. **D. Bonthius:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

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Program#/Poster: 612.04/V10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 NS63605

Title: Altered microglial gene expression suggests impaired microglial function in HIV infection, even in the absence of detectable virus in brain

Authors: *T. FISCHER-SMITH[1], S. D. GINSBERG[2], M. J. ALLDRED[2], S. GUNNAM[1]; [1]Neurosci., Temple Univ. Sch. of Med., Philadelphia, PA; [2]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY

Abstracts: HIV-associated neurocognitive disorders (HAND) are a common complication of HIV. The neuropathogenesis of HAND is poorly understood, however, HIV-infected and non-infected activated macrophages (M)s and microglia are significant to HIV-related neuropathogenesis. Previously, we reported considerable accumulation of $CD163^+$/$CD16^+$ Ms and microglia in brains of patients with HIV encephalitis (HIVE), the neuropathological correlate of the most severe form of HAND, HIV-associated dementia (HIV-D). More recently, we have found HIV^+ subjects without encephalitis (HIV/noE) also show increased $CD163^+$ and $CD16^+$ brain Ms and microglia, even in the absence of detectable virus production in the brain. Accordingly, we hypothesize that microglial activation is a common mechanism between lesser and more severe HIV-associated neurodegenerative processes, regardless of virus production in the brain. To begin to explore this hypothesis, we investigated gene expression changes of specific classes of transcripts in parenchymal Ms and microglia from archival brain tissue of patients with HIVE, HIV/noE, and age-matched seronegative (HIV^-) controls. Microarray analyses were performed on ~2,500 laser capture microdissected $CD163^+$, $CD16^+$ or $CD68^+$ Ms/microglia per case, using terminal continuation (TC) RNA amplification and a custom-designed array platform. Preliminary data demonstrates massive changes in gene expression between HIV^- and the two HIV studied conditions, HIVE and HIV/noE. Consistent with our hypothesis, altered expression of several classes of transcripts, including cell death genes, immediate-early genes, and inflammatory-based markers, suggestive of progressive M/microglial activation with disease severity, is seen in Ms/microglia recovered from HIVE brain, relative to HIV/noE and HIV^- subjects. We also see evidence for transcripts that are typically associated

with neurons and neurotransmission enriched in microglia, suggesting an important support role of these cells for proper neuronal activity in human brain. These preliminary results indicate the utility of profiling Ms/microglia in the brain in HIV infection to ascertain alterations in specific genes, signaling pathways, and presumably, encoded proteins that may be amenable to targeted treatment modalities. In addition, these studies provide additional insight into the diverse roles microglia play in "normal" brain homeostasis, as well as alterations in microglial function that likely contribute to neuronal injury and cognitive impairment.

Disclosures: T. Fischer-Smith: None. **S. Gunnam:** None. **S.D. Ginsberg:** None. **M.J. Alldred:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1F32NS083426-01

NIH Grant AG043384

NIH Grant MH062962

Title: Alterations in mitochondria biogenesis during HIVE: Implications for mechanisms of neurodegeneration

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Abstracts: Approximately 50% of HIV+ persons are afflicted by some variant of HIV-associated neurocognitive disorders (HAND), and prevalence is increasing as patients live longer with disease. HIV enters the brain causing infection of resident CD4+ cells, viral replication, viral protein/toxin production and neuroinflammation. Some, or all, of these mechanisms culminate in neurodegeneration and HAND. We hypothesized that HIV proteins released from infected CNS cells enter bystander neurons and affect mitophagy and mitochondria biogenesis. To test this we assayed brain tissues from a well-characterized cohort of HIV- and HIV+ donors for expression of key mitophagy and mitochondria biogenesis proteins and complimented these studies with *in vitro* assays using HIV proteins, lentiviral vectors and neuroblastoma cells. The

mitochondrial fusion protein mitofusin (MFN) 1 was significantly increased in brains of HIVE donors, while dynamin-related protein 1 (DRP1) levels were decreased. Immunostaining showed altered DRP1 association with mitochondria and enlarged and abnormal mitochondria in neurons of HIV versus HIVE brains. These findings were recapitulated in the brains of transgenic mouse expressing gp120. HIV gp120 caused increased MFN 1 expression accompanied by changes in mitochondria morphology; overexpressing DRP1 reversed this effect. These data suggest HIV gp120 may interact with CNS neurons and subsequently disrupt mitochondria biogenesis leading to neurodegeneration and HAND. Interfering with this neurodegenerative mechanism may provide relief for HAND patients.

Disclosures: **J.A. Fields:** None. **E. Masliah:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.06/V12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 NS050621

R25 MH81482-6

Title: Cysteinyl leukotrienes are essential to neurotoxicity of HIV-infected macrophages

Authors: ***A. B. SANCHEZ**[1], C. M. DE ROZIERES[1], K. E. MEDDERS[1], R. MAUNG[1], M. KAUL[1,2];
[1]Sanford-Burnham Med. Res. Inst., La Jolla, CA; [2]Univ. of California San Diego, San Diego, CA

Abstracts: Macrophages are the principal target of HIV-1 in the central nervous system (CNS). Activation of macrophages and microglia is critical for neuronal injury which is thought underlie the initiation and progression of neurodegenerative diseases such as HIV-1 associated neurocognitive disorders (HAND). Simultaneously, as a part of the innate and adaptive immune response, macrophages produce proinflammatory cytokines, chemokines and soluble proinflammatory lipid mediators, such us cysteinyl leukotrienes (cysLTs). Several studies have reported that leukotrienes, and their receptors, are expressed in the brain contributing to neurological disorders. A better understanding of the role of these proinflammatory lipid mediators in the neuronal toxicity will lead us to design new targets for protection of the CNS

from neurodegenerative diseases like HAND. In this study, we demonstrated that HIV-1 infection increased production of proinflammatory cysLTs by monocyte-derived macrophages (MDM). Next, neurotoxicity of HIV-infected MDM was assessed in the presence and absence of an inhibitor of cysteinyl leukotriene receptor-1 (cysLTR-1). Neuronal injury was evaluated after 24 hours treatment in fixed cells using specific markers for neuronal dendrites (MAP-2) and pre-synaptic terminal (synaptophysin) in combination with nuclear DNA staining and fluorescence microscopy. When rat cerebrocortical cells (RCC) were exposed to supernatant from HIV-infected MDM, but not uninfected cells, neuronal survival was significantly reduced. In contrast, the presence of the cysLTR-1 inhibitor completely prevented the neurotoxicity of supernatants from HIV-infected MDM. Altogether, our findings indicated that cysLTs originated in HIV-infected MDM and played a critical role in HIV-associated neuronal injury, since Montelukast, an inhibitor of cysLTR-1, protected cerebrocortical neurons against the toxicity of HIV-infected MDM

Disclosures: **A.B. Sanchez:** None. **C.M. de Rozieres:** None. **K.E. Medders:** None. **R. Maung:** None. **M. Kaul:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.07/V13

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Peter Deane Trust

Title: TIA-1 orchestrates antiviral granule formation in vesicular stomatitis virus-induced encephalitis *in vivo* and *in vitro*

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Abstracts: Stress granules (SGs) are known for sequestering mRNA or reprogramming translation during stress, such as oxidative stress and heat shock. Viral infection is also known to induce a number of cellular stress responses and modulate stress granule (SGs) formation for maximizing replication efficacies, but mechanism of viral infection-induced SGs formation in the brain is still elusive. In the present study, we examine whether neurons could form SGs in

response to vesicular stomatitis virus (VSV) infection and its underlying mechanism. Immunohistochemical and immunoprecipitation results showed that VSV interacted with TIA-1 or G3BP and formed non-canonical SGs formation in E(t)C cerebellar granule neuronal culture. In VSV-infected olfactory bulb of *Cd11c/eyfp* transgenic mice, VSV also interacted with TIA-1 and co-localized with neuronal TIA-1 expression in glomeruli and granule cell layer. These TIA-1 positive granules were located in perinuclear region and sustained to 14 day of post-infection (d.p.i). In addition, TIA-1 positive granules were colocalized with cytoplasmic sensors for RNA viruses, such as retinoic acid inducible gene-1 (RIG-1) and melanoma differentiation-associated protein-5 (MDA-5), in the olfactory bulb of VSV-infected mice. These results suggest that TIA-1 positive granules might be an important place for antiviral immune response.

Disclosures: Y. Chung: None. **K. Bulloch:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

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Program#/Poster: 612.08/V14

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01MH097476

Title: Impact of SIV inoculation on cognitive performance in aged female rhesus monkeys

Authors: K. GURNSEY[1], N. NANIA[2], H. P. JEDEMA[1], *S. J. BISSEL[2], C. A. WILEY[2], C. W. BRADBERRY[1,3];
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Abstracts: As part of ongoing studies into the effect of chronic SIV infection and antiretroviral therapy on cognition in the elderly, a cohort of 23 aged female rhesus macaques (16-23 years old) were trained and underwent neurocognitive assessments using a touch screen based stimulus response task designed to measure response latency and accuracy. Two performance-matched groups were established. One group was inoculated intravenously with SIVmac251 while the other group received saline. (Each group will be split later in the study to receive antiretroviral therapy or placebo). There were no measurable physiological consequences of acute inoculation; no differences in body temperature, animal activity or temperament. When performance immediately prior to infection (mean of 5-9 sessions) was compared to that following infection

(mean of 4-7 sessions after a one week hiatus) using a two way repeated measures ANOVA with group and time, there was no significant difference in response latency, but there was a significant difference in accuracy $F(1,21) = 9.2$, $p = 0.006$. Within each group, accuracy was not significantly different over time in control animals ($n = 11$, $p = 0.111$), but accuracy diminished over time in the inoculated group ($n = 12$, $p=0.015$). Thus the impact of SIV inoculation on cognition would not appear due to clinically measurable effects known to regulate body temperature and activity. The mechanism whereby cognitive performance was altered remains unclear. Whether these cognitive changes will persist or be impacted by antiretroviral therapy remains to be determined.

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Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH T32 AI060523

NIH R01 CA021776

Title: Early host-pathogen interactions influence outcomes of herpes simplex encephalitis in the developing brain

Authors: *D. R. WILCOX[1], N. R. WADHWANI[3], D. E. ALEXANDER[4], D. A. LEIB[4], B. HE[5], R. M. LONGNECKER[2], W. J. MULLER[1];
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Abstracts: Background: Infection with herpes simplex virus (HSV) in adults is often subclinical, but is a significant cause of mortality and morbidity in the newborn. Although differences in the immune response have been implicated in the increased severity of disease in the neonate, the precise reasons remain unknown. The type I interferon response is important for controlling viral

replication in an adult mouse model of HSV encephalitis. This response is countered by the multifunctional HSV protein γ34.5, which acts to reverse host translational arrest, inhibit TBK1 function, and inhibit autophagy. The interactions between this viral protein and host responses to CNS infection, and their dependence on developmental age of the animal, have not been studied. Methods: We used mutant viruses and their corresponding rescue viruses in a model of HSV encephalitis to study host responses to CNS infection and their relationship to the different functions of HSV γ34.5 in newborn and adult mice. Mice were inoculated intracranially and followed over time for development of symptoms and mortality. Viral replication in the CNS was assessed by plaque assay. Results: Adult mice lacking an intact type I interferon response are significantly more susceptible to HSV CNS disease compared to wild-type (WT) mice, with a higher overall mortality, a shorter time to mortality, and increased viral replication. In contrast, newborn mice lacking a type I interferon response had no difference in mortality or viral replication compared to WT newborn mice. Based on these results, we hypothesized that HSV-1 γ34.5 would be dispensable for pathogenesis in the neonate. However, γ34.5 was required for disease in both adult and newborn mice, suggesting that modulation of the host by γ34.5 is a critical mechanism of pathogenesis. We dissected the specific functions of γ34.5 to determine the pathways in the developing brain important for viral disease. We have shown that the autophagy-inhibiting function of γ34.5, which is critical to pathogenesis in the adult, is dispensable in the newborn. In contrast, here we show that mutations in γ34.5 that disrupt the ability of HSV to counteract host translational shutoff, and mutations abrogating interaction with the host signaling protein TBK1, individually attenuate virulence in WT newborns. Conclusion: Host-translational shutoff and TBK1 activity are important innate responses to HSV infection in the newborn brain. Identification of factors important for HSV pathogenesis in the CNS provides insight into the unique innate immune responses of the developing brain, and offers a mechanism into the increase susceptibility of this population to viral encephalitis.

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Poster

612. Neuroinflammation: HIV and Infections

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Program#/Poster: 612.10/V16

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Foundation for Morristown Medical Center

Overlook Foundation

Title: Inflammatory profile in encephalopathy patients with a history of Lyme disease

Authors: *E. A. ECKMAN[1,2], J. PACHECO-QUINTO[1,2], J. J. HALPERIN[2,3];
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[3]Neurosciences, Overlook Med. Ctr., Summit, NJ

Abstracts: As in other infections, some patients with Lyme disease have persistent nonspecific symptoms following appropriate treatment. The etiology remains to be identified. Emphasis on cognitive aspects of these symptoms has resulted in patients' fears that this reflects CNS infection. Despite absence of any evidence supporting this, such individuals often receive additional and inappropriate antibiotics. Controlled trials indicate such treatment is ineffective and has adverse effects. It is possible that a subset of patients recovering from Lyme disease may have an abnormal inflammatory response that could, in the absence of ongoing infection, cause symptoms ranging from fatigue to altered cognition. Improved diagnosis and differentiation between symptoms resulting from active nervous system Lyme infection, systemic infection, or an abnormal immune response could clarify pathogenesis and guide appropriate treatment. Published studies have attempted to correlate the initial type and extent of the inflammatory response to Lyme disease with the development of post-treatment symptoms. However, interpretation of the data is often complicated by the absence of an appropriate control population. The goal of our study was to characterize the inflammatory profile in CSF and serum from patients with encephalopathy or headache occurring after antibiotic treatment for Lyme disease. The levels of 18 cytokines and chemokines, including markers of Th1, Th2, and Th17-type inflammatory responses, were measured using multiplex assays. Results were compared to those from patients with similar symptoms unrelated to Lyme disease, to healthy controls, and to patients with active CNS infection. Consistent with published studies, we observed significant elevations in CSF inflammatory markers including CXCL13 in patients with nervous system infection. These elevations correlated with CSF pleocytosis and were not specific to Lyme disease. CSF inflammatory markers were not elevated in the majority of patients with post-treatment encephalopathy or headache, consistent with other evidence that such symptoms do not result from CNS infection. A subset of both Lyme seropositive and seronegative encephalopathy patients did show elevations in serum IL-23 and related cytokines indicative of a Th17-type inflammatory response. However, the relationship between this response and the type and duration of symptoms is unclear. Further studies with sera from a larger number of asymptomatic patients are required to investigate the possible significance of this finding.

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Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.11/V17

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: CCL5 activates a orphan G-protein coupled receptor 75 in human neuroblastoma SH-SY5Y cell line

Authors: *S. DEDONI[1,2], V. AVDOSHINA[2], I. MOCCHETTI[2];
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Abstracts: The chemokine CCL5 inhibits entry of M-tropic HIV strains into macrophages/microglia by affecting the binding of the envelop protein gp120 to the co-receptor CCR5. Interestingly, CCL5 also prevents neuronal cell death mediated by the T-tropic gp120 and the viral protein Tat, which have no affinity for CCR5. Thus, CCL5 could be used to reduce HIV-associated neurocognitive disorder (HAND). Nevertheless, the mechanism of action of CCL5 remains to be fully characterized. Recent studies have shown that CCL5 activates a G-protein coupled receptor 75 (GPR75) which encodes for a 540 amino-acid orphan receptor of the Gq α family. In the present study, we examined the interaction of CCL5 and GPR75 in neuroblastoma SH-SY5Y cells that do not express other receptors for CCL5, such as CCR5, CCR3, and CCR1. CCL5 then promoted GPR75 internalization within few minutes. In addition, CCL5 elicited a significant dose-dependent increase in pro-survival pathways, such as the phosphatidylinositol 3-kinase (PI3K) and the extracellular signal-regulated kinases (ERK1/2). Akt and ERK1/2 phosphorylation were blocked by the specific pathway inhibitors, Wortmannin and U73 122, respectively, but not by pertuxin toxin, suggesting that CCL5 activate a Gq-coupled receptor. In conclusion, we hypothesize that CCL5-GPR75 signaling could further activate a neuroprotective mechanism that could explain the multiple pro-survival roles of CCL5 in reducing gp120 and Tat cell death.

Disclosures: S. Dedoni: None. **V. Avdoshina:** None. **I. Mocchetti:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Program#/Poster: 612.12/V18

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIMH 1R01MH098737-02

Title: Efavirenz promotes β-secretase expression and increased Aβ1-40,42 via oxidative stress and reduced microglial phagocytosis: Implications for HIVassociated neurocognitive disorders (HAND)

Authors: *D. FERRELL[1], L. BROWN[2], J. JIN[2], B. GIUNTA[2];
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Abstracts: Efavirenz (EFV) is among the most commonly used antiretroviral drugs globally, causes neurological symptoms that interfere with adherence and reduce tolerability, and may have central nervous system (CNS) effects that contribute in part to HIV associated neurocognitive disorders (HAND) in patients on combination antiretroviral therapy (cART). Thus we evaluated a commonly used EFV containing regimen: EFV/zidovudine (AZT)/lamivudine (3TC) in murine N2a cells transfected with the human "Swedish" mutant form of amyloid precursor protein (SweAPP N2a cells) to assess for promotion of amyloid-beta (Aβ) production. Treatment with EFV or the EFV containing regimen generated significantly increased soluble amyloid beta (Aβ), and promoted increased β-secretase-1 (BACE-1) expression while 3TC, AZT, or, vehicle control did not significantly alter these endpoints. Further, EFV or the EFV containing regimen promoted significantly more mitochondrial stress in SweAPP N2a cells as compared to 3TC, AZT, or vehicle control. We next tested the EFV containing regimen in Aβ - producing Tg2576 mice combined or singly using clinically relevant doses. EFV or the EFV containing regimen promoted significantly more BACE-1 expression and soluble Aβ generation while 3TC, AZT, or vehicle control did not. Finally, microglial Aβ phagocytosis was significantly reduced by EFV or the EFV containing regimen but not by AZT, 3TC, or vehicle control alone. These data suggest the majority of Aβ promoting effects of this cART regimen are dependent upon EFV as it promotes both increased production, and decreased clearance of Aβ peptide.

Disclosures: **D. Ferrell:** None. **L. Brown:** None. **J. Jin:** None. **B. Giunta:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.13/V19

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS074916

NIH NS079172

NIH MH096625

Title: Helix-A peptide blocks HIV gp120 neurotoxicity by restoring microtubular transport in neurons

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Abstracts: The HIV envelop protein gp120 can be secreted by infected cells and internalized by neurons. This phenomenon promotes reduction of dendritic and synaptic connections both *in vitro* as well as in HIV-positive individuals who develop cognitive and motor abnormalities. One of the mechanisms that could explain gp120-mediated synaptic damage is the blockade of the intracellular transport of mitochondria. Such transport relies on microtubule (MT) integrity, a critical step of synaptic stability. Neuronal MTs contain tubulin-β3 (TUBB3), a specific isoform of β-tubulin expressed only in neurons. Using *in vitro* studies we have found that various strains of gp120 bind to recombinant TUBB3, tubulin dimers, and assembled MTs. However, gp120 did not bind to any other isoforms of tubulin. Gp120 binding occurs by a twenty amino acid domain of the α-helix region (Helix-A). The aim of the present study was to evaluate the neuronal protective effect of Helix-A peptide from gp120 toxicity. To address this hypothesis we examined the displacement of gp120 from TUBB3, tubulin dimers, and assembled MTs by synthetic Helix-A peptide. Because this peptide does not penetrate cell membrane we have synthetized Helix-A peptide linked to porous silica nanoparticles (nHelix-A peptide) for intracellular delivery. In primary rat cortical neurons nHelix-A peptide prevented gp120-mediated decrease in mitochondrial function (MTT) as well as alteration in mitochondrial morphology. In addition, nHelix-A peptide prevented gp120-mediated neuronal loss (Hoechst/PI). Since Helix-A peptide did not bind to gp120 the proposed mechanism of neuroprotection of Helix-A involves its competitive binding to TUBB3 and MTs. Indeed, nHelix-A peptide restored mitochondrial transport that was impaired in neurons exposed to gp120. Thus, neuroprotection conferred by Helix-A peptide strongly suggests that the direct interaction of gp120 with tubulin is one of the main central mechanisms by which gp120 promotes axonal degeneration. The interaction of gp120 with MTs could be a new target for the

development of alternative therapeutic strategies to reduce synaptic simplification observed in HIV positive subjects.

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Poster

612. Neuroinflammation: HIV and Infections

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 NS038932

R01 NS087539

NIH 8T32OD011089-36

NIMH P30 MH075673-06

1R03 DA032470

Title: Delayed virus clearance and immune cell infiltration in a mouse model of alphavirus encephalomyelitis treated with a glutamine antagonist

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Abstracts: Encephalomyelitis induced by arthropod-borne alphaviruses is an increasingly important cause of significant disease and disability in people, and no treatments beyond supportive care or licensed vaccines are currently available. Infection of mice with the TE strain of Sindbis virus (SINV) produces a model of nonfatal alphavirus encephalomyelitis with which to study the immune response to infection and the mechanisms of virus clearance. While infectious virus is cleared within a week of infection, viral RNA persists in the brain beyond recovery from clinical disease. Neuronal damage during SINV infection is due primarily to inflammation and glutamate toxicity rather than the virus itself. 6-diazo-5-oxo-l-norleucine

(DON), a glutamine antagonist, inhibits both of these mechanisms, providing a tool with which to examine the pathogenesis of disease. In this study, our objective was to examine the effect of DON administration on CNS pathology and SINV clearance. Five-week-old male C57BL/6 mice were infected intranasally with 10^5 pfu SINV or mock-infected with PBS and then treated for seven days intraperitoneally with either a high (0.6 mg/kg) or low (0.3 mg/kg) dose of DON or PBS vehicle. Tissues were collected both during treatment at 5 and 7 days post infection (DPI) and following treatment at 9 and 11 DPI. Brains were examined for infectious virus titers by plaque assay and viral RNA by RT-qPCR, and immune cell infiltration was evaluated by flow cytometry and histopathology. Infectious virus and viral RNA levels were increased in DON-treated mice at 7, 9, and 11 DPI in a dose-dependent manner compared to untreated SINV-infected mice, indicating impaired virus clearance. Inflammation and cell death in the brains of DON-treated, SINV-infected mice were reduced compared to untreated mice. Mononuclear immune cell infiltration was markedly decreased in the brains of treated mice compared to those of untreated mice throughout the course of DON administration. T lymphocyte numbers increased and virus clearance was initiated in DON-treated, SINV-infected mice following cessation of treatment. These findings demonstrate the dual-role the immune response plays in alphavirus encephalomyelitis: while inflammation contributes to the neuronal damage and CNS pathology seen during infection, the immune response is also required for effective virus clearance.

Disclosures: V. Baxter: None. **M.C. Potter:** None. **B.S. Slusher:** None. **D.E. Griffin:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R21NS066130

NIH Grant 1P20GM103643

Title: Glial M1/M2 balance in morphine-potentiated LP-BM5 murine AIDS

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Abstracts: Of the 1.1 million people infected with human immunodeficiency virus (HIV-1) in the United States, nearly 20% will develop cognitive deficits ranging from mild memory impairment to dementia. Over 30% of HIV-1 patients abuse opiates such as heroin, which increases the risk and severity of HIV-associated neurocognitive disorders. Morphine mediates this effect through its influence on glia, the initiators of innate immune defense against viral infection in the central nervous system (CNS). Through the LP-BM5 murine acquired immunodeficiency syndrome (MAIDS) model, we have shown that morphine suppresses proinflammatory (M1) cytokine RNA expression, correlating to a significant increase in viral RNA in the hippocampus. We hypothesize that this increase in viral RNA is the result of the synergistic effects of morphine and LP-BM5 viral infection on the M1/M2 balance of the CNS. To investigate this, we infected male C57BL/6 mice with LP-BM5 (5e4 plaque-forming units, intraperitoneal injection). At 7 weeks post-infection, animals received 1 week of subcutaneous morphine (25 mg) or placebo pellet implantation. We measured expression of anti-inflammatory (M2) markers and type 1 interferons in key regions of interest - hippocampus, striatum, and frontal cortex - through quantitative real-time PCR and immunohistochemistry. We observed region-specific decreases in expression of M1 (c-c motif ligand 5, CCL5) markers and type 1 interferons (interferon-β). Morphine treatment increased the proportion of M2 to complementary M1 marker expression (arginase-1/inducible nitrous oxide synthase; interleukin (IL)-10/IL-12 p40; IL-1 receptor antagonist/IL-1β), suggesting a trend towards M2 activation, which could favor faster viral replication. Through the LP-BM5/MAIDS model, we aim to provide a fresh perspective on the role of morphine in HIV-1 infection in the CNS and identify new pathways for the prevention of opiate-accelerated neurocognitive disorders.

Disclosures: **V.D. McLane:** None. **L. Cao:** None. **C.L. Willis:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Program#/Poster: 612.16/V22

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R01NS077873

Title: HIV-1 Tat protein induces microglial neurotoxic activity via potassium channel Kv1.3

Authors: J. LIU[1], H. LIU[1], J. ZHANG[1], *H. (. XIONG[2];
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Abstracts: Microglia playa an important role in the pathogenesis of HIV-1-associated neurocognitive disorders (HAND). Increasing evidence indicates the voltage-gated potassium (K_V) channels are involved in the regulation of microglia function. We hypothesize that microglia K_V channels are involved in microglia-mediated neurotoxic activity in HIV-1-infected brain. To test this hypothesis, we examined the involvement of Kv channels in microglia response to HIV-1 Tat protein. Treatment of rat microglia with HIV-1 Tat protein (200ng/ml) resulted in microglia activation, reflected by an increased production of TNF-α, IL-1β, reactive oxygen species, and nitric oxide, which were associated with enhanced outward K^+ current and K_V1.3 channel expression. Suppression of microglial K_V1.3 channel activity, either with Kv1.3 channel blockers Margatoxin, 5-(4-Phenoxybutoxy)psoralen, or broad-spectrum K^+ channel blocker 4-aminopyridine, or by knockdown of K_V1.3 expression via transfection of microglia with K_V1.3 siRNA, was found to abrogate microglia neurotoxic activity induced by HIV-1 Tat exposure. Further studies revealed an involvement of Erk1/2 mitogen-activated protein kinase signaling in HIV-1 Tat - microglia K_V1.3 - microglia neurotoxicity. These results indicate that microglial K_V1.3 may be a potential therapeutic target for HAND and other inflammation-associated neurological disorders.

Disclosures: **J. Liu:** None. **H. Liu:** None. **J. Zhang:** None. **H.(. Xiong:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Program#/Poster: 612.17/V23

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01NS079166

NIH Grant R01DA036165

Title: Mechanism of astrocyte activation during the pathogenesis of HIV-associated pain

Authors: *Y.-M. ZHANG[1,2], Y. SHI[2], B. LI[2], W. RU[2], S.-J. TANG[2];
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Abstracts: Chronic pain is one of the most common neurological disorders, affecting over 60% of HIV-1-infected patients, but the underlying pathogenic mechanism is unclear. Our recent

work reveals astrocyte activation in the spinal cord dorsal horn (SDH) specifically from the HIV patients with chronic pain but not from the patients without pain. This finding indicates a critical role of reactive astrocytes in the pathogenesis of HIV-associated pain. How astrocytes are activated under this condition is not known. We are interested in testing the role of Wnt signaling in the astrocyte activation using a gp120 mouse model. Our preliminary data indicate that Wnt5a, which is predominantly expressed in SDH neurons and secretes in response to neuronal activation, up-regulates GFAP in the SDH; conversely, Wnt5a antagonist blocks gp120-induced GFAP up-regulation. Since Wnt5a receptor ROR2 is expressed in astrocytes, we reason that the Wnt5a/ROR2 signaling pathway plays an important role in gp120-induced astrocytes activation. We will use conditional knockout approaches to determine the contribution of neuronal Wnt5a and astrocytic ROR2 to the gp120-induced astrocyte activation. Results from our studies are expected to improve the mechanistic understanding of HIV-associated pain pathogenesis.

Disclosures: **Y. Zhang:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **Y. Shi:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **B. Li:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **W. Ru:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **S. Tang:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 612.18/V24

Topic: B.11. Glial Mechanisms

Title: Effect of 17-β estradiol and progesterone on Toxoplasma gondii infection in astrocytes *in vitro*

Authors: *A. F. GUTIÉRREZ MALDONADO, J. DUEÑAS JIMENEZ, L. RODRÍGUEZ–PÉREZ, M. GALVÁN-RAMÍREZ;
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Abstracts: INTRODUCTION. Toxoplasmosis is a disease caused by an obligate intracellular parasite called Toxoplasma gondii. In humans, proliferating tachyzoites have been detected in glial cells in patients developing toxoplasmic encephalitis. The progesterone and 17-β estradiol have different effects on infection, may exacerbate or reduce parasite replication, however the participation of these hormones in astrocytes infected is no known. OBJECTIVE. Know the

effect of 17-β estradiol and progesterone on Toxoplasma gondii infection in astrocytes *in vitro*. MATERIALS AND METHODS. Astrocytes were obtained from rat cortex and were pre-treated with 17-β estadiol and progesterone at concentrations (10, 20, 40 and 80 nM/mL) for 48 hours, then were infected 24 hours with 14,375 Toxoplasma gondii tachyzoites. The effect of hormones on Toxoplasma gondii infection in astrocytes was evaluated by immunocytochemistry using anti-Toxoplasma antibody to identify the parasite and anti-GFAP to identify astrocytes. Cellular viability was measured with MTT. RESULTS. The 17-β estradiol increased the number of intra and extra cellular parasites at concentrations of 20 and 80 nM/mL versus control. Progesterone decreased the number of intra and extra cellular parasites at all concentrations compared to the control. Finally, the parasite viability was reduced to concentrations of 20, 40 and 80 nM/mL of 17-ß estradiol by MTT. CONCLUSIONS. 17-β Estradiol exacerbates Toxoplasma gondii infection at 20 and 80 nM/mL, while that progesterone at 10, 20,40 and 80 nM/mL had a reduced effect on T. gondii proliferation in astrocytes infected.

Disclosures: A.F. Gutiérrez Maldonado: None. **J. Dueñas Jimenez:** None. **L. Rodríguez–Pérez:** None. **M. Galván-Ramírez:** None.

Poster

612. Neuroinflammation: HIV and Infections

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Topic: B.11. Glial Mechanisms

Support: NIH R01NS079166

NIH R01DA036165

Title: Microglia Contribute to HIV1-gp120Bal- induced synapse loss

Authors: *W. RU[1], S. TANG[2];
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Abstracts: HIV-1 infection of the central nervous system (CNS) may lead to synaptic degeneration that is implicated in the pathogenesis of cognitive impairments in HIV-1/AIDS patients. However, the mechanism(s) by which HIV-1 causes the synaptic degeneration is unclear. We hypothesize that microglia, which are the major phagocytes in the CNS, contribute to removing the damaged synapse on live neurons after HIV-1 infection. As an initial step to test

this hypothesis study, we have determined the effects of HIV-1 coat protein gp120 on synapses of cortical neurons in culture. Using western blotting analysis, we found that gp120Bal decreased the protein levels of pre- and post-synaptic makers synapsin I and PSD95 in a time-dependent manner. Confocal imaging revealed a decrease of synapses following gp120 application. These data suggest that gp120 induces synaptic loss on the cultured neurons. Interestingly, we found that pretreating the primary cultures with minocycline, an inhibitor of microglia activation, abolished the gp120Bal-caused synapse loss. In addition, we also observed that pharmacological blockers of either NMDA or AMPA receptor abolished gp120-induced decrease of PSD-95 and synapsinI. These preliminary findings indicate that HIV-gp120 induces synapse loss via a biological process that depends on microglial activation and synaptic activity. We will perform additional studies to test this hypothesis.

Disclosures: W. Ru: None. **S. Tang:** None.

Poster

612. Neuroinflammation: HIV and Infections

Location: Halls A-C

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Program#/Poster: 612.20/V26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HHMI Grant 52007563

RCMI Grant G12MD007585-23

Title: Amyloid beta reduces cytopathic effects of Herpes Simplex virus type I

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Abstracts: Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by memory loss and other types of dementia. Amyloid β (Aβ) is a main cause of senile plaques detected in the brains of patients with AD and other forms of dementia. Recent evidence suggests that the buildup of Aβ is may be in response to microbial infections in the central nervous system. Aβ has been shown to inhibit the growth of bacteria and yeast; however, little work has tested its role in reducing viral activity. The present work tests the hypothesis that Aβ suppresses the cytopathic effects induced by the neurotropic Herpes Simplex Virus Type I (HSV-1). To test

this hypothesis, we treated SH-SY5Y neuroblastoma cells with varying concentrations of Aβ one hour before HSV-1 infection (1716 strain). Forty-eight hours after infection, cell viability and morphology was assayed. First we determined cell viability by utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay indicated that pretreatment of Aβ (100nM, 1μM, 10μM) reduced HSV-1 induced cell death by 40% ($p<0.01$). Photomicrographs of neuroblastoma cells infected with HSV-1 (multiplicity of infection; MOI=1) also demonstrated that Aβ pretreatment reduced cytopathic effects of HSV-1. To more directly test HSV-1 activity, plaque formation assays are ongoing. These data indicate that the antimicrobial activities of Aβ are not limited to bacteria. Overall, the results provide another link connecting HSV-1 infection and the progression of AD.

Disclosures: **J. Merritt:** None. **A. Angajala:** None. **G. Griffin:** None. **J.R. Babu:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.01/V27

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Capes

FPA

Title: Rats reared in isolation from weaning show decreased expression of NMDA receptors in the medial prefrontal cortex

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Abstracts: Dysfunction of the prefrontal cortex (PFC), where the excitability of the microcircuits has been demonstrated to occur through the NMDA glutamate (Glu) receptors, has been reported to contribute to symptoms of the schizophrenia such as cognitive deficits, thought disorders, delusions and hallucinations. It has been shown that Glu neurotransmission is decreased in the PFC of both schizophrenics and rats reared in isolation from weaning, a model

of this disease. The aim of this study was to evaluate the changes on the expression of NMDA receptors (NR1 and NR2) in the PFC (primary somatosensory cortex), sub-regions infralimbic (IL) and prelimbic (PrL) of the medial PFC (mPFC) and in entorhinal cortex (EC) induced by isolation rearing. Two groups of Wistar rats (n=5-8/each) were used. In both groups the pups remained with their mothers (6/mother) until weaning (21 days - 40g) when they were allocated randomly to one of two conditions: grouped (housed 3/cage, handled 3 times/week) or isolated (housed individually, handled once/week for cleaning purpose) for 10 weeks. The animals were anaesthetized, perfused and their brains sectioned (40μm) in the PFC and EC for immunohistochemistry. The number of immunopositive cells (IC) or the optical density (OD) was quantified bilaterally in 3 sections/rat. Data were compared by Student t-test ($p<0.05$). In contrast, the expression of NR2 was decreased significantly in both mPFC-PrL (25%, $p=0.003$) and EC (25%, $p<0.001$) of rats reared in isolation from weaning. The number of NR2-IC did not change in the PFC and mPFC-IL of rats reared in isolation when compared to grouped rats ($p>0.05$). The decreased expression only in NR2 found in the mPFC-PrL and EC of rats reared in isolation suggest that specific subtypes of Glu receptors underlie the alterations on the Glu neurotransmission reported in schizophrenia. These findings also suggest that Glu hypofunction occur in specific brain regions in schizophrenia as previously reported for sub-regions of the PFC. Understanding the altered PFC circuits in schizophrenia may improve the knowledge of the pathophysiology of this disease and contribute to the development of treatments that can retard the onset of the symptoms. **Support:** Capes; FPA.

Disclosures: **G.G. Borges:** None. **M. Ferreira:** None. **J. Rodrigues:** None. **K. Moriyama:** None. **M. Santos:** None. **H. Fachim:** None. **M. Iyomasa:** None. **M. Rosa:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.02/V28

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: The NMDA receptor GluN2C subunit regulates behavioral and cellular phenotypes relevant to schizophrenia

Authors: *A. RAVIKRISHNAN, B. G. HILLMAN, S. C. GUPTA, R. PAVULURI, D. J. STAIRS, S. M. DRAVID;
Creighton Univ., Omaha, NE

Abstracts: The NMDA receptor (NMDAR) hypofunction hypothesis in schizophrenia posits that reduced NMDAR function leads to behavioral abnormalities observed in schizophrenia. Lower expression of GluN2C subunit of NMDAR has been reported in the cortex and thalamus in postmortem brains from schizophrenic patients, however its exact functional implications remain unknown. With the GluN2C heterozygous and knockout (GluN2C HET and KO) mouse model we tested whether a deficit in GluN2C expression leads to schizophrenia-like phenotypes. Moreover, using a GluN2C/GluN2D-subunit selective potentiator (CIQ) together with the GluN2C genetic model we tested whether facilitation of GluN2C-containing receptors can prevent schizophrenia-like behavior. GluN2C HET and KO mice were more sensitive to phencyclidine (PCP)-induced hyperlocomotion and working memory deficit in a Y-maze test compared to wildtype (WT) mice. CIQ attenuated PCP-induced hyperlocomotion and working memory deficit in WT and GluN2C HET but not in GluN2C KO. Furthermore, GluN2C KO had a modest deficit in prepulse inhibition and CIQ prevented dizocilpine (MK-801)-induced prepulse inhibition deficit in WT and GluN2C HET but not in GluN2C KO. Moreover, social isolation-induced stress led to emergence of hyperlocomotion and social interaction and working memory deficits in GluN2C KO. GluN2C KO also exhibited lower dendritic spine density in pyramidal neurons and higher expression of a marker for oxidative stress, 4-hydroxy-2-noneal in the medial prefrontal cortex. These results demonstrate that a deficit in GluN2C-containing NMDARs leads to schizophrenia-like phenotypes, and indicate that pharmacologic enhancement of GluN2C-containing NMDARs may lead to beneficial effects for behavioral and cognitive deficits in schizophrenia.

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Poster

613. Schizophrenia: Glutamate

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.03/V29

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH R01MH085666

Title: Epigenetic regulation of NMDAR expression in prefrontal cortex and hippocampus during development in the methylazoxymethanol model and Disrupted-in-Schizophrenia-1 model for schizophrenia

Authors: *Y. GULCHINA[1], M. A. SNYDER[1], M. V. PLETNIKOV[2], W.-J. GAO[1];
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Abstracts: The glutamate hypothesis demonstrates the importance of the NMDA receptor in the pathological process of schizophrenia, however the question of how mis-regulation of the NMDA receptor occurs remains unclear. One potential mechanism underlying aberrant NMDA receptor function is epigenetic control of transcription wherein gene product levels are altered without direct modification of the DNA sequence. To explore this possibility, we have employed two animal models: the neurodevelopmental methylazoxymethanol (MAM) acetate rat model and the transgenic mutant Disrupted-in-Schizophrenia-1 (mDISC1) mouse model. We have characterized NMDA receptor subunit protein levels through development, and we observed reduced NR2B protein levels in the prefrontal cortex (PFC) of both MAM-treated rats and mDISC1 mice in juvenile stage (p21) of development. Corresponding to the reduced NR2B, we identified an upregulation of H3K27me3, a histone marker indicative of repressive transcription, in the PFC of juvenile (p21) MAM-treated animals. In addition, HDAC4, a protein involved in regulating synaptic plasticity, was upregulated in adolescent (p45) MAM-treated hippocampus, where NR2B levels were similarly decreased as was observed in the PFC. We are currently evaluating the role of repressive transcriptional proteins, such as repressor element 1 silencing transcription factor (REST/NRSF), as well as histone marks of global changes in gene expression in the nuclei of PFC and hippocampus neurons in these animal models. In addition, we are investigating whether these epigenetic modifiers actively repress NR2B expression in juvenile and adolescent MAM-treated and mDISC1 animals, respectively.

Disclosures: Y. Gulchina: None. **M.A. Snyder:** None. **M.V. Pletnikov:** None. **W. Gao:** None.

Poster

613. Schizophrenia: Glutamate

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Program#/Poster: 613.04/V30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: FAPESP (2011/09548-3)/ Brazil

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UFABC/ Brazil

Title: Reversion of behavior deficits with nitric oxide synthase inhibitor and gene expression in MAM schizophrenia model

Authors: *C. SALUM[1,2], M. C. BROSCO[2], O. M. LIMA-FILHO[2], G. S. V. HIGA[2], A. H. KIHARA[2];
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Abstracts: Gestational methylazoxymethanol acetate (MAM) exposure has been suggested to produce neural and behavioral abnormalities which model some aspects of schizophrenia. Some of the behavior deficits observed are reduced prepulse inhibition (PPI) and social interaction. Nitric oxide (NO) is altered in schizophrenic patients and we have shown that NO synthase (NOS) inhibitor, NG-nitro-L-arginine (LNO), was able to prevent PPI deficits caused by dopamine agonists and NMDA antagonists in rats. Our aims were to investigate if LNO was able to improve the behavior deficits of PPI and social interaction observed in MAM offspring and investigate gene expression of receptors DRD2, DRD1, NMDAR1 and enzymes nNOS and iNOS. Nine pregnant Wistar rats were treated with ip injection of either Saline or 22mg/kg of MAM on the 17th day of pregnancy. Male rats of the offspring (N=38) were used with approximately 90 days of age. Twelve of them, after the behavioral testing, were euthanized and their brains were removed and for dissection of four brain regions: prefrontal cortex (PFC), striatum (CPU), midbrain, and hippocampus. After RNA extraction, the expression of DRD2, DRD1, NMDAR1, NOSn, NOSi were examined by quantitative reverse transcription polymerase chain reaction. Other 26 rats of that offspring received an ip injection of Saline or LNO (40mg/kg) 1h before testing and were tested on PPI and then on social interaction. PPI test consisted on 64 stimuli presentations: pulse (120 dB), prepulse (PP, 69, 73 and 81dB), PP+P and null (no stimuli). %PPI and amplitude startle response (ASR) were registered. Social interaction test consisted on the exposure of two unfamiliar rats for 5min to the open field registering: sniffing, following and genital inspection. MAM-saline group presented significant lower %PPI and higher ASR to all stimuli compared to Saline-Saline group also showed significantly. Analyses detected reduced social behaviors for MAM-saline rats compared to Saline-Saline animals. In all behaviors there was no difference between MAM-LNO rats compared to Saline-Saline ones. PCR results showed lower expression of DRD1 at CPU and hippocampus, of DRD2 at CPU and midbrain and of NMDAR1 at hippocampus and midbrain in MAM rats compared to control ones. No difference was found in expression of nNOS and iNOS. Results suggest that LNO is able to restore some behavior deficits in MAM rats which may not be accounted by higher levels of nNOS. These findings and the genes expression alterations may be relevant for the study of schizophrenia. Financial **Support:** FAPESP (2011/09548-3), CNPq 476162/2011-4 and UFABC

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(2011/09548-3)/ Brazil, CNPq 476162/2011-4 / Brazil, UFABC/ Brazil. **M.C. Brosco:** None. **O.M. Lima-Filho:** None. **G.S.V. Higa:** None. **A.H. Kihara:** 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.; FAPESP/ Brazil.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.05/V31

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH R01MH085666

Title: Untangling GSK3β, DISC1 and NMDAR interactions in prefrontal cortical neurons

Authors: *S. MONACO[1], W.-J. GAO[2];
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Abstracts: Cognition is a fundamental neurological process mandatory for successfully navigating through a persistently changing environment. The ability to perceive, filter, prioritize, update and ultimately respond to incoming stimuli requires efficient cognitive processing. NMDA receptors are considered the cellular constituents associated with learning, memory, and higher order cognition. Disruption of NMDA receptors is strongly implicated in several disorders characterized by cognitive impairments, particularly schizophrenia. Schizophrenia is a neurodevelopmental disease with combined environmental and genetic factors that converge and thus affect susceptibility. DISC1 is recognized as a candidate risk gene linked to schizophrenia and has been found to directly interact with GSK3β as well as regulate NMDA receptors. The goal of this study is to further elucidate the signaling mechanism at play among DISC1, GSK3β, and NMDA receptors with a particular focus on prefrontal cortical neurons. We hypothesize that interaction of GSK3β and DISC1 affects the expression of NMDA receptors via regulation of β-catenin signaling. Preliminary results indicated that pharmacological inhibition of GSK3β lead to increased NR2B, NR2A, and β-catenin expression levels at 4 hours post-treatment, with no change in NR3A protein levels. pGSK3βser9 levels were reduced both at 4 and 24 hours following SB216763 treatment. We predict that DISC1 serves as an intermediate factor, linking environmental perturbations to genetic alterations. Gaining a better grasp on this cellular

signaling cascade will help provide an essential framework for understanding how the DISC1/GSK3β complex regulates NMDA receptor changes in prefrontal cortical neurons.

Disclosures: S. Monaco: None. **W. Gao:** None.

Poster

613. Schizophrenia: Glutamate

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: All authors are employees of AbbVie. The design, study conduct, and financial support for this work was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Title: "Two-hit" developmental model of schizophrenia (prenatal Poly I:C and neonatal PCP): Transient effects on microglia activation

Authors: C. SCHIFANI, A.-L. RELO, *C. KLEIN, A. Y. BESPALOV;
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Abstracts: The neurodevelopmental hypothesis of Schizophrenia describes the disease onset as an interplay of at least two genetic or environmental 'hits' during development. Present research focused on an animal model combining two environmental factors known to be at risk for schizophrenia, maternal immune activation during pregnancy (polyinosinic-polycytidylic acid, Poly I:C, infusion on gestation day 15) and early life insult (neonatal exposure of offsprings to glutamatergic insult by repeated treatment with the NMDA receptor antagonist phencyclidine, PCP, on postnatal days 7, 9 and 11). This treatment leads to changes in behavior and metabolic brain activity in puberty and adulthood. More specifically, treated rats showed increased engagement in play behavior during puberty as well as increased exploratory activity during adulthood. Furthermore, adult hippocampal brain activity was reduced. At least some of the observed effects in the adult animals were correlated with the alterations in play behavior during late adolescence. Therefore, a separate series of studies addressed effects of Poly I:C/PCP treatment in late adolescent rats. To assess the potential neuroinflammatory state, hippocampal brain slices of 48-day old rats were stained with Iba-1, a marker for microglia. Treated rats showed a very strong increase of the number of microglia compared to controls. To characterize this effect further, OX-42 staining was used for the same slices to measure potential elevated

microglia activation. However, this was not shown to be different between groups. One possible explanation for this outcome could be that the microglia activation is already gone in late adolescent rats but number of microglia is still increased. To conclude, combined prenatal Poly I:C and early postnatal PCP treatment increases the number of microglia but, in this model, pathophysiological contribution of neuroinflammation appears to decline as the animals become adult.

Disclosures: **C. Schifani:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & Co.KG. **A. Relo:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & Co.KG. **C. Klein:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & Co.KG. **A.Y. Bespalov:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & Co.KG.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.07/W1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Dainippon Sumitomo Pharma Co., Ltd.

Title: Which acetylcholine receptors participate to the lurasidone-induced improvement in novel object recognition subchronic phencyclidine model of cognition in schizophrenia?

Authors: *M. MIYAUCHI[1,2], L. RAJAGOPAL[1], M. HUANG[1], S. KWON[1], Y. OYAMADA[2], H. Y. MELTZER[1];
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Abstracts: Background: Both nicotinic acetylcholine (ACh) receptors (nAChR) and muscarinic Ach receptors (mAChR) are critical components of cognitive function. Under normal condition, nAChR and mAChR agonists generally improve cognition, while antagonists have the opposite effect. Lurasidone is a novel atypical antipsychotic drug, with high affinity for dopamine (DA) D2, serotonin 5-HT2A, 5-HT7, and 5-HT1A receptors. We have previously shown that acute lurasidone significantly reversed declarative memory deficits induced by subchronic treatment with the N-methyl-D-aspartate receptor (NMDAR) antagonist, phencyclidine (PCP) in a novel object recognition (NOR) paradigm, and also increased DA and ACh release in the medial prefrontal cortex (mPFC). The role of DA (via D1 receptor stimulation) in reversing the deficit in

NOR resulting from subchronic PCP is well established but the contribution of cholinergic mechanisms to this effect requires further study. Thus, the aim of the present study was to investigate the role of nAChR and mAChR in the ameliorating effect of acute lurasidone treatment in subchronic PCP treated rats. Materials and Methods: Female Long-Evans rats received vehicle or PCP (2 mg/kg, b.i.d.) for 7 days, followed by a 7-day washout. Groups of six normal rats received a single dose of the non-competitive nAChR antagonist, mecamylamine (MEC) (4 mg/kg) or the mAChR antagonist, scopolamine (SCO) (0.1 mg/kg) 15 min prior to acquisition. Another group of rats, following washout of subchronic PCP, received MEC (4 mg/kg) or SCO (0.1 mg/kg) 15 minutes prior to an effective dose of lurasidone (0.1 mg/kg). Results: Normal rats who received acute MEC (4 mg/kg) or SCO (0.1 mg/kg) showed significant NOR deficits. MEC (4 mg/kg) 15 minutes prior to lurasidone showed significant deficits; i.e. the ameliorating effect of lurasidone was blocked by pretreatment with MEC. SCO (0.1 mg/kg) pretreatment did not prevent the effect of lurasidone. Discussion: This study demonstrates that: 1) both MEC and SCO induce significant deficits in NOR in normal rats; 2) MEC, a nAChR antagonist, but not SCO, a mAChR antagonist, blocked the ameliorating effect of lurasidone following subchronic PCP treatment in rats; 3) endogenous ACh efflux in cortex and probably hippocampus induced by acute lurasidone plays a crucial role in its effect on subchronic PCP-induced NOR deficits via nAChR stimulation. Further studies with selective nAChR agonists and antagonists in subchronic PCP-treated rats in combination with lurasidone is needed to establish which nAChRs are involved in its ability to improve NOR.

Disclosures: **M. Miyauchi:** A. Employment/Salary (full or part-time):; Dainippon Sumitomo Pharma Co. Ltd. **L. Rajagopal:** None. **M. Huang:** None. **Y. Oyamada:** A. Employment/Salary (full or part-time):; Dainippon Sumitomo Pharma Co., Ltd. **H.Y. Meltzer:** 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.; Dainippon Sumitomo Pharma Co., Ltd.. F. Consulting Fees (e.g., advisory boards); Dainippon Sumitomo Pharma Co., Ltd.. **S. Kwon:** None.

Poster

613. Schizophrenia: Glutamate

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Program#/Poster: 613.08/W2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: BBHI-COBRE 12-100899-HSC

Title: Examining the effects of a chronic exposure to phencyclidine on a rodent system: An analysis of functional network connectivity, behavioral performance, and mRNA expression

Authors: *C. M. MAGCALAS[1], N. I. PERRONE-BIZZOZERO[2], V. D. CALHOUN[4], J. BUSTILLO[2], E. E. PEREZ[2], D. A. HAMILTON[3];
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Abstracts: Chronic administration of phencyclidine (PCP) generates neurobiological and behavioral changes that mimic symptoms and micro-levels changes found in schizophrenia (sz). PCP impairs spatial learning and memory performance and flexibility of learning in addition to decreasing mRNA expression of markers including parvalbumin, NR2B, mGluR2, and $GAD_{65/67}$. The neuropathology responsible for the onset of sz is still unknown. Thus, establishing valid animals models plays a vital role in characterizing the processes involved. A growing interest in quantifying functional network connectivity (FNC) has yielded clinical studies, which have identified abnormal activation in sz patients. The current study aims to evaluate the effects of chronic PCP exposure on resting state FNC in the rat. Adult male rats (N=40) were pre-trained in the Morris Water Task (MWT) prior to a 4-week injection regimen. Rats received 14 intraperitoneal injections of either PCP (2.58 mg/kg) or 0.9% saline solution (1 mL/kg). Rats were anesthetized 72-hrs after their final injection & imaged in a 4.7T Bruker Biospin MRI scanner. Resting state fMRI BOLD data were collected after which rats were retested in the MWT to investigate long-term spatial memory and behavioral flexibility. Tissue punches from the medial and ventral frontal cortex, cerebellum, and parietal cortex were collected following the MWT retest. cDNA was synthesized from total RNA and RT-PCRs were performed in order to examine gene expression for parvalbumin, calbindin, GAD67, ErbB4, NR2A, and NR2B. EPI image sequences were normalized. Group independent component analysis implemented in Group ICA of fMRI Toolbox (GIFT) was used to identify resting state networks. A total of 27 non-artifactual components were retained and consisted of 14 cortical, 7 amygdala/hippocampal, 2 thalamic, 2 striatal, and 2 midbrain components. PCP exposed rats displayed more negatively correlated hippocampal-cortial and midbrain-cortial components, primarily for cortical components localized in the frontal cortex. Increases in positive correlations were observed in PCP exposed rats within hippocampal-hippocampal components. Performance during the MWT retesting phase indicated that PCP exposure induced a long-term spatial memory deficit but did not impair subsequent spatial learning. RT-PCR analysis determined that rats exposed to PCP show decreased expression for GAD67 in the ventral frontal cortex. These results indicate that chronic PCP exposure causes widespread alterations in FNC including constituents of the default mode network and other subcortical and cortical regions implicated in sz.

Disclosures: C.M. Magcalas: None. **N.I. Perrone-Bizzozero:** None. **V.D. Calhoun:** None. **J. Bustillo:** None. **E.E. Perez:** None. **D.A. Hamilton:** None.

Poster

613. Schizophrenia: Glutamate

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Program#/Poster: 613.09/W3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 232855

155255

Title: Psychosis-Like behaviors in rats induced by toluene exposure: Role of NMDA receptors

Authors: *M. T. RIVERA, C. LÓPEZ-RUBALCAVA, S. CRUZ;
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Abstracts: Toluene is a volatile hydrocarbon found in a variety of chemical compounds that is commonly misused by inhalation for recreational purposes. The misuse of this solvent exacerbates psychosis symptoms in patients but the mechanism through which it exerts this effect is unknown. Toluene has a complex mechanism of action that includes NMDA receptor antagonism, among other effects. It has been described that other NMDA receptor antagonists like phencyclidine or ketamine induce psychotic like symptoms in healthy people. In preclinical research, these compounds are used to mimic a psychotic-like state. The main objective of the present study was to analyze if toluene could induce psychosis-like behaviors and to characterize the participation of the NMDA receptors in these actions. To this aim, male Wistar rats were exposed to toluene (500 -8000 ppm) for 30 min and immediately after were evaluated in social interaction and prepulse inhibition (PPI) tests. On the other hand, head-twitch response (HTR), a behavior related to hallucinogenic-like effects, was analyzed during toluene exposure. Our results show that there were a concentration-dependent decrease of cumulative interaction time and percent inhibition of the startle response in PPI test, while there was an increase in the number of HTR. In a second part of the study we analyzed the role of NMDA receptor antagonism in toluene´s effects, for this purpose rats were treated with D-serine 20 min before toluene exposure and tested in different trials. Results showed that co-activation of NMDA receptors prevent the deficit induced by toluene in social interaction and PPI test, and reduce the HTR. In conclusion, toluene exposure induces psychotic-like behaviors; the NMDA receptor antagonism is relevant in toluene's effects.

Disclosures: **M.T. Rivera:** None. **C. López-Rubalcava:** None. **S. Cruz:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.10/W4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Changes in NMDA receptor subunits following developmental ketamine administration

Authors: *V. JEEVAKUMAR[1], S. KROENER[2];
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Abstracts: The NMDAR-hypofunction theory postulates that schizophrenia is associated with alterations of NMDARs in parvalbumin (PV)-expressing GABAergic interneurons, causing deficits in the function of those interneurons, leading to disinhibition of glutamatergic cells and cortical desynchronization. We recently characterized a developmental NMDAR antagonism model of the disorder in which mice are treated with ketamine (KET) (30 mg/kg) on PND 7, 9 and 11. We observed enduring cognitive deficits and negative-like symptoms of schizophrenia in adult animals. In the medial prefrontal cortex (mPFC) NMDAR blockade increased excitatory inputs onto PV interneurons, but did not change GABAergic inputs onto pyramidal (PYR) cells. Unexpectedly, we also observed a homeostatic upregulation of NMDARs in PV cells. Here, we further explore the alterations at NMDARs in PV cells of adult animals by examining subunit-specific changes in NR2A and NR2B contribution. We used whole-cell patch-clamp recordings to study pharmacologically isolated NR2A and NR2B currents by bath-applying either the NR2B blocker Ro25-6981 or the NR2A blocker PEAQX, respectively. The percentage change in amplitude of the response gave an estimate of the contribution of each subunit. We found that in KET-treated animals NR2A subunit contribution was unaltered (SAL = -46.38 ± 5.21%, n = 5; KET = -50.81 ± 4.01%, n = 5; p = 0.52); whereas NR2B-mediated currents were increased (SAL = -20.49 ± 4.4%, n = 7; KET = -35.35 ± 5.22%, n = 8; p = 0.03). This indicates that the upregulation of NMDAR currents in PV cells caused by developmental KET treatment is mainly due to an increase in NR2B subunits. Because we saw no enduring changes in IPSCs onto PYRs in adult animals, we investigated whether disinhibition of PYRs occurs transiently following KET administration. Thus mice treated with KET or saline on PND 7, 9 and 11 were sacrificed on PND 12 for sIPSC recordings from layer 5 PYRs in the mPFC. In a second experiment, the acute effects of KET were studied by recording sIPSCs from layer 5 PYR cells following a single injection of KET (or saline) on PND 7. Surprisingly, under both conditions we observed no changes in the amplitude or frequency of sIPSCs. Ongoing experiments explore the possibility that disinhibition of PYR cells in the PFC following both acute and chronic NMDAR

antagonism is layer-specific. Specifically, selective disinhibition of PYR cells in layers 2/3 may contribute to the increase in sEPSCs seen in layer 5 PV cells. Taken together, our results demonstrate paradoxical effects of developmental KET treatment on PV cells that alter mPFC network function and lead to persistent schizophrenia-like behavior in adult animals.

Disclosures: V. Jeevakumar: None. **S. Kroener:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NSERC Discovery Grant 402642

Title: Dose-dependent effects of repeated ketamine administration on novelty detection and cognitive processes

Authors: *A. SCHUMACHER, E. C. TOLLEDO, B. SIVANANDAN, J. WOLDEGABRIEL, R. ITO;
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Abstracts: Ketamine is a non-competitive antagonist of the NMDA receptor, known to elicit strong psychotomimetic effects. Repeated exposure to sub-anesthetic doses of ketamine in rats has previously been shown to induce cognitive deficits, as well as behavioural changes akin to the negative symptoms of schizophrenia, giving much validity to the use of ketamine administration as a pharmacological model of schizophrenia. This study sought to further characterize the behavioural effects of two different ketamine pre-treatment regimens, focusing primarily on the effects of repeated ketamine administration on novelty processing, which is known to be dependent on the integrity of the hippocampus. Given existing evidence of reduced hippocampal recruitment in schizophrenia, we predicted that repeated ketamine pre-treated rats would demonstrate impaired novelty detection. Male Long Evans rats received either 5 or 14 intra-peritoneal injections of 30mg/kg ketamine or saline. After a withdrawal period of 10 days, rats were tested in an associative mismatch detection task. Rats were habituated to two audiovisual sequences, and subsequently presented with novel and familiar stimulus configurations to examine their ability to detect novel sequences. Furthermore, rats underwent a novel object detection task, in which they were habituated to four objects presented in a plus

maze. Subsequently, one object was replaced, and the spatial locations of two familiar objects were switched. Rats also underwent testing in various other tasks such as the delayed matching to place T maze task, sucrose preference task and locomotor tests involving administering a challenge dose of amphetamine (AMPH). As predicted, we found that the high-dose ketamine pre-treatment regimen elicited impairments in mismatch detection, spatial novel object detection and working memory. In contrast, the low-dose ketamine pre-treatment regimen improved performance of novelty detection. In addition, low-dose ketamine pre-treated rats showed enhanced locomotor activity following an AMPH challenge, indicative of locomotor sensitizaton, while the high-dose ketamine pre-treated rats showed decreased locomotor activity, compared to saline rats. These data demonstrate that different regimens of repeated ketamine administration can induce behavioural alterations in opposite directions, and that neural adaptations occurring in the mesolimbic dopamine system may underlie these effects. These findings have important implications not only for the use of repeated ketamine as an animal model of schizophrenia, but also for recent studies demonstrating ketamine to have antidepressant properties.

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Poster

613. Schizophrenia: Glutamate

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.12/W6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Philanthropic gift to JHU Brain Science Institute

Title: Inhibition of D-amino acid oxidase does not increase D-serine plasma levels in monkey or dog

Authors: J. ALT[1], N. ATOR[2], T. TSUKAMOTO[1], C. ROJAS[1], *B. S. SLUSHER[1];
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Abstracts: D-serine administration has been shown to be effective for the treatment of schizophrenia symptoms. However, D-serine has to be administered at high doses in order to observe clinical effects. This is thought to be due to D-serine undergoing oxidation by D-amino

acid oxidase (DAAO) before it reaches the brain. Consequently, co-administration of D-serine with a DAAO inhibitor has been suggested as a way to lower the dose of D-serine required to treat schizophrenia. Early studies to evaluate this hypothesis showed that concomitant administration of DAAO inhibitors with D-serine significantly enhanced D-serine plasma levels in rodents compared to administration of D-serine alone. In a follow-up effort we wanted to demonstrate a corresponding enhancement of D-serine levels in larger mammals. We evaluated D-serine plasma levels in baboons for 24 h after 30 mg/kg oral administration in the presence or absence of 5- chloro-benzo[d]isoxazol-3-ol (CBIO), a prototype DAAO inhibitor. CBIO was administered by intravenous infusion at 0.1 mg/kg/h for 24 h. Even though CBIO reached concentrations in plasma at about 4-6 μM, well above its binding affinity for DAAO (200 nM), plasma D-serine levels were the same in the presence or absence of CBIO. Similar results were obtained when the same experiment was carried out with dogs instead of monkeys or when using other DAAO inhibitors instead of CBIO. We conclude that in contrast to rodents, DAAO inhibition in monkeys or dogs does not increase levels of D-serine in plasma. The difference in results among the different species could be due to differences in D-serine metabolism and/or clearance mechanisms. It is also possible that the role of DAAO is different in rodent versus non-rodent mammalian species.

Disclosures: J. Alt: None. **N. Ator:** None. **T. Tsukamoto:** None. **C. Rojas:** None. **B.S. Slusher:** None.

Poster

613. Schizophrenia: Glutamate

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Program#/Poster: 613.13/W7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01MH085666

Title: Age-dependent effects of mGluR2 agonist LY395756 on NMDA receptor expression and function in the rat prefrontal cortex

Authors: *M. LEE[1,2], B. XING[1], W.-J. GAO[1], X.-Q. HU[2], F. LI[3];
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Abstracts: Pharmacologically targeting the group II metabotropic glutamate receptor (mGluR2/3) has been reported to be effective in alleviating symptoms of multiple neurological and psychiatric disorders, including persistent pain, drug addiction, and schizophrenia. It is widely believed that mGluR2/3 agonists mainly affect presynaptic release to regulate synaptic function. However, we recently reported that mGluR2/3 agonist also exhibit strong postsynaptic regulation of both AMPA and NMDA receptor function. Because its antipsychotic effect is mainly through activation of mGluR2, we explored the effects of LY395756 (LY39), a compound that is selective for mGluR2 as an agonist and mGluR3 as an antagonist, on NMDAR expression in the prefrontal cortex (PFC) of both juvenile and adult rats. We found that LY39 induced dose-dependent changes of synaptic membrane proteins in NMDAR subunits. It significantly increased the expression of NR1, NR2A, NR2B and NR2B phosphorylations Tyr1472, Ser1303 at high dose of 3 mg/kg in adult rats, but had no significant effect on NMDAR subunits at all 3 doses (0.3, 1, 3 mg/kg) in juvenile rats. Moreover, there were significant decreases in total protein level of mTOR and pGsk3β-Ser9/Gsk3β ratio at a high dose of 3.0 mg/kg LY39 in adult rats. It is therefore likely that LY39 exhibits distinct actions in NMDAR expression and function in young vs. adult animals. We are currently investigating this possibility and the potential mechanisms by using electrophysiological recording.

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Poster

613. Schizophrenia: Glutamate

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.14/W8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Characterization of a subchronic PCP rat model of shizophrenia and evaluation of effects of Bitopertin and Tolcapone

Authors: *V. ANGLADE[1], D. PARACHOU[1], E. CAYRE[1], E. R. DETRAIT[2], C. DRIEU LA ROCHELLE[1];
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Abstracts: Classical antipsychotics such as haloperidol are known to treat only the positive symptoms of schizophrenia (hallucinations, paranoid delusion, language disorder) whereas certain atypical antipsychotics have been proposed for the treatment of negative (social withdrawal, anhedonia) and cognitive (impaired attention, memory, executive function)

symptoms. In humans, the non-competitive NMDA antagonist phencyclidine (PCP) produces a schizophrenic-like psychosis including positive and negative symptoms and cognitive dysfunction. In animals, repeated PCP treatment produces long term behavioral and pathophysiological deficits that correlate with those reported in schizophrenia. We recently presented data (405.09, SFN 2013) on an animal model of schizophrenia, evaluating social withdrawal using the social interaction (SI) test and memory impairment using the novel object recognition (NOR) test in rats treated subchronically with PCP followed by withdrawal. The goal of the present study was to extend validation of this model by characterizing the duration of the PCP-induced deficits and evaluating the efficacy of bitopertin (RG1678, a highly potent GlyT1 inhibitor) and tolcapone (a COMT inhibitor) in reversing the deficits. Long Evans rats received a 7-day subchronic treatment with PCP (5 mg/kg twice a day, ip). In the first experiment, animals were tested in social interaction 1, 4 and 9 weeks after the end of PCP treatment or in the NOR test 1, 2 and 4 weeks after the end of PCP treatment. In the second experiment, the efficacy of acute treatment with bitopertin (1, 3 and 10 mg/kg, ip) and tolcapone (7.5, 15 and 30 mg/kg, ip) in reversing the deficits were assessed. The results show that PCP-induced reproducible social withdrawal lasts around 9 weeks post-treatment and the deficit in the NOR test lasts around 4 weeks post-treatment. Moreover, bitopertin (3 and 10 mg/kg) dose-dependently reversed the PCP-induced SI deficit and all three doses of tolcapone reversed the recognition memory impairment. In conclusion, subchronic treatment with PCP followed by withdrawal induces a deficit in novel object recognition and social withdrawal in rats which last up to four and nine weeks, respectively. These symptoms can be reversed by treatment with pharmacological agents such as the GlyT1 inhibitor bitopterin and the COMT inhibitor tolcapone.

Disclosures: V. Anglade: None. **D. Parachou:** None. **E. Cayre:** None. **E.R. Detrait:** None. **C. Drieu la rochelle:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.15/W9

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Korea Healthcare Technology R&D Project Grant HI12C1470

Title: Effects of electroconvulsive seizure on the neonatal MK-801 treatment-induced long-term changes in behaviors and protein translation signal pathway in the rat frontal cortex

Authors: *S. KIM[1], H. PARK[2], Y. KIM[3];
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Abstracts: Systemic injections of MK-801, a selective NMDA receptor antagonist, into neonatal rats induce long-term neurochemical and behavioral changes. It has been suggested that these changes form the neurodevelopmental basis for schizophrenia-like behavior in rats. In this study, postnatal 7-day (PN7) rats were treated with MK-801, and their frontal cortices at PN60 were examined to investigate the long-term effects on the molecules in signal pathway of protein translation. At PN60, the rats treated with MK-801 at PN7 showed increased locomotor activity and deficits in prepulse inhibition, as reported previously. Accompanied with the behavioral changes, the phosphorylation level of S6 at S240/244, which promotes protein translation initiation, was increased, and the phosphorylation of raptor at S792, which inhibits the activity of mTOR signal pathway, was reduced in the rat frontal cortex at PN60. Repeated treatments of electroconvulsive seizure (ECS) from PN51 to PN60 ameliorated the increased locomotor activity and prepulse inhibition deficits of PN7 MK-801-treated rats. In addition, ECS treatments recovered the PN7 MK-801-induced increase in the phosphorylation of S6 at S240/244 and decrease in the phosphorylation of raptor at S792. In summary, long-term behavioral changes induced by neonatal MK-801 treatment was accompanied with the increased phosphorylation of S6 in the brain, which were recovered by ECS treatments. These findings could suggest an important role of aberrant long-term activation of protein translation machinery in the MK-801 neurodevelopmental animal model of schizophrenia.

Disclosures: S. Kim: None. **H. Park:** None. **Y. Kim:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.16/W10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NSERC

FRQS

Title: Effects of chronic prenatal MK-801 treatment on behaviour and dopamine functioning in the adult rat offspring

Authors: *S. GALLANT, L. WELCH, P. MARTONE, U. SHALEV;
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Abstracts: Patients with schizophrenia display impaired cognitive functioning, increased social withdrawal, and increased sensitivity to psychomimetic drugs. The neurodevelopmental hypothesis of schizophrenia posits that disruption of the developing brain predisposes neural networks to lasting structural and functional abnormalities resulting in the emergence of such symptoms in adulthood. Given the critical role of the glutamatergic system in early brain development, we investigated whether chronic prenatal exposure to the glutamate NMDA receptor antagonist, MK-801, induces behavioural and neurochemical changes in adult rats. Pregnant Long-Evans rats were administered saline or MK-801 (0.1 mg/kg; s.c.) at gestation day 7-19. Object recognition memory and cognitive flexibility were assessed in the adult male offspring using a novel object preference task and a maze-based set-shifting procedure, respectively. Social behaviour was assessed in pairs of rats interacting in an open-field box. Locomotor-activating effects of acute amphetamine (0.75, 1.0, 1.5 mg/kg) and MK-801 (0.1, 0.4 mg/kg) were also assessed. To investigate differences in dopamine (DA) utilization, rats were decapitated following an amphetamine challenge (0.75 mg/kg) and levels of DA and DA metabolites were assessed in the nucleus accumbens, striatum, and prefrontal cortex using HPLC. Adult, prenatally MK-801-treated rats failed to show novel object preference after a 90 min delay, suggesting that they did not recognize the familiar object. In addition, the set-shifting task revealed impaired acquisition of a new rule in prenatally MK-801-treated rats compared to controls. This deficit appeared to be driven by regression to the previously learned behaviour. Furthermore, in the social interaction task, prenatally MK-801-treated rats spent less time sniffing and more time wrestling with a novel rat compared to controls. There were no significant differences in locomotor activity following acute amphetamine challenges. Unexpectedly, MK-801-induced locomotor activity in prenatally MK-801-treated rats was lower compared to controls. Prenatally MK-801-treated rats appeared to have lower DA utilization in the nucleus accumbens and dorsal medial striatum compared to saline rats, though these differences were not statistically significant. These findings suggest that glutamate dysfunction during early development may mediate behavioural deficits in adulthood and may therefore shed light into the role of neurodevelopmental adaptations in schizophrenia.

Disclosures: **S. Gallant:** None. **L. Welch:** None. **P. Martone:** None. **U. Shalev:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.17/W11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant DA029252

Title: Sub-chronic ketamine effects in the rodent odor span task

Authors: D. PANOZ-BROWN, C. ANDERSON, R. WELCH, M. DEAL, K. GOBENCIONG, S. HANNAH, S. HESS, C. MYERS, K. E. BRUCE, A. PRICHARD, *J. GALIZIO;
Univ. North Carolina, Wilmington, NC

Abstracts: A number of studies have shown that sub-chronic exposure to NMDA antagonists (e.g., ketamine) impairs performance on a variety of cognitive tasks and may provide a model for the cognitive impairments in schizophrenia. The present study evaluated the effects of sub-chronic ketamine in a rodent model of working-memory capacity, the odor span task (OST). Rats were trained to stability on the OST and then exposed to five daily injections of saline, 10 mg/kg or 30 mg/kg ketamine. After exposure to sub-chronic ketamine, OST performance was evaluated along with the capacity to learn an olfactory discrimination reversal. Some reduction in span length and longest run of consecutive responses was observed in ketamine-treated rats, but these effects were relatively small and short lived. Overall OST accuracy did not show significant impairment and there was no effect on either the performance of a simple discrimination or on the reversal learning task. The present results were not consistent with previous studies showing large and persistent effects of sub-chronic ketamine and indicate the importance of task variables in the assessment of residual effects of NMDA antagonist treatment.

Disclosures: D. Panoz-Brown: None. **C. Anderson:** None. **R. Welch:** None. **M. Deal:** None. **K. Gobenciong:** None. **S. Hannah:** None. **S. Hess:** None. **C. Myers:** None. **K.E. Bruce:** None. **J. Galizio:** None. **A. Prichard:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.18/W12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: P51OD11132 (Yerkes National Primate Research Center)

Title: Ketamine-induced brain activation determined by fMRI in conscious nonhuman primates as a translational model to evaluate the CNS effects of antipsychotics

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Abstracts: The present study extends the successful development of an apparatus and methodology to conduct fMRI studies in conscious rhesus monkeys in order to evaluate the CNS effects of antipsychotics. The dosing regimen employed a single bolus i.v. injection of ketamine followed by a continuous i.v. infusion over one hour in five adult female subjects. Prior to the fMRI protocol, multiple doses of ketamine were administered on separate occasions and plasma levels of ketamine were determined to establish dosing that approximated plasma levels (93-106 ng/mL at the low dose, 120-216 ng/mL at the high dose) reported in human studies. Behavioral observations indicated that dosing was well below the anesthetic range and all subjects were responsive to tactile and auditory stimulation. MRI scans were conducted in a Siemens Trio 3 Tesla magnet using a custom-designed transmit-receive volume NHP head coil. The monkeys lay prone in a custom-built restraint cradle optimized for acquiring MRI data from conscious monkeys attached to the NHP head coil, and physiological data, including heart rate, mean arterial blood pressure, and respiratory rate were monitored and collected continuously. BOLD fMRI images were collected utilizing a whole-brain gradient echo single-shot echo planar imaging sequence (TR/TE/FA = 3000ms/32ms/90; 1.5mm X 1.5mm X 1.5mm resolution). Each monkey underwent one 55-minute fMRI scan (2 minute baseline; followed by 53 minute infusion of ketamine). High-resolution (0.5mm X 0.5mm X 0.5mm) T1-weighted MPRAGE scans were acquired for anatomic reference. Statistical parametric maps were obtained with appropriate GLM models incorporating motion and hemodynamics of ketamine infusion. Ketamine induced a very robust ($p < 10\text{-}7$), dose-dependent activation in several brain regions, including dorsolateral prefrontal cortex, anterior cingulate, inferior parietal lobule and thalamus, and regional specificity declined with increased dose. Clinical fMRI studies have reported activation by ketamine in each of these brain regions, documenting the remarkable concordance between the nonhuman primates and human subjects. Ongoing studies are evaluating the interaction of ketamine with a standard antipsychotic drug in clinical use, risperidone. The results obtained will provide a metric to evaluate novel antipsychotics.

Disclosures: **E. Maltbie:** None. **K. Gopinath:** None. **N. Urushino:** None. **L. Howell:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.19/W13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH grant NS058710

NIH grant NS075338

NIH Grant NS062097

American Heart Association grant AHA 12GRNT12060222

Title: Impaired Social activity in NMDA receptor GluN3A (NR3A) subunit knockout mice

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Abstracts: The N-methyl-d-aspartate receptor (NMDAR) has been implicated in the pathophysiology of neuropsychiatric disease, including schizophrenia and autism spectrum disorders (ASD), which has impaired social behaviors as a unique feature. For example, the glycine binding site in NMDARs can regulate social behavior in schizophrenia and ASD. In addition, the level of NR2 subunit tends to increase after administration of antipsychotic drugs for patients with schizophrenia and ASD. These results suggest that NMDA receptors play important roles in social activity. GluN3A or NR3A is a unique inhibitory subunit in the NMDAR complex. The role of GluN3A in social behavioral activities is unclear. In this study, we sought to determine altered social activities in GluN3A knockout (KO) mice. Wild type (WT) and GluN3A KO mice were tested for social transmission ability in a food preference task, the social interaction test, and the three-chamber social interaction test. Although GluN3A KO mice displayed normal social transmission of food preference, these mice spent less time in reciprocal social interaction in the social interactions test compared to WT mice. The three-chamber social test confirmed that mice lacking GluN3A had lower sociability and did not show a preference for social novelty. In addition, using a home cage monitoring system we observed reduced grooming and sniffing behaviors in GluN3A KO mice. To determine cellular signals that might mediate the altered social behaviors in GluN3A KO mice, we examined the expression of some social behavior-related genes including serotonin receptor, serotonin transporter, vasopressin, vasopressin receptor, oxytocin, oxytocin receptor, brain-derived neurotropic factor (BDNF), and cluster of differentiation 73 (CD73) in the prefrontal cortex, hippocampus, and thalamus. Among

these genes, the expressions of oxytocin and its receptor were significantly lower in the prefrontal cortex in GluN3A KO mice than that in WT mice. These initial findings suggest that GluN3A expressed in the adult prefrontal cortex plays a significant regulatory role in the social activity. Reduced expression levels of oxytocin and its receptors may contribute to the impaired social behavior and imply a link between GluN3A, oxytocin system and some psychiatric disorders such as schizophrenia and ASD.

Disclosures: **J. Lee:** None. **X. Ji:** None. **L. Wei:** None. **S.P. Yu:** None.

Poster

613. Schizophrenia: Glutamate

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 613.20/W14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 102-2420-H-002-008-MY2 from the Ministry of Science and Technology

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Grants Drunken Moon Lake Integrated Scientific Research Platform

Aim for Top University Project from NTU

Title: Evaluation of the effect of sarcosine, an endogenous glycine transporter 1 inhibitor, on behavioral performance and the glutamate hypothesis of schizophrenia in mice

Authors: ***W.-L. HUNG**[1], W.-S. LAI[1,2,3];
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Abstracts: The hypofunction of the NMDA glutamate transmitter system is emerging as a potentially more promising hypothesis for schizophrenia, especially for cognitive deficits. There is a growing interest in the development of pharmacological agents with potential antipsychotic properties that enhance the activity of the NMDA receptor via glycine modulatory site. Glycine is a coagonist at the NMDA receptor complex and glycine levels are regulated by glycine type I transporters, which serve to maintain low sub-saturating glycine levels in the vicinity of the NMDA receptor. Sarcosine (N-methylglycine), a naturally occurring selective glycine type I transporter inhibitor, can increase the concentration of glycine within the synaptic cleft and

potentiate NMDA receptor function. Emerging studies suggest that sarcosine might be useful for the restoration of NMDA hypofunction and the treatment of schizophrenia. However, the effective dose and the mechanism for the therapeutic effect of sarcosine remains much unclear. In this study, a series of experiments was conducted to evaluate the effect of sarcosine in male mice and MK-801 was used to induce acute NMDA receptor hypofunction in these mice. We found that (1) a single dose of sarcosine (except the highest does) did not affect basic locomotor activity in mice; (2) a single administration of sarcosine (except the highest dose) had no effect on MK-801 induced hyperlocomotion and stereotypy; (3) among these doses we selected, no obvious toxic effect was found in the blood biochemical analysis except the highest dose; (4) a reduction of brain activity was found after a single injection of sarcosine using microPET scan with 18F-fluorodeoxyglucose; and (5) MK-801 induced behavioral deficits in the prepulse inhibition and holeboard task can be normalized by sarcosine pre-injection. Behavioral data collection and further analyses are still in progress. Findings from this study provide some clues to understanding the effect of sarcosine and its therapeutic potential in the treatment of schizophrenia.

Disclosures: **W. Hung:** None. **W. Lai:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.01/W15

Topic: F.01. Human Cognition and Behavior

Support: Netherlands Organization for Scientific Research (NWO) Grant 056-23-011

Title: Neural mechanisms of empathy in autism and aggressive conduct disorder

Authors: ***E. T. KLAPWIJK**[1,3], M. AGHAJANI[1,3], O. F. COLINS[1,3], N. D. J. VAN LANG[1,3], N. J. A. VAN DER WEE[3,2], R. R. J. M. VERMEIREN[1,3];
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Abstracts: Empathy, the ability to share and understand the feelings of other people, is a crucial aspect of human social interactions. Diminished empathy is thought to be central in autism spectrum disorders (ASD) and may also underlie aggressive and antisocial behavior in individuals with conduct disorder (CD). Using an fMRI paradigm, we investigated the neural

mechanisms underlying empathy in a sample of adolescents with ASD, a group of aggressive CD adolescents and a group of matched healthy controls. Thirteen male adolescents (15-19 years old) with ASD, 26 male adolescents with aggressive CD and 33 healthy control subjects were scanned using fMRI while completing an explicit empathy task (Schulte-Ruther et al., 2007). In this task emotional faces (angry, fearful and neutral) from the Radboud Faces Database (Langner et al., 2010) were presented. Participants were asked to either infer the emotional state from the face (other-task) or to judge their own emotional response to the face (self-task). A perceptual decision on the width of neutral faces was included as a control condition. FMRI data processing was carried out using FEAT Version 6.00, part of FSL (www.fmrib.ox.ac.uk/fsl). Behavioral results suggested that all participants were faster and better at recognizing other's fear compared to other's anger in the other-task ($p < .001$). No significant group differences in behavior were found. Neuroimaging results (all $p < .05$ cluster-corrected) showed higher activation in the dorsal anterior cingulate cortex (dACC) in the control group compared to the ASD group and in the posterior cingulate cortex (PCC) in the CD group compared to the ASD group during the self-task. Furthermore, higher activation in the left and right amygdala and thalamus was found for the control group compared to the CD group in this condition. The dACC is an important region for higher-order social cognition and empathy. Decreased activity in this area and in the PCC in the ASD group is in line with previous studies suggesting problems with cognitive processing of empathy in ASD. The amygdala and thalamus are important affective regions and decreased activity in these regions in the CD group is thought to reflect diminished affective reactions to other's emotions. Our results underline the importance of distinguishing between cognitive and affective aspects of empathy in ASD and CD groups.

Disclosures: **E.T. Klapwijk:** None. **M. Aghajani:** None. **O.F. Colins:** None. **N.D.J. van Lang:** None. **N.J.A. van der Wee:** None. **R.R.J.M. Vermeiren:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.02/W16

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: ZonMW (grant numbers: 3160007, 91676084, 31160003, 31180002, 31000056, 2812412, 100001002, 100002034)

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Competence Network Schizophrenia

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Title: Enigma schizophrenia working group findings from 2,028 cases and 2,540 controls

Authors: *T. G. VAN ERP[1], D. P. HIBAR[2], P. M. THOMPSON[2], J. A. TURNER[3], T. ENIGMA SCHIZOPHRENIA WORKING GROUP[4];
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Abstracts: Introduction The profile of brain structural abnormalities in schizophrenia is not fully determined, despite years of structural brain imaging research. To avoid the low statistical power obtainable with small samples analyzed using a variety of methods, here we analyzed brain magnetic resonance imaging (MRI) scans from 15 centers worldwide using standardized methods. Our ultimate goal is to identify biomarkers for use in schizophrenia imaging-genetics studies. Here we ranked subcortical brain abnormalities by effect size and identified sources of effect size heterogeneity across samples. Methods This ENIGMA (Stein et al. 2011) Schizophrenia Working Group project pooled, via meta-analysis, structural magnetic resonance imaging (MRI) brain scans from 15 sites across the globe. Data from 4,568 individuals, including 2,028 patients and 2,540 controls, were analyzed using standardized image analysis (FreeSurfer), quality assurance, and statistical methods. Bilateral accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus as well as ventricular volume and total intracranial volume (ICV) were obtained. Group contrast effect sizes were estimated controlling for age, sex, and intracranial volume (ICV). Meta-regression analyses were performed to identify effect size moderators. Results Compared to healthy controls, patients with schizophrenia had smaller hippocampus (Cohen's d=-0.46), amygdala (=-0.31), thalamus (d=-0.31), accumbens (d=-0.25), and intracranial volumes (d=-0.12) and larger pallidum (d=0.21) and lateral ventricle

volumes (d=0.37). Putamen and pallidum volume exacerbations were positively associated with duration of illness; hippocampal deficits scaled with the proportion of not-medicated patients in each cohort. Conclusion This first ENIGMA Schizophrenia Working Group cooperative analysis of brain imaging data supports a consistent profile of subcortical abnormalities in schizophrenia. The largest effect size was observed for hippocampal deficits followed by lateral ventricle enlargement. Overall, the profile of subcortical abnormalities is consistent with a large meta-analysis of structural brain abnormalities in schizophrenia (Haijma et al. 2012). Patients with schizophrenia showed significantly smaller hippocampus, amgydala, thalamus, accumbens, and intracranial volumes and significantly larger pallidum and lateral ventricle volumes.

Disclosures: **T.G. van Erp:** F. Consulting Fees (e.g., advisory boards); Roche Pharmaceuticals, Inc.. **D.P. Hibar:** None. **P.M. Thompson:** None. **J.A. Turner:** None. **T. ENIGMA Schizophrenia Working Group:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.03/W17

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: ENIGMA Bipolar disorder working group findings from 1,745 cases and 2,613 controls

Authors: *D. P. HIBAR[1], L. T. WESTLYE[2], P. M. THOMPSON[1], O. A. ANDREASSEN[2], .. FOR THE ENIGMA BIPOLAR DISORDER WORKING GROUP[3];
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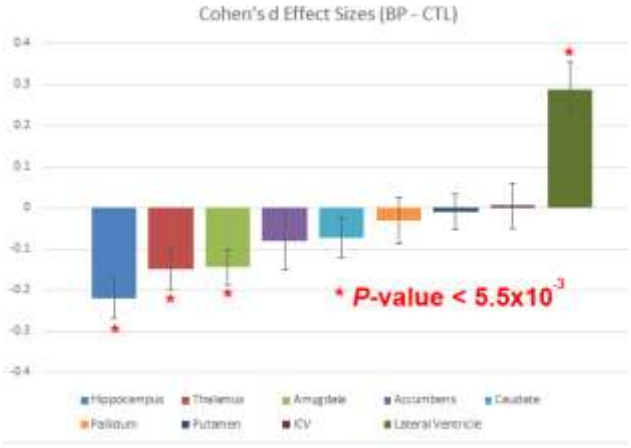
[3]http://enigma.ini.usc.edu/ongoing/enigma-bipolar-working-group/, Los Angeles, CA

Abstracts: Introduction The pattern of effects on the brain in bipolar disorder (BP) has proven heterogeneous, and volumetric comparisons of brain structures theorized to be involved in the pathophysiology of BP have yielded mixed results. To investigate sources of uncertainty, we have formed an international collaboration for the study of BP as part of the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) Consortium (Stein et al., 2012). In this initial ENIGMA-Bipolar effort, we perform the largest ever study of subcortical brain volumes in BP cases and healthy controls, by analyzing MRI scans from a total of 4,358 participants. Methods The ENIGMA Bipolar disorder working group brings together structural

MRI brain scans from 4,358 subjects - 1,745 cases and 2,613 healthy controls. We examined the mean volumetric differences between BP cases and healthy controls in seven subcortical brain structures: nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus as well as ventricular volume and total intracranial volume (ICV). All effect sizes were estimated while controlling for age, sex, and differences in head size (ICV). Results BP cases had significantly lower volumes of the hippocampus (d = -0.221 ± 0.049; P = 6.62x10-6), thalamus (d = -0.150 ± 0.051; P = 3.21 x10-3), and amygdala (d = -0.143 ± 0.043; P = 9.44 x10-4) but larger lateral ventricles (d = 0.289 ± 0.066; P = 1.29x10-5) than healthy controls. None of the other five structures was significantly different between BP cases and controls using a Bonferroni corrected significance threshold p* < 0.05/9 = 5.6x10-3 (Figure 1). Conclusions Here we performed the largest ever study of neuroimaging measures in BP, enabling robust estimates of brain structure abnormalities. Patients with BP had significantly enlarged ventricles, which is the most consistently reported finding in the BP literature (Hallahan et al., 2010). Patients with BP have significantly smaller hippocampus, amygdala, and thalamus volumes - findings that have not been consistently shown in previous



reports.

Disclosures: D.P. Hibar: None. **L.T. Westlye:** None. **P.M. Thompson:** None. **O.A. Andreassen:** None. **.. for the ENIGMA Bipolar Disorder Working Group:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.04/W18

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH

NIBIB

NICDH

NIA

Title: Study of candidate gene effects on white matter microstructure in 4000+ individuals - from the ENIGMA-DTI working group

Authors: *N. JAHANSHAD[1], P. KOCHUNOV[2], E. SPROOTEN[3], E. DTI WG[1], P. THOMPSON[1], D. GLAHN[3], T. E. NICHOLS[4], R. C. W. MANDL[5], R. M. BROUWER[5], B. LANDMAN[6], H. LEMAITRE[7], A. DEN BRABER[8];
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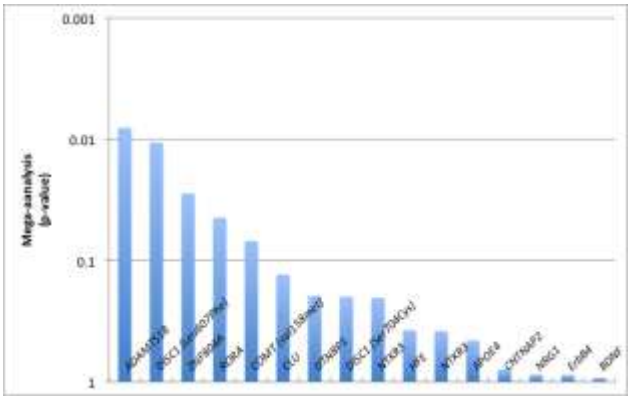
Abstracts: The role of genes on risk for changes in brain anatomy is a challenge faced by neuroscientists and geneticists. To date it has been found that the majority of common genetic variants exert only a small effect on anatomical traits derived from brain imaging, if at all. Discovering the specific role of genes in these highly heritable brain traits is particularly confounded by the fact that study populations vary greatly across basic demographics, neurological conditions, and imaging acquisition methods, making critical replication and validation attempts a near impossible task. The Diffusion Tensor Imaging (DTI) Working Group in the Enhancing Neuro-Imaging Genetics through Meta-Analysis Consortium (ENIGMA-DTI) has developed image processing protocols and analysis methods that reliably pool data from heritable imaging phenotypes across various cohorts regardless of demographic and technical variability. To evaluate the effects of 16 common genetic variants previously reported to associate to white matter microstructural variation, we pooled the average fractional anisotropy data from 4314 scans from 6 various cohorts. Individuals ranged in age from 11 to 85. Data was pooled across populations through a mega analysis (all data were considered simultaneously in

the model) as well as a meta- analysis (results from individual cohort analyses were pooled) after covarying for linear and quadratic effects of age, sex, their interaction, as well as components from multi-dimensional scaling to reduce population stratification effects. Mega analysis showed more promise for detection of effects than meta-analysis. Polymorphisms for three genes showed nominally significant associations with global FA values *ADAMTS18*, *DISC1*, and *ZNF804A*. Despite this being the largest study of its kind for DTI, no variant survived multiple comparisons correction for the number of variants tested, let alone at the genome-wide statistical threshold for significance. This study validates the need for large-scale collaborative efforts for discovering genetic influences over brain connectivity.



Disclosures: **N. Jahanshad:** None. **P. Kochunov:** None. **E. Sprooten:** None. **E. Dti wg:** None. **P. Thompson:** None. **D. glahn:** None. **T.E. Nichols:** None. **R.C.W. Mandl:** None. **R.M. Brouwer:** None. **B. Landman:** None. **H. Lemaitre:** None. **A. den Braber:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.05/W19

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Subcortical brain volume abnormalities in major depressive disorder: Prospective meta-analytic findings from the enigma major depressive disorder working group

Authors: *L. SCHMAAL[1,2], D. J. VELTMAN[2], D. P. HIBAR[3], ;. FOR THE ENIGMA-MDD WORKING GROUP[4];
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Netherlands; [3]Imaging Genet. Ctr., USC, Los Angeles, CA;
[4]http://enigma.ini.usc.edu/ongoing/enigma-mdd-working-group/, Amsterdam, Netherlands

Abstracts: Despite overwhelming evidence that major depressive disorder (MDD) is heritable, intensive efforts to elucidate genetic variation underlying MDD have yielded only modest success, perhaps partly because genetic effects do not directly translate into dichotomous clinical phenotypes. Biomarkers, for instance measures of brain structure and function, may represent an intermediate step in the causal pathway from genetic variation to distal clinical phenotypes and may therefore help to resolve questions about the etiology of depression. The goal of our ENIGMA-Major Depressive Disorder (ENIGMA-MDD) consortium, a network of 14 research institutes from 7 different countries (for a full list of participating institutes, see: http://enigma.ini.usc.edu/ongoing/enigma-mdd-working-group/) with overlapping neuroimaging data from around 1800 MDD patients and 7200 controls, is to identify imaging markers that robustly discriminate MDD patients from healthy controls using a prospective meta-analytic approach employing the same analysis techniques and statistical models across all participating centers. Here we present the results of the first meta-analysis on differences in subcortical volumes between MDD patients and controls. MDD patients showed robust hippocampal volume reductions compared to healthy individuals (Cohen's d=-0.14), whereas other subcortical volumes seemed to be preserved. However, when examining subcortical volume differences between healthy controls and patients with recurrent MDD and patients experiencing their first MDD episode at time of scanning, more widespread subcortical volume abnormalities were observed in recurrent MDD patients including the hippocampus (Cohen's d=-0.14), amygdala (Cohen's d=-0.11), caudate (Cohen's d=0.21) and lateral ventricles (Cohen's d=0.16). In contrast, no differences between first episode patients and healthy individuals were observed. No associations between age of onset and use of antidepressant medication and subcortical volumes were observed. In conclusion, this currently largest worldwide effort to identify subcortical structural brain alterations showed robust reductions in hippocampus volume in MDD patients, with more widespread subcortical volume abnormalities in MDD patients with more than one depressive episode.

Disclosures: L. Schmaal: None. **D.J. Veltman:** None. **D.P. Hibar:** None. **;. for the ENIGMA-MDD Working Group:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.06/W20

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Dutch Organisation for Scientific Research Vici grant to Barbara Franke

Title: Brain structure in ADHD across the life span: The ENIGMA ADHD working group

Authors: *B. FRANKE[1], M. HOOGMAN[2], M. ZWIERS[2], M. MENNES[2], .. FOR THE ENIGMA-ADHD WORKING GROUP[3];
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Abstracts: Introduction Neuroimaging studies in ADHD show structural alterations of various brain regions in affected children and adults (Frodl and Skokauskas, 2012; Nakao et al., 2011; Valera et al., 2007). It is unclear, however, how these differences develop across the lifespan, and whether they are global effects or area-specific. To clarify brain changes across the lifespan, an ADHD Working Group was formed within the ENIGMA consortium (http://enigma.ini.usc.edu/). Within the working group, we are sharing brain imaging data from children and adults with ADHD and healthy comparison subject. Our first aim is to study subcortical brain differences in ADHD across the lifespan, and the potential effects of medication and co-morbidity. A second aim is to investigate effects of gender. **Methods** The ENIGMA-ADHD Working Group has adopted a rolling mega-analysis design: new groups can join at any time, and fixed data "freezes" allow analysis at different time points. Images are analyzed using fully automated and validated neuroimaging segmentation algorithms (FSL FIRST or FreeSurfer), for which protocols are available on the ENIGMA website. For all subjects, we collect volumetric data for hippocampus and the following subcortical structures: nucleus accumbens, amygdala, caudate nucleus, putamen, pallidum, and thalamus. We also share information on co-morbidity, ADHD symptoms, IQ, and medication use. The database currently includes 1640 cases and 1503 controls. **Results** So far, 22 international sites have joined the working group (see the ENIGMA website for a full list). Data from 452 ADHD cases and 405 controls have been analyzed to date. This sample has an age range of 6-63 years and includes 60% males. Our first analysis showed subtly but significantly smaller volumes for the left and right nucleus accumbens (*d*: 0.16 and 0.26), left amygdala (*d*: 0.16), right caudate nucleus (*d*: 0.16), and left putamen (*d*: 0.15) for cases compared to controls. These differences were independent of age. **Conclusions** The developmental trajectory of alterations in brain structure of individuals with ADHD remains largely unknown. This is due to the scarcity of large, well-powered longitudinal studies. Through data sharing, the ENIGMA-ADHD Working Group, with a sample of over 3000 cases and controls across the lifespan, will begin to address this gap. With sample sizes already similar to those of previous neuroimaging meta-analyses upon first data-freeze and analysis of the enlarged sample currently ongoing, our mega-analysis will help to

clarify outstanding questions regarding age, medication and gender effects on brain-developmental trajectories.

Disclosures: B. Franke: None. **M. Hoogman:** None. **M. Zwiers:** None. **M. Mennes:** None. **.. for the ENIGMA-ADHD Working Group:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.07/W21

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Intramural Program

Title: Neural correlates of implicit face-emotion processing in youth with severe irritability

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Abstracts: Background: Pathologically irritable youths exhibit impairments in face-emotion processing. However, the neural basis of this impairment is unknown. One report found amygdala hyperactivity to increasingly angry affect during implicit face-emotion processing in pathologically irritable youths compared to healthy comparison youths (HC). Here, we examine the neural correlates of implicit face-emotion processing in youths with chronic and severe irritability [operationalized as severe mood dysregulation; SMD]. Methods: During fMRI, 85 youths (ages 8-18 years) completed an implicit face-emotion processing task by identifying the gender of faces varying in affect and intensity of expression. Acquisition occurred on a 3T750 GE scanner. Participants were excluded for excessive motion (5 SMD, 2 HC) and low behavioral accuracy <65% (3 SMD, 7 HC), leaving 34 SMD and 34 HC for group analysis. Group differences in BOLD signal response to affects (angry, fearful, or happy) at different intensities (0=neutral affect, 50%, 100%, and 150%) were compared by ANOVA. We used standard procedures in Analysis of Functional Neuroimages to identify significant clusters, thresholded voxelwise at $p<.005$ and cluster corrected to $\alpha \leq 0.05$ whole-brain. Results: Relative to HC youths, SMD were less accurate ($p=0.03$) and slower to respond ($p<0.001$), but did not differ in accuracy

or reaction time by type of emotion or emotional intensity. HC and SMD youths differed in BOLD responses to facial affects at different intensities in bilateral fusiform gyri [right: $F(6,396)=8.3$, $p<0.001$, $k=170$, peak coordinates=44,59,-11; left: $F(6,396)=7.2$, $p<0.001$, $k=114$, peak coordinates=-49,56,-11], right precuneus [$F(6,396)=5.1$, $p<0.001$, $k=76$, peak coordinates=24,61,-29], and two cerebellar areas. In the fusiform gyri, SMD youths had a greater BOLD response to angry faces at 150% emotional intensity, relative to both HC youths ($p's \leq 0.05$) and themselves at other intensities of angry ($p's \leq 0.01$), while HC youths had more BOLD responses in the fusiform gyri to happy faces at the 150% intensity relative to both SMD youths ($p's<0.05$) and themselves at all other intensities of happy ($p's<0.03$). Conclusions: In regions mediating attention to facial features, SMD youth show hyperactivation in response to intensely angry affect, whereas HC youth show such hyperactivation in response to intensely happy affect. These results expand the prior literature of atypical neural responses to increasing angry affect in pathologically irritable youths and suggest a neural correlate to the social threat attention bias found in these youths.

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Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.08/W22

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: the National Basic Research Program of China 2011CB707805

Title: Aberrant striatal functional connectivity in schizophrenia patients and unaffected first-degree relatives

Authors: ***P. LI**[1], R.-J. ZHAO[2], L. SHI[1], Y.-L. FAN[2], H.-Q. SUN[3], L. LU[3];
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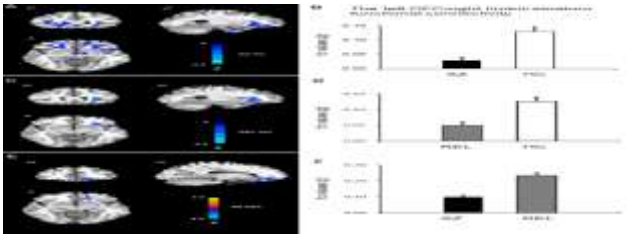
Abstracts: The striatum, where antipsychotic drugs mainly act, interacts with cortical areas involved in the affective and cognitive control. A large body of research has revealed aberrant

functional and structural connectivity of striatum in schizophrenia. However, it remains unclear whether connectivity between subdivisions of striatum and cortex is differentially affected in schizophrenia, and whether certain altered striato-cortical connectivity represents a valid intermediate phenotype of the disorder. We examined spontaneous functional connectivity between striatal subdivisions and the cortex using resting-state functional MRI (rs-fMRI) in patients with schizophrenia (n=50), unaffected first-degree relatives of patients (n=25), and healthy controls (n=48). Patients with schizophrenia mostly exhibited decreased functional connectivity between nearly all striatal subregions and the prefrontal cortex, temporal lobe and limbic cortex. Furthermore, both patients with schizophrenia and their unaffected first-degree relatives showed reduced functional connectivity between limbic striatum and orbitofrontal cortex compared with healthy controls, and meanwhile the reduction level in patients was higher than that in unaffected first-degree relatives. Our findings demonstrated that schizophrenia was characterized in part by abnormalities in the functional connectivity within the striato-cortical system. Especially, altered functional connectivity between limbic striatum and orbitofrontal cortex was related to the genetic risk of schizophrenia, which makes it a potential biological marker for schizophrenia.



Disclosures: **P. Li:** None. **R. Zhao:** None. **Y. Fan:** None. **H. Sun:** None. **L. Lu:** None. **L. Shi:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.09/W23

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Erasmus

Title: Probing electrophysiological and behavioral correlates of working memory deficits in schizophrenia

Authors: S. FISH[1,2], E. PAPPA[1,2], M. TOUMAIAN[1,2], T. DAVIES[1,2], *C. G. THELERITIS[3], C. SAVILLE[2], M. ECONOMOU[4], C. KLEIN[2], N. SMYRNIS[4,1];
[1]Lab. of Sensorimotor Control, Univ. Mental Hlth. Res. Inst., Athens, Greece; [2]Sch. of Psychology, Bangor Univ., Bangor, United Kingdom; [4]Psychiatry, [3]Univ. Athens Med. Sch., Athens, Greece

Abstracts: Schizophrenia has previously been associated with a number of cognitive deficits. Research has consistently demonstrated deficits in working memory (WM) including increased error rate as well as higher and more variable reaction time (RT) in schizophrenia patients. Furthermore, patients have been shown to demonstrate decreased amplitude and increased latency of the P3b event-related potential (ERP). The present study combines these behavioural and electrophysiological findings in schizophrenia patients (n=22) and healthy controls (n=19). Accuracy, RT and variability of RT, as well as P3b amplitude and latency were measured for all participants during an n-back WM task consisting of two conditions (0-back and 1-back). ERP measures were obtained via 64 channel electroencephalographic (EEG) recording. Patients' responses were significantly more erroneous ($F(1, 39) = 7.42$, $p = .01$), RTs were greater ($F(1, 39) = 19.95$, $p < .001$), and more variable ($F(1, 39) = 13.36$, $p < .001$) than controls. Patients' accuracy ($F(1, 39) = 8.34$, $p = .006$) and RT ($F(1, 39) = 5.92$, $p = .02$) decreased more drastically than controls as WM load increased. Patients' P3b latencies were significantly more prolonged for both tasks in comparison to controls ($F(1, 39) = 7.07$, $p = .01$). The results demonstrate a WM deficit as well as increased P3b latency in patients with schizophrenia. Additionally, an increase in WM load was associated with increased latency for all participants. However, the absence of a significant effect of WM load on latency between the two groups, suggests that P3b may not explain the confirmed WM deficits in schizophrenia. Future research may investigate alternative electrophysiological markers for this cognitive deficit and have implications for the development of more specific endophenotypes of psychotic disorders.

Disclosures: S. Fish: None. **C.G. Theleritis:** None. **E. Pappa:** None. **T. Davies:** None. **M. Toumaian:** None. **C. Saville:** None. **C. Klein:** None. **N. Smyrnis:** None. **M. Economou:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.10/W24

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Swiss National Science Foundation grant number: PZ00P3_126363 (to S. Spinelli)

Title: Enhanced regional homogeneity in the anterior cingulate cortex contributes to adaptive rumination in major depressive disorder

Authors: *S. SPINELLI[1], J. SPÄTI[2], J. HÄNGGI[3], J. ERNST[4], N. DOERIG[5], F. SAMBATARO[6], J. BRAKOWSKI[7], L. JÄNCKE[8], M. GROSSE HOLTFORTH[9], E. SEIFRITZ[4]; [1]Psychiatric Univ. Hosp. Zurich, Zurich, Switzerland; [2]Natl. Ctr. of Neurol. and Psychiatry, Tokio, Japan; [3]Inst. of Psychology, Univ. of Zurich, Zurich, Switzerland; [4]Psychiatric Hospital, Univ. of Zurich, Zurich, Switzerland; [5]Dept. of Psychology, Univ. of Zurich, Zurich, Switzerland; [6]Inst. Italiano di Tecnologia, Trento, Switzerland; [7]Psychiatric Hospital, Univ. of Zurich,, Zurich, Switzerland; [8]Inst. of Psychology, Univ. of Zurich,, Zurich, Switzerland; [9]Dept. of Psychology,, Univ. of Bern, Bern, Switzerland

Abstracts: Background: Major depressive disorder (MDD) is associated with structural and functional alterations in fronto-cingulate regions of the default mode and the task-positive networks (DMN, TPN). Enhanced activity in the anterior cingulate cortex (ACC) at rest is found in treatment-responsive patients, and may foster higher adaptive rumination. However, how structural changes affect functional coupling between the TPN and DMN, ACC activity, and how these functional changes are related to adaptive rumination is still unclear. Methods: Cortical thickness and regional homogeneity (ReHo) maps were calculated and compared between unmedicated depressed patients and healthy controls. Regions with reduced cortical thickness defined seeds for subsequent functional connectivity (FC) analyses. Results: Compared to healthy controls, depressed patients showed bilateral thinning of the dorsolateral prefrontal cortex (DLPFC) extending into the right frontopolar cortex (FPC), higher ReHo of the supragenual ACC and increased FC of the FPC with the midcingulate cortex (MCC) and the orbitofrontal cortex/ACC. In patients, higher supragenual ACC ReHo values were related to higher levels of adaptive rumination and stronger FPC - MCC connectivity. Moreover, in depressed patients greater adaptive rumination was also associated with stronger connectivity between the right DLPFC and the dorsal nexus, and higher connectivity strength correlated with increased ACC ReHo, even though DLPFC - dorsal nexus connectivity did not differ between patients and controls. Conclusions: Our findings suggest that bolstering the function of the supragenual ACC via the FPC/MCC and via the right DLPFC/dorsal nexus may foster adaptive rumination and potentially help treatment's response.

Disclosures: **S. Spinelli:** None. **J. Späti:** None. **J. Hänggi:** None. **J. Ernst:** None. **N. Doerig:** None. **F. Sambataro:** None. **J. Brakowski:** None. **L. Jäncke:** None. **M. grosse Holtforth:** None. **E. Seifritz:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.11/W25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01DA027680

NIH Grant R01MH085646

Title: Impact of smoking and age on WM compared to GM of schizophrenia

Authors: *H. SAMPATH[1,2], P. KOCHUNOV[1,2], F. MUELLERKLEIN[1,2], L. E. HONG[1,2]; [1]Neuroimaging Res. Program, Maryland Psychiatric Res. Ctr., Catonsville, MD; [2]Univ. of Maryland Ctr. for Brain Imaging Res., University of Maryland School of Medicine, MD

Abstracts: Patients with schizophrenia (SZ) have steeper age-related decline in white matter (WM) (Kochunov et al 2013; Wright et al 2014). This finding may share some common pathways linked to the 20% shorter life expectancy in SZ compared with the general population. SZ are also associated with 3-fold increase in the rate of smoking. We hypothesized that the abnormally steeper WM decline in SZ may be associated with abnormal aging and smoking. In comparison, cortical gray matter(GM) is known to be reduced early in the disease, and the impact of aging and smoking during adulthood could be less obvious. The study included 30 SZ smokers, 30 SZ nonsmokers, 30 normal control (NC) smokers, 30 NC nonsmokers. They underwent a MPRAGE T1 protocol for calculating GM voxel-based morphometry (VBM) and a diffusion tensor imaging protocol (DTI) for fractional anisotropy (FA), as indices of GW and WM integrity, respectively. Nicotine addiction severity was assessed by cigarette per day (CPD). GW VBM was assessed by SPM8+ DARTEL algorithm. FA was calculated by tract-based-spatial-statistics. Regression analyses were performed where brain index (VBM or FA) was the dependent variable, and age, CPD the predictors. Compared with NC, SZ showed reduced VBM in 9 cortical areas (corrected $p<0.05$). Age was associated with whole brain VBM in NC ($R^2=0.22$, $p<0.001$) and SZ ($R^2=0.25$, $p<0.001$), which were not significantly different ($z=0.26$, $p=0.80$), suggesting that VBM does not follow a precipitous decline in SZ. In NC smokers, the whole brain VBM was contributed by age ($t=-7.0$, $p<0.001$) but not CPD ($t=-0.76$, $p=0.45$). In SZ smokers, it was also contributed by age ($t=-2.8$ $p=0.008$) but not CPD ($t=-0.12$, $p=0.90$). For WM, we found age ($t=-6.92$, $p<0.001$) and a trend of CPD ($t=-1.79$, $p=0.076$) effect on whole brain FA in NC smokers. In SZ smokers, we similarly found age ($t=-4.18$, $p<0.001$) and a trend

of CPD (t=-1.89, p=0.076) contribution to FA. Previously we reported that the genu of the corpus callosum (GCC) was most severely associated with SZ and aging (Kochunov et al 2013), hence we re-test this in GCC. Here, we found significant effect of age (t=-4.11, p<0.001) but not CPD (p=0.74) on GCC FA in NC smokers. In SZ smokers, we found significant age (t=-3.23, p=0.003) and CPD (t=-2.26, p=0.032) effect on GCC FA. Age remains a key factor in the GM and WM decline overtime. Increased age-related decline was seen only in WM but not GM in SZ. In WM, nicotine addiction appeared to play a modest additional role to the FA reduction besides age. The effect was stronger in SZ, especially in GCC that provides connecting fibers between the frontal lobes. The potential underlying neurobiology of the joint age and smoking effects are considered.

Disclosures: H. Sampath: None. **P. Kochunov:** None. **F. Muellerklein:** None. **L.E. Hong:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.12/W26

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Investigator initiated grant from Astra-Zeneca to R. Ramasubbu

Title: The impact of onset age on intrinsic functional connectivity of amygdala in major depression

Authors: *D. L. CLARK[1], A. KEMP[1], N. KONDURU[1], F. CORTESE[1], S. BRAY[2], I. GAXIOLOA-VALDEZ[1], B. GOODYEAR[1], R. RAMASUBBU[1];
[1]Univ. of Calgary, Calgary, AB, Canada; [2]Alberta Children's Hosp., Calgary, AB, Canada

Abstracts: Major depressive disorder (MDD) is the leading cause of disability worldwide. Advances have been made in the treatment of MDD, but it is not a homogenous disease, and because of its many subgroups - each with a unique etiology and most likely a distinct optimal treatment - it is difficult to achieve patient remittance. Early-onset MDD is associated with more illness burden and an increased risk of relapse. Previously our group found reduced resting state functional connectivity of the amygdala in MDD. Since there is limited data on the effect of MDD onset age on the intrinsic connectivity (IC) of the amygdala in adult patients, we examined the relationship between age of onset and IC of amygdala in MDD. Resting state functional

magnetic resonance data were collected from 55 medication-free, right handed Caucasian adults with MDD and 19 healthy controls. Patients were separated into two groups based on the age of onset of MDD: Early (≤18 years of age; n=21) and Adult (>18 years of age; n=33). Amygdala seed based resting state functional connectivity or IC maps were generated for patients with MDD using age, sex, depression duration and number of episodes as covariates. Similar maps were generated for healthy controls using age and sex as covariates. Overall, compared to healthy controls, patients with early onset MDD showed reduced IC in only two regions: the caudate and cerebellum; whereas, patients with adult onset depression showed widespread reduced IC of the amygdala with fusiform, temporal and occipital regions, subgenual cingulate, insula, caudate, and the cerebellum and compensatory increases in IC with prefrontal and premotor regions. Early onset MDD, when compared to adult onset, resulted in extensive increases in amygdala IC with areas related to both top-down and bottom-up regulation of emotional processing, including the dorsolateral prefrontal cortex, supplementary motor area, insula, temporal, fusiform and occipital regions, anterior cingulate and thalamus. The results were corrected at $P<0.05$ for multiple comparisons using false discovery rate estimations. Here we show that overall reduction in IC of amygdala in MDD is primarily driven by adult-onset MDD patients, while early onset MDD appears to result in a distinctive pattern of amygdala IC. Our data, together with previous structural findings, support that early onset MDD is a distinct subgroup with unique neural features. Our study is limited by a small and unbalanced sample size, but we hope it will motivate examination of onset age in larger clinical samples. Characterization of early-onset MDD might serve to inform targeted treatment strategies in the future.

Disclosures: D.L. Clark: None. **A. Kemp:** None. **N. Konduru:** None. **F. Cortese:** None. **S. Bray:** None. **I. Gaxioloa-Valdez:** None. **B. Goodyear:** None. **R. Ramasubbu:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.13/W27

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Probing speeded decision processing in schizophrenia patients with fMRI

Authors: C. GRIGORAS[1], E. THRAPSANIOTI[1], G. PANAGIOTAROPOULOU[2], L. MANTONAKIS[3], E. KARAVASILIS[4], T. SOLDATOS[4], E. ANGELOPOULOS[3], *N. P.

SMYRNIS[3,1];
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Abstracts: There is a wealth of evidence that decision processing is impaired in patients with schizophrenia. In previous studies we have confirmed that these patients present significant differences in Reaction Time distribution in simple visuomotor tasks that have been interpreted as deficits in speeded decision processing. In this study 14 patients and 14 age and sex matched healthy individuals performed 300 trials of a forced 2-choice RT task (a version of the Eriksen Flanker task) in two conditions of easy and difficult perceptual decision while fMRI data were acquired. Our results confirmed that patients had slower ($F_{1,25} = 5.6$, $p=0,02$) and more variable ($F_{1,25}=15,7$, $p=0,0005$) RTs compared to controls while there were no error differences ($F_{1,25} =0,1$, $p=0,7$) between the two groups indicating that the difference in the speed of decision processing in patients was not related to performance differences. A specific difference in decision processing between patients and controls was also confirmed by the neuroimaging results showing that the contrast between difficult and easy decision trials resulted in an increased activation only at the Right Precuneus in controls while in patients a large network of areas were significantly more active, including the Left Precentral Gyrus, the Left Supplementary Motor Area, the Left and Right Occipital Middle Area and the Right Inferior Frontal Gyrus. These results favor the hypothesis that speeded decision processing is a very demanding task for patients with schizophrenia resulting in slowed and variable RT as well as the increased activation of a network of sensory, motor and frontal cortical areas.

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Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.14/W28

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: EKVAN 97

Title: Smooth eye pursuit and fixation endophenotypes in schizophrenia and obsessive compulsive disorder

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Abstracts: It is well established that smooth eye pursuit deficits are present in patients with schizophrenia and other psychotic disorders. It has also been shown that patients with schizophrenia present deficits in sustaining visual fixation especially in conditions of increased difficulty (such as in the presence of visual distracting stimuli). This study examines the specificity of these oculomotor endophenotypes for psychotic disorders by examining the performance of male patients with schizophrenia (N=38) and male patients with Obsessive Compulsive Disorder(OCD) (N=34) versus normal male controls(N=50).The participants performed smooth eye pursuit with increasing speed (10,20 and 30deg/sec) and a sustained visual fixation task (50sec duration) in three conditions (simple fixation, fixation with visual distractors and fixation in the dark with no fixation target).Smooth eye pursuit gain decreased with increasing pursuit speed ($F_{2,238} = 53.5$ $p<10\text{-}5$) and it was lower for patients with schizophrenia than in both OCD patients and healthy controls, while the later groups did not differ from each other ($F_{2,238} = 6.8$ $p<10\text{-}5$). The specific decrease in gain that was observed in schizophrenia was evident only for the higher pursuit speeds of 20 and 30degrees/sec as indicated by the significant speed by group interaction ($F_{4,238} = 2.9$ $p<10\text{-}5$). Saccade frequency in the fixation tasks was significantly higher for patients with OCD and patients with schizophrenia compared to controls ($F_{2,238} = 12.8$ $p<10\text{-}5$)and it was higher for the fixation condition with distraction and for the no target fixation condition compared to the simple fixation condition ($F_{2,238} = 7.8$ $p<10\text{-}5$). Finally a highly significant group by condition interaction ($F_{4,238} = 3.6$ $p<10\text{-}5$) was related to the fact that there was a specific increase in saccade frequency observed in the simple fixation task for the OCD patient group while patients with schizophrenia behaved as normal controls in the same fixation condition. In contrast both patients with schizophrenia and patients with OCD had higher saccade frequencies compared to healthy controls for the other two fixation conditions. These results indicate specific deficits in the pursuit system in schizophrenia and specific deficits in the visual fixation system for OCD that are different from those observed in schizophrenia, suggesting that oculomotor endophenotypes for schizophrenia and OCD are dissociable.

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Poster

614. Biomarkers in Serious Mental Illness

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Program#/Poster: 614.15/W29

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIAR01AG021476

Vanderbilt CTSA grant UL1 TR000445 from NCRR/NIH

Title: Cognitive bias for negative information in women with and without past depression correlates with differences in brain network activity and functional connectivity

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Abstracts: Women have a greater risk of depression than men and women with a past history of major depressive disorder (MDD) have a high risk of recurrence, even after menopause. Cognitive biases towards negative information that persist during remission may contribute to cognitive vulnerability to depressive symptoms or MDD recurrence. In this study we examined whether attentional bias for negative information differs between postmenopausal women with and without a history of MDD. 19 euthymic postmenopausal women were screened and MDD history was determined using the SCID. 11 women had no history of MDD (*Control*), and 8 women had a history of unipolar MDD (*MDD-Hx*), but were euthymic for at least 1 year. All of the women were at least one year from final menses, with no hormone or antidepressant treatment for at least one year. Subjects then completed an Emotion Dot Probe (EDP) task and resting state scan during an fMRI session. Average Beck Depression Index was 1.86 (SD = 2.41), with no significant difference between groups. Behavioral results for the EDP task revealed that the MDD-Hx group had greater negative facilitation (faster RT for negative than neutral images) than the Control group. During the EDP task the MDD-Hx group had greater left amygdala activity for negative images and greater activity in a number of brain areas than the Control group during negative switch trials, including the temporal parietal junction (TPJ). For all subjects, the functional connectivity strength between the left amygdala and medial orbital prefrontal cortex (moPFC) was inversely correlated with left amygdala activity during negative images. The Control group had greater functional connectivity between the left amygdala and moPFC than the MDD-Hx group. By contrast, the MDD-Hx group had greater functional

connectivity between right anterior insula and the TPJ (salience network). The strength of connectivity between these two regions was highly correlated with activity of the TPJ during "negative switch" trials of the EDP and negative facilitation only in the MDD-Hx group. In this study, women with past depression showed an attentional performance bias and increased limbic activity for negative images. These differences were correlated with functional connectivity differences in salience and emotional response brain networks, suggesting that changes in brain network connectivity may underlay a continued cognitive bias towards negative information in women with past depression. This persistent bias in women with past depression may indicate an underlying cognitive processing deficit that may increase salience of negative information conveying vulnerability to MDD recurrence.

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Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.16/W30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH DIRP

Title: Abnormal functional interactions of sensorimotor and social association networks in childhood-onset schizophrenia

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Abstracts: In recent years, the hypothesis of schizophrenia as a disorder of brain connectivity has regained considerable attention. Studies of adult-onset schizophrenia have shown mixed alterations in "functional connectivity" as measured by the covariation of spontaneous brain activity. Here we used resting-state functional magnetic resonance imaging to study network interactions in childhood-onset schizophrenia (COS), a rare, severe form of the disease that may have more salient neurobiological abnormalities. We addressed two major questions: 1) Do alterations in functional connectivity reveal disrupted interactions among specific brain networks in COS? 2) If so, do these alterations correspond to behavior? We used a data-driven approach

(Gotts et al., 2012) to analyze data from 19 COS patients and 26 healthy controls, group matched for age, sex, and handedness (all $p>.05$). Analysis produced 12 data-driven "seeds" with decreased functional connectivity in COS compared to controls (voxelwise threshold $p<0.0005$, cluster-corrected $p<.05$). A conjunction of resulting seed maps identified an additional 14 regions of altered connectivity in COS. All 26 ROIs had decreased functional connectivity in COS; we found no evidence for hyperconnectivity. Multi-dimensional scaling and k-means clustering of ROIs identified two major clusters. The first was comprised of social/limbic brain regions, including areas of the "default mode network" such as the posterior cingulate and medial prefrontal cortex. This social cluster also included higher-order association areas in parietal and dorsolateral prefrontal cortex. The second cluster was comprised of somatosensory and motor areas, including pre- and post-central gyrus, putamen, and inferior cerebellum. Matrix plots of ROI x ROI interactions revealed negative coupling of these two clusters in COS but not controls. Analysis of unaffected COS siblings (n=22) indicated that these alterations are state-specific, as siblings did not exhibit the decreased connectivity seen in COS. We then asked whether the network structure was related to positive and negative symptom scores in COS patients. Positive symptoms were associated with decreased across-cluster correlations, whereas negative symptoms were associated with decreased correlations within the social cluster. In summary, resting-state networks in COS are distinguished by a negative coupling between basic somato-motor areas and higher-order limbic / association areas. This opposing interaction suggests an abnormal integration of sensorimotor and social/cognitive processes and may relate to deficits in self-monitoring that are central to the disease.

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Poster

614. Biomarkers in Serious Mental Illness

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: T32 Grant T32MH067533

R01 Grant R01MH094520

Title: Effectiveness of fast mapping to promote learning of novel stimuli in schizophrenia

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Abstracts: Research on fast mapping (FM), a process that promotes expeditious incidental learning of information, is thought to support rapid vocabulary acquisition in young children through the extra-medial temporal lobe (MTL) regions. Recent research suggests that patients with amnesia and profound MTL damage are able to learn novel word-image associations using an FM paradigm (Sharon et. al, PNAS 108:1146-1151, 2011), but also see (Smith et. al, PNAS 111:475-479). The present study aims to determine whether FM would be an effective learning strategy for individuals suffering from schizophrenia, a severe mental illness that is associated with compromised MTL functionality. Twenty-three patients with schizophrenia and 23 healthy control subjects between the ages of 18-55 years completed two tasks: FM encoding and an episodic encoding control. For visit 1, participants were shown novel word-image associations with the FM paradigm and tested for recognition after a 10 minute break. A delayed recognition trial was completed one week later (± 2 days). During the same visit as the delayed recognition trial for FM, participants were evaluated on their ability to retain novel word-image associations using a control episodic encoding condition that also included a 10 minute delay trail and a one week (± 2 days) delay recognition trial. Results indicated that both groups performed better on the explicit encoding recognition trials when compared to FM recognition trials (p's < 0.05). For the FM recognition trials, both groups performed similarly. However, participants with schizophrenia performed significantly worse on the explicit encoding recognition trials than control participants (p's < 0.05). While participants with schizophrenia did not perform significantly worse on FM recognition trials when compared to healthy controls, these results do not provide enough evidence to suggest that FM promotes learning in schizophrenia. Whether FM benefits select individuals with schizophrenia is currently a focus of further investigation.

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Poster

614. Biomarkers in Serious Mental Illness

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Program#/Poster: 614.18/W32

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: BUMED W168

Title: Left frontal peak alpha frequency correlates with depression in male combat veterans with mTBI or mTBI+PTSD

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Abstracts: Depression is a common comorbid disorder in combat veterans with posttraumatic stress disorder (PTSD) and/or mild traumatic brain injury (mTBI). Studies show that combined PTSD and depression results in more severe illness burden, poorer prognosis and increased suicidal ideation compared to depression alone. Much work has suggested frontal alpha power asymmetry correlates with depression, but little attention has focused on alpha frequency to address speed of processing. High density EEG was recorded from 33 OEF/OIF veterans (16 mTBI only; 17 mTBI+PTSD) during several behavioral tasks, between which were periods of rest when subjects sat quietly starring at a fixation cross. EEG data from the entire session was submitted to independent component analysis (ICA) to find areas of high variance independent activity. Independent components (ICs) were clustered across subjects based on scalp map similarity. Periods of rest were concatenated and spectral power was calculated for all clustered ICs. Three clusters showed slower peak alpha frequency in the mTBI groups compared to Controls. These clusters localized to bilateral occipital, mid-parietal and left fronto-temporal regions. None of the peak alpha frequencies correlated with PTSD severity, but the left fronto-temporal region peak alpha frequencies were significantly negatively correlated with depression severity according to the Beck Depression Inventory (BDI) ($p = 0.01$). Lower alpha frequency may contribute to compromised thalamo-cortical network activity resulting in greater difficulty reversing negative thought patterns or other depressive symptoms.

Disclosures: J.A. Onton: None. **I. Shu:** None. **S.C. Matthews:** None.

Poster

614. Biomarkers in Serious Mental Illness

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: University of Luxembourg -- Institute for Systems Biology Strategic Partnership

NIH Grant GM076547

Title: Risk variants for bipolar disorder influence multiple cellular and molecular mechanisms leading to altered neuronal excitability

Authors: *S. A. AMENT[1], K. ROULEAU[2], J. PEARL[3], C. FUNK[2], M. SHELTON[2], R. GELINAS[2], J. C. ROACH[2], L. HOOD[2], N. D. PRICE[2];
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Abstracts: Very little is known about the genetic and neuronal mechanisms underlying risk for bipolar disorder. We recently discovered through whole-genome sequencing that multiply affected pedigrees with bipolar disorder have an elevated rate of rare coding and non-coding variants in calcium channels and other genes related to neuronal excitability. Here, we test the hypothesis that genomic variation found in bipolar disorder genomes influences neuronal excitability and cellular transcriptomic states. We tested our hypothesis at two levels: (i) by characterizing a novel role in calcium signaling for a top candidate gene, humanin, that had not previously been linked to calcium channel function; and (ii) by characterizing the functional effects of rare calcium channel variants found in bipolar disorder families in cultured neurons. Our approach combines RNA interference, CRISPR/Cas9 genome editing, pharmacology, neurophysiology, and molecular systems biology. We found that humanin influences calcium homeostasis by inhibiting neuroinflammatory cytokines, at least in part via interactions at the level of transcriptional regulation. Some calcium channel variants found in bipolar disorder genomes disrupt protein synthesis; mimicking disrupting variants in the Cav1.2 channel CACNA1C using RNAi suggested that this gene has shared transcriptomic effects with humanin but does not interact with cytokines. Other variants in calcium channels disrupt enhancer and promoter sequences recognized by transcription factors important for neuronal cell type specificity and plasticity. Based on our results and recent findings from other groups we propose that altered neuronal excitability may be a general mechanism underlying risk for bipolar disorder.

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Poster

614. Biomarkers in Serious Mental Illness

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Program#/Poster: 614.20/W34

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: ICM P10-001-F

ICM P09-015-F

FONDEF CA121-10061

Title: Pupillary reactivity in subjects affected by Schizophrenia during free viewing of natural images of different complexity

Authors: C. ACEVEDO[1], K. MUÑOZ[2], S. MADARIAGA[2], R. MAYOL[1], *J. I. EGANA[3,4], P. MALDONADO[1];
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Abstracts: Subjects affected by schizophrenia (SCZ) presents a wide range of dysfunctions in both cognitive and non-cognitive processes. The activity of the Autonomous Nervous System's (ANS) is one of non-cognitive mechanisms reported as altered in SCZ. However, cognitive processes are known to modulate ANS responses in healthy subjects. Most of these reports, both in SCZ and healthy people have used artificial experimental settings. We tested whether ANS activity, measured as pupillary reactivity, is modulated by cognitive load in SCZ. We used an ecological setting using free viewing of natural images. Different properties of pupillary dynamics were evaluated. 20 subjects with DSM-IV-R criteria for SCZ and 11 controls were evaluated. After written informed consent was obtained participants were exposed to a set of 70 natural images of 7 different visual complexities. All sets of images exhibited same luminance distribution. Images were displayed in a 21" screen located at 57 cm (40ºx30º visual field). Each image was presented between 4.5-5.5 seconds. Pupillary response was assessed using and EyeLink II/1000 eye-tracking device with a 500 Hz resolution. Participants were instructed to freely explore images. In order to proceed to the next image the subject had to perform a drift correction and press a button. Data was artifact-corrected and Z-score normalized. We found that SCZ group exhibited, in general, a smaller response (less amplitude) given mainly by a more contracted pupil at the start of trials (baseline). Patients also showed more pronounced maximum contractions and delays (time to max. contraction). Dilatation velocity after max contraction (1 sec after max contraction) was faster in the SCZ group and then slows down to catch-up with the control group by the end of the trial. When different images categories were, we found that complex images enhanced the differences in max contraction, delay and dilatation velocity between SCZ and control groups. When 500 ms before image start were considered (button pressing), two phenomena were observed. First, the baseline pupil diameter was the same for

SCZ and healthy control groups. Second, the dilatation velocity in control was faster so that at the start of image presentation baselines are separated. Taken together these findings support the idea that an SCZ patient exhibits both abnormal ANS basal tone and responses. Results also show that cognitive a process modulates ANS functioning in SCZ patients but in an abnormal way that increases differences with the control population. It remains open to further explore these and other ANS dysfunctions, as biomarkers of this condition.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH

NARSAD

Title: Blood glutathione predicts cortical glutamate levels and cognitive functions

Authors: *T. TANAKA[1], J. M. COUGHLIN[1], A. MARSMAN[2], H. WANG[1], S. BONEKAMP[2], P. K. KIM[1], C. HIGGS[1], S. POSPORELIS[1], M. VARVARIS[1], R. A. E. EDDEN[2], M. POMPER[2], D. SCHRETLEN[1], N. CASCELLA[1], P. B. BARKER[2], A. SAWA[1];
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Abstracts: Aberrant oxidative stress and glutamatergic pathways may underlie the pathophysiology of schizophrenia. In this study, we measured the level of total glutathione (GSH), a marker of oxidative stress, in plasma and lymphoblasts of patients with schizophrenia (SZ) and age-matched, healthy controls (HC). We also examined the levels of glutathione and Glx (the sum of glutamate and glutamine) in anterior cingulate cortex (ACC), expressed as ratios to creatine (Cr), using 3T brain magnetic resonance spectroscopy (MRS). In addition, we systematically assessed cognitive function in all study participants. First, we observed significant reduction in the levels of total GSH in plasma and lymphoblasts of patients with SZ compared with those levels in HC. Total GSH in plasma showed significantly positive correlation with the Glx/Cr in the ACC after adjusting for age and smoking status. Furthermore, the reduction in

peripheral GSH correlated with specific deficits in cognitive function, namely those within the domain of processing speed. These results suggest that blood measurement of GSH may be useful in predicting levels of brain chemicals in metabolically-related pathways (in particular glutamate) and neuropsychological deficits associated with SZ. We are further investigating the potential of GSH as a peripheral marker of the disease. In contrast, we did not observe a significant difference in ACC GSH/Cr ratios between SZ and control groups, and ACC GSH/Cr did not correlate with the levels of blood GSH. We conclude that (1) peripheral GSH is a promising candidate biomarker for key endophenotypes relevant to SZ, and (2) biochemical assays currently provide more reliable GSH measure than MRS at low magnetic field (e.g., 3T).

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Poster

614. Biomarkers in Serious Mental Illness

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 31-116689

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51AU40_125759

Title: Evaluation of redox dysregulation in the pathology of schizophrenia using induced pluripotent stem cell technology

Authors: *B. GIANGRECO[1], P. STEULLET[1], J.-H. CABUNGCAL[1], L. BARTESAGHI[2], N. TONI[3], R. CHRAST[2], K. Q. DO[1];
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Abstracts: Background: Schizophrenia (SZ) is a disorder that involves genetic and environmental factors. A decrease of glutathione (GSH), a major cellular antioxidant, was shown in patient's brain and CSF. Furthermore, polymorphisms in the key synthetizing enzyme for

GSH were found associated with the disease. These observations lead to the hypothesis that redox dysregulation is a main hub in this disorder. In this study, we set up a method based on fluorescence imaging to identify the redox state of thiol residues in a GSH deficient mouse model (Gclm-/-). Our long-term objective is to use induced pluripotent stem cells (iPSC) to examine the impact of oxidative stress on neurons derived from a well-characterized cohort of SZ patients. Methods: We established the conditions for thiol labelling by fluorescence in WT mice brain slices and evaluated its sensitivity. Then, we investigated redox state of cells in WT and GBR-treated Gclm-/-mice, GBR being a dopamine reuptake inhibitor that induces additional oxidative stress. In parallel, we have started to generate iPSC from patient's fibroblasts and to derive them into neurons. Results: The ratio between oxidized and reduced thiols was increased in GBR-treated Gclm-/-compared to WT mice, suggesting a more oxidized cellular environment. This ratio will be measured in iPSC-derived neurons from patient's fibroblasts that we are currently producing. Conclusions: This method together with other approaches will allow to assess whether the redox state is also altered in iPSC-derived neurons from patients. Ultimately, application of this method to iPSC may pave the way to individualized therapies.

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Poster

614. Biomarkers in Serious Mental Illness

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Brain and Behavior Research Foundation

Title: Self-disturbances in schizophrenia: Models of hippocampal-dopamine interactions and their phenomenological correlates

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Abstracts: Background: Human-self is an important but elusive topic in neuroscience and its clinical application. Schneider (1959) described self-disturbances (Ichstörungen) as belonging to the first-rank symptoms and therefore pathognomic of schizophrenia, e.g., thought-withdrawal, thought-insertion, somatic-passivity, delusions of reference, mental/motor automatisms, and

break-down of unitary self-experience (Mishara et al., 2014). Nevertheless, whether self-disturbances are heterogeneous, specific to schizophrenia, and the underlying neurobiological mechanisms remain unclear (Mishara et al., 2014). Conrad subsequently found disrupted self-experience in early-schizophrenia: 1) random occurrences are seen as having special meaning directed towards self; 2) delusions spread from a few salient-events to ever greater scope of the patient's experience related back to the self; 3) the self is experienced as passive center-point of the Ichstörungen (Mishara, 2010). These observations provide a useful heuristic for considering plausible neurobiological mechanisms. Methods: Recent neurobiological-models of hippocampal dysfunction in psychotic-symptoms including Ichstörungen, were examined to see to what extent they could be mapped onto the phenomenology: 1) positive feedback in a thalamus-hippocampus-VTA circuit acts as trigger when NMDA-receptor deficiency is combined with a transient increase in dopamine release (Lisman); 2) Diminished Pattern-Separation/Increased Pattern-Completion (Tamminga, Stan and Wagner); 3) Aberrant salience mediated by dopaminergic dysfunction disrupts encoding of the delusional memories (Mishara and Fusar-Poli, 2013). Results: Each model provides plausible explanations for some Ichstörung-symptoms. However, the roles of neuroplasticity mechanisms, dopamine and antipsychotic medications in reducing symptom-phenomenology and improving memory performance in schizophrenia patients remain largely undetermined. Conclusions: Phenomenologic-analysis of self-disturbances in beginning-schizophrenia provides an initial step for developing neurobiological models, which implicate hippocampal dysfunction, and help explain and treat these disturbances. Mishara, AL (2010). Klaus Conrad (1905-1961): Delusional mood, psychosis and beginning schizophrenia. Schizophrenia Bulletin 36, 9-13. Mishara AL, Fusar-Poli P (2013) The phenomenology and neurobiology of delusions. Schizophrenia Bulletin 39, 278-86. Mishara AL, Lysaker PH, Schwartz MA (2014) Self-disturbances in schizophrenia. Schizophrenia Bulletin 40, 5-12.

Disclosures: A.L. Mishara: None.

Poster

614. Biomarkers in Serious Mental Illness

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH R01 EB009666

Title: Resting-state brain networks predict severity of auditory hallucinations in schizophrenia

Authors: E. S. FINN[1], F. TOKOGLU[2], X. SHEN[2], R. E. HOFFMAN[3], *R. T. CONSTABLE[2]; [1]Interdepartmental Neurosci. Program, [2]Dept Diagnos. Radiol, Yale Univ., New Haven, CT; [3]Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstracts: Background: Auditory verbal hallucinations (AVHs), or "hearing voices," are a prominent symptom of schizophrenia. We sought to identify biomarkers of various dimensions of AVH severity using functional connectivity analysis of resting-state fMRI data. **Methods:** Resting-state fMRI scans were acquired from hallucinating patients (HP, n = 28), non-hallucinating patients (NHP, n = 21), and healthy controls (C, n = 21). The two patient groups were matched on important demographic and clinical variables. Each patient received a clinical rating using the auditory hallucination rating scale (AHRS), a composite AVH scale that includes frequency, loudness, length, realness, number of voices, distress associated with voices, and degree to which voices alter actions and thoughts. To conduct connectivity analysis, a functional brain parcellation consisting of 162 regions was used to define network nodes across the whole brain. Matrices of correlation coefficients ("edges") between the timecourses of each pair of nodes were calculated for each subject, and the network-based statistic--a spatial correction algorithm yielding network results that are both biologically and statistically valid--was used to identify networks of fully connected edges whose strength differed significantly between groups. The two main contrasts of interest were HP vs NHP and HP vs C. Having identified group-difference networks, we then performed within-group correlations to relate connectivity strength in these networks to measures of AVH severity. **Results:** Overall, the HP group showed higher connectivity relative to the NHP group in a network of approximately 200 edges. Nodes with higher connectivity for HPs included the left inferior parietal lobule, right supplementary motor area, left superior temporal gyrus (STG) and left anterior cingulate cortex. Summing values of all edges within the HP > NHP network in individual HPs revealed that increased connectivity in this network predicted a higher total AHRS score ($r = 0.47$, $p = 0.01$), and especially the subscore for number of voices ($r = 0.57$, $p = 0.002$). The HP vs C contrast revealed differential connectivity patterns for left and right thalamus, primary sensorimotor and supplementary motor cortices, left posterior cingulate cortex, and right caudate, among other nodes. Interestingly, connectivity between the thalamus and left STG--a classic language region, and one that has been previously implicated in AVHs--was higher in HPs than Cs, yet *negatively* predicted AHRS score ($r = -0.57$, $p = 0.002$), suggesting that increased connectivity between these regions might reflect a compensatory mechanism in patients.

Disclosures: E.S. Finn: None. **R.E. Hoffman:** None. **R.T. Constable:** None. **F. Tokoglu:** None. **X. Shen:** None.

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.25/X3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: US Navy Bureau of Medicine and Surgery

Title: The development of a computerized neurocognitive test as an objective functional biomarker of emotional distress

Authors: L. A. KING[1], *E. B. ROACH[2], C. E. LATHAN[3], J. L. SPIRA[1];
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Abstracts: We examined the potential for using computerized neurocognitive tests (CNTs) as objective biomarkers of functional impairment in a functioning, non-clinical, military population in order to complement the subjective reporting of post-deployment distress symptoms. Due to difficulties in utilizing traditional neuropsychological assessments, there is a paucity of research on neurocognitive functioning in military members with post-deployment emotional distress. CNTs that use precise speed and accuracy measures hold the promise of being more sensitive than traditional paper and pencil tests and are more easily utilized in a variety of healthcare settings. The Defense Automated Neurobehavioral Assessment (DANA) was used to assess neurobehavioral functioning in 646 United States Marines, all of whom were fit for duty and currently functioning in their assigned duties. This research aimed to determine if neurocognitive functioning was associated with emotional and somatic distress in this sample of combat veterans. The marines were assessed for concussion history, postconcussive symptoms, emotional distress, neurocognitive functioning, and deployment history. Differences in those with and without significant symptoms of PTSD, Depression, Insomnia, and Anger were compared across seven neurocognitive tests, and sensitivity and specificity were also determined. Medium to small effect sizes were found on cognitive performance for all psychological factors. Marines with elevated PTSD scores had significantly slower and less accurate performance in Simple Reaction Time (SRT), Choice Reaction Time (CRT), Go-No/Go, Spatial Discrimination, and Symbol-Digit Delayed Memory, and decreased accuracy in Running Memory (all $p<.005$ with moderate effect sizes); elevated Depression and Anger scores were associated with similar findings (Depression: all but Choice RT, $p<.05$ with moderately small effect sizes; Anger: all but

Spatial Discrimination p<.05, moderate- small effect size). The DANA Attentional-Discrimination Composite Score was positive for PTSD, Depression and Anger (p<.05 with a small effect size). A receiver operator curve (ROC) analysis showed that the DANA Composite Score has adequate sensitivity and specificity for PTSD, Depression, and Anger (p<.05, Sensitivity and Specificity all >.80). These results suggest that the DANA NCT is a reliable, sensitive, and objective measure of the cognitive sequelae of emotional distress that has the potential for being utilized by clinicians for initial diagnosis, treatment disposition, and potentially assessing improvement and return to work determinations.

Disclosures: L.A. King: None. **E.B. Roach:** None. **C.E. Lathan:** F. Consulting Fees (e.g., advisory boards); AnthroTronix Inc. **J.L. Spira:** F. Consulting Fees (e.g., advisory boards); AnthroTronix Inc..

Poster

614. Biomarkers in Serious Mental Illness

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 614.26/X4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH 5R01MH093398-03

Title: An automated toolkit to generate Cerebral Blood Volume (CBV) maps of the hippocampal circuit applied to prodromal schizophrenia

Authors: *F. A. PROVENZANO[1], R. R. GIRGIS[3], N. BRUNO[1], U. A. KHAN[1], J. A. LIEBERMAN[2], S. A. SMALL[1];
[1]Taub Inst., [2]Dept. of Psychiatry, Columbia Univ., New York, NY; [3]New York State Psychiatric Inst., New York, NY

Abstracts: Introduction. Using Cerebral Blood Volume (CBV) fMRI, we have previously reported that selective hypermetabolism in the anterior CA1 region of the hippocampal circuit occurs in prodromal stages of schizophrenia and related psychotic disorders. To date, a manual approach was used to generate CBV maps of the hippocampal circuit. This approach is time consuming and introduces sources of noise. To address these limitations, over the last few years we have been developing and optimized an analytic toolkit that generates CBV maps of the hippocampal circuit in an automated fashion. Here, we unveil this toolkit and test its ability to detect anterior CA1 hypermetabolism in prodromal stages of disease. Methods. Recruited

patients (N=19) and controls (N=16) underwent a series of MRI scans, including a structural T1-weighted image (1x1x1 mm) as well as two T1-weighted scans (.68x.68x3 mm), acquired in the coronal plane along the long axis of the hippocampus prior to and after a bolus injection of a gadolinium based contrast agent. A template was created using the pre-contrast scans of these patients and a trained anatomist drew regions of interest of the hippocampus, including the anterior, middle and posterior left CA1. Our software was able to generate CBV maps for each patient and apply the template drawn ROIs to template co-registered CBV. Mean value differences reveal a significant increase in CBV signal, concordant with extant literature, in the prodromal group versus the age matched controls. Discussion. By detected anterior CA1 hypermetabolism, this study validates the capabilities of this automated toolkit. Achieving this goal is important as CBV-fMRI is being applied in different research centers, investigating a range of disorders that target the hippocampal circuit. Additionally, an automated toolkit is required if CBV-fMRI will turn out to become a biomarker of prodromal schizophrenia, as we are currently testing, and potentially used in clinical trials.

Disclosures: F.A. Provenzano: None. **R.R. Girgis:** None. **N. Bruno:** None. **U.A. Khan:** None. **J.A. Lieberman:** None. **S.A. Small:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.01/X5

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Development of real-time fMRI neurofeedback attention training for depression

Authors: *D. M. SCHNYER[1], M. T. DEBETTENCOURT[2], C. G. BEEVERS[1], S. SHERMAN[1], J. D. COHEN[2], K. A. NORMAN[2], N. B. TURK-BORWNE[2];
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Abstracts: We applied a real-time fMRI attention-training (rtAT) intervention to help depressed individuals improve attentional control over negative information. Poor regulatory control over negative cognition is a central feature of the disorder. The rtAT task used a modified sustained attention to response task (SART) and multivoxel pattern analysis (MVPA) in real-time to provide timely feedback about moment-to-moment fluctuations in attentional states. Seven adults with high levels of depressive symptoms were trained across 3 days. Behavioral performance was measured in five separate sessions: a pre-training session, three fMRI neurofeedback

sessions, and a post-training session. Resting state fMRI was collected before and after rtAT training. Performance (d') in a pre-training laboratory-based SART task was associated with a standard measure of attentional bias for negative stimuli. Individuals with greater attention bias for negative stimuli (measured with eye tracking in a standard dot probe task) had poorer performance on the SART with sad face distracters (r = -.45) and attention bias for sad faces was uncorrelated with performance with neutral face distracters (r = -.02). Sustained attention improved over the course of 3 rtAT sessions and 5 of the 7 participants experienced significant improvement from the pre-training to post-training laboratory based assessments. Over the course of 4 weeks (rtAT plus weekly follow-up for three weeks), we observed a significant decline in depression symptoms and decreases in depression over time were strongly correlated with improved SART task performance over the same time period (r = .50). Across participants there was a relationship between the severity of the depression and the behavioral improvements observed in the task, such that participants who showed higher levels of depression improved more. Despite the small sample size, from pre to post training, all participants revealed increased resting state functional connectivity between the right middle frontal gyrus and left and right supramarginal gyrus - a key portion of a previously identified attention control network (ACN). These pilot data indicate that real-time attention training shows high promise for altering the neural networks that support the control of attention to affective stimuli. With only three sessions of rtAT we are seeing symptom improvement, reductions in attention bias, and functional connectivity changes within the ACN. Ongoing work will add additional patients and an appropriate control group.

Disclosures: D.M. Schnyer: None. **M.T. deBettencourt:** None. **C.G. Beevers:** None. **S. Sherman:** None. **J.D. Cohen:** None. **K.A. Norman:** None. **N.B. Turk-Borwne:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.02/X6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH085734

NIH Grant MH097978

NARSAD Young Investigator Award

VA Merit Grant

Swedish Research Council 350-2012-303

Swedish Society of Medicine SLS-244671

Sweden American Association

Title: Emotion-dependent functional connectivity of the default mode network in adolescent depression

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Abstracts: Functional magnetic resonance imaging (fMRI) research suggests that both adult and adolescent major depressive disorder (MDD) is marked by aberrant connectivity of the default mode network (DMN) during resting-state (Greicius, 2008; Connolly et al., 2013). However, no studies to date have examined the influence of emotional processing on DMN pathology in adolescent depression. Here, we collected fMRI data from 27 medication-free adolescents (13-17 years) with MDD and 37 well-matched healthy controls (HCL) during an emotional identification task and also during rest. Emotion-dependent and resting-state functional connectivity analyses were assessed using functionally defined seeds of the primary nodes of the DMN: medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC). MDD showed comparatively elevated emotion-dependent functional connectivity between mPFC and PCC with regions in the default mode (precuneus), cognitive executive (cingulate gyrus, inferior parietal lobule), and salience and affective (striatum/subcallosal cingulate gyrus) networks (Figure 1). Furthermore, PCC-subcallosal cingulate connectivity remained elevated in MDD compared to HCL during rest ($F(1,54)=5.410$, $p=0.023$). Lastly, emotion-dependent functional connectivity patterns significantly correlated with depression severity ($r=0.436$, $p=0.029$; $r=0.483$, $p=0.014$) and age of depression onset ($r=-0.527$, $p=0.014$). Together, our findings suggest that depression may be characterized by non-adaptive functional coupling of brain networks that reflect fundamental differences in processing internal and external environments. Given that our study sample of depressed adolescents is free from possible confounds of chronic illness and medication usage, these patterns point to possible neural substrates underlying the cognitive processes initiating and maintaining depression and offer potential insight into how depression may alter the normal development of intrinsic brain networks.

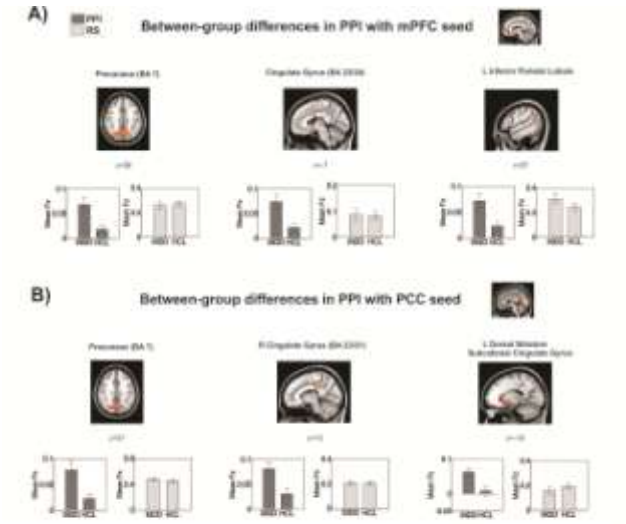

Figure 1. Regions showing significant between-group differences in emotion-dependent functional connectivity as measured using the psychophysiological interaction (PPI) method. Results are shown for the medial prefrontal cortex (mPFC) seed **(A)** and posterior cingulate cortex (PCC) seed **(B)**. All areas survived correction for multiple comparisons at a cluster-wise threshold of $p<0.05$. Mean functional connectivity values are reported as Fisher's z-scores (Fz). Mean resting-state (RS) Fz were extracted from the regions exhibiting significant between-group differences on the PPI analysis. Locations are reported in MNI coordinates (radiological convention).

Disclosures: T.C. Ho: None. **C.G. Connolly:** None. **J. Wu:** None. **M. Chan:** None. **K.Z. LeWinn:** None. **E. Henje Blom:** None. **A.N. Simmons:** None. **T. Yang:** None.

Poster

615. Depression Biology

Location: Halls A-C

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Program#/Poster: 615.03/X7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: a Health and Labor Science Research Grant from the Japanese Ministry of Health, Labor and welfare

SENSHIN Medical Research Foundation

Title: Blood diagnostic biomarkers for major depressive disorder using dna methylation profiles

Authors: *S. NUMATA[1], K. ISHII[2], A. TAJIMA[3], J.-I. IGA[1], M. KINOSHITA[1], S. WATANABE[1], H. UMEHARA[1], M. FUCHIKAMI[4], S. OKADA[4], S. SHIMODERA[5], I. IMOTO[3], S. MORINOBU[5], T. OHMORI[1];

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Abstracts: BACKGROUND: Aberrant DNA methylation in the blood of patients with major depressive disorder (MDD) has been reported in several previous studies. However, no comprehensive studies using medication-free subjects with MDD have been conducted. Furthermore, the majority of these previous studies has been limited to the analysis of the CpG sites in CpG islands (CGIs) in the gene promoter regions. The aim of the present study is to identify DNA methylation markers that distinguish patients with MDD from non-psychiatric controls. **METHODS**: Genome-wide DNA methylation profiling of peripheral leukocytes was conducted in two set of samples, a discovery set (20 medication-free patients with MDD and 19 controls) and a replication set (12 medication-free patients with MDD and 12 controls), using Infinium HumanMethylation450 BeadChips. **RESULTS**: Significant diagnostic differences in DNA methylation were observed at 363 CpG sites in the discovery set. We were able to distinguish patients with MDD from the control subjects with a sensitivity of 100% and a specificity of 100% in the discriminant analysis using the DNA methylation markers. We validated these selected DNA methylation markers with 100% accuracy in the replication set. **CONCLUSION**: Our results indicate that multiplex DNA methylation markers may be useful for supporting the diagnosis of MDD.

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Poster

615. Depression Biology

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Program#/Poster: 615.04/X8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH Grant MH067234

NIMH Grant MH079299

NIMH Grant MH01741

NIMH Grant MH52339

NIMH Grant MH073630

Title: Dermal fibroblasts bring molecular insights into Major Depressive Disorder

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Abstracts: Understanding the biological bases for Major Depressive Disorder (MDD) may be facilitated by the use of peripheral tissue. Primary dermal fibroblasts are easy to obtain and propagate, are not immortalized, and most importantly, after several cell divisions in controlled cell culture conditions, the effects of patients' environment and medication use are practically eliminated. In addition, fibroblast cultures allow us to study the cellular response to various challenges, which are particularly valuable for uncovering the disturbed molecular processes related to the disease. Primary fibroblast cultures were obtained from skin biopsies of subjects with MDD and matched healthy controls (n=16). Cultures were maintained in standard (STD) medium. Metabolic stress was evoked by glucose substitution with galactose (GAL) and with lipid reduced (RL) media. In all conditions mRNA and miRNA expression levels were simultaneously measured by gene expression microarrays and qPCR. In STD conditions fibroblasts from MDD patients, when compared to matched controls (CNTR), revealed a strong disease-associated mRNA expression pattern of genes related to molecular pathways involved in cell communication, immunity and cell proliferation. Furthermore, the MDD-associated miRNA expression signature appeared to be functionally connected with the mRNA expression pattern. Next, to evaluate the response of MDD and CNTR fibroblasts to a metabolic stress (and reveal molecular deficits that are not obvious under resting conditions), we compared the mRNA/miRNA profile of MDD and CNTR cells grown in galactose and reduced lipids media. Culturing mammalian cells in GAL is a mitochondrial oxidative phosphorylation challenge, while RL exposure strongly engages de novo lipid biosynthesis. GAL challenge in MDD patients resulted in enrichment of molecular pathways involved in cell cycle regulation, apoptosis, cell survival and inflammation. RL exposure in MDD caused increased expression of genes participating in cell survival, migration, proliferation, and regulation of metabolism, but also decreased expression in pathways regulating energy production. Many of these disturbed processes have been also previously observed in postmortem brain tissue of subjects with MDD. From these experiments we conclude that dermal fibroblasts are useful and informative biomaterial for studying psychiatric disorders. Furthermore, it appears that challenging patient

cells with stressors is essential for revealing the underlying molecular deficits that characterize the disorder.

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Poster

615. Depression Biology

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Program#/Poster: 615.05/X9

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: FAPERGS

CNPq

CAPES

Title: Genetic variation in adenosine A2A receptor gene (rs2298383) is associated with peripheral levels of TNF-α and major depression in women

Authors: *M. P. KASTER[1,2], M. GAZAL[2], F. N. KAUFMANN[2], K. JANSEN[2], J. P. OSES[2], L. D. M. SOUZA[2], R. A. SILVA[2], D. C. MOREIRA[3,4], D. R. LARA[4], G. C. GHISLENI[2,3]; [1]Dept. de Bioquímica, Univ. Federal De Santa Catarina, Florianópolis, Brazil; [2]Life and Hlth. Sci., Univ. Católica de Pelotas, Pelotas, Brazil; [3]Hosp. de Clínicas de Porto Alegre, Univ. Federal do Rio Grande do Sul, Porto alegre, Brazil; [4]Pontifícia Univ. Católica do Rio Grande do Sul, Porto Alegre, Brazil

Abstracts: Introduction: Major depressive disorder (MDD) has been a growing public health concern estimated to become the second leading cause of disability by the year 2020. Recently, polymorphisms in genes involved in adenosine metabolism and adenosine receptors were associated with vulnerability to psychiatric disorders including depression (Biochim. Biophys. Acta. 1808:1380-99, 2011). Objective: The aim of our work is to identify a possible association between the 23155511C/T single nucleotide polymorphism (SNP) in exon 1 of the adenosine A2A receptor gene, ADORA2A (rs2298383), located in a potential promoter region, peripheral inflammatory cytokines (TNF-α, IL-1β and IL-6) and MDD in a southern Brazilian population. Methods: This work is part of a population-based study including 750 subjects (18 to 24 year-old) from the urban area of Pelotas, RS (Brazil). MDD diagnosis was made with the Mini

International Neuropsychiatric Interview 5.0. DNA was extracted from peripheral leucocytes and genotyping was performed using Real Time PCR. Serum cytokines were evaluated by ELISA. Results: Of the 750 subjects evaluated, we found 256 with MDD. Most of the MDD subjects were women (54%), caucasian (78.9%) and with a low use of psychiatric medication (8.3%). No differences were detected according to diagnosis and genotypic distribution ($\chi 2=0.211$) or levels of TNF-α (control: 104.90±15.41 vs MDD: 135.71±25.94 pg/mL, p=0.278), IL-1β (control: 9.09±1.77 vs MDD: 15.40±4.74 pg/mL, p=0.14) or IL-6 (control: 20.17±2.36 vs MDD: 18.15±1.25 pg/mL, p=0.45). However, after stratification by gender we observed an association between carriers of the T allele (C/T homo- and T/T heterozygotes) and MDD in women ($p<0.05$, Pearson's chi-squared test). In addition, there was a tendency to increased TNF-α levels in MDD women vs control (63.72±16.50 and 135.71±25.94 pg/mL, respectively, p=0.08, using Student' t test). No changes were observed IL-1β and IL-6 levels in women according to diagnosis. In addition, two-way ANOVA revealed significant differences for the interaction between genotype and diagnosis in the levels of TNF-α ($p<0.05$), but not IL-1β (p=0.56) and IL-6 (p=0.36). The post-hoc analysis indicated that in healthy control women the levels TNF-α were similar according to the genotype (CC: 60.05±42.90 pg/mL, vs CT/TT 77.75±23.64). However, the levels of TNF-α were significantly higher in MDD women with the CC genotype (241.32±50.15 pg/mL) when compared CT/TT genotypes (91.95±30.52 pg/mL). Conclusion: We concluded that there is an association between allele T of the ADORA2A SNP, lower levels of TNF-α and decreased risk for MDD in women.

Disclosures: **M.P. Kaster:** None. **M. Gazal:** None. **F.N. Kaufmann:** None. **K. Jansen:** None. **J.P. Oses:** None. **L.D.M. Souza:** None. **R.A. Silva:** None. **G.C. Ghisleni:** None. **D.C. Moreira:** None. **D.R. Lara:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.06/X10

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

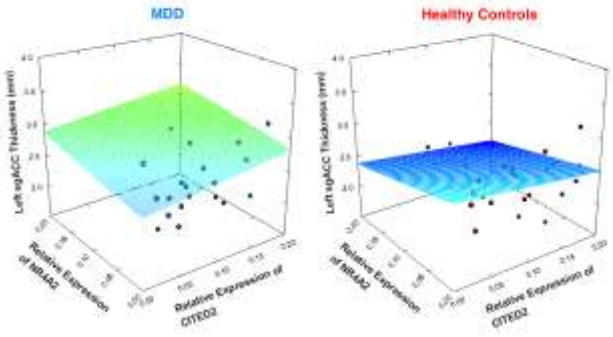
Support: NIH Grant MH098099

Title: The inflammation-related gene CITED2 modulates the relationship between the dopamine-related gene NR4A2 and subgenual anterior cingulate cortical thickness in Major Depressive Disorder

Authors: *H. SUZUKI[1], J. MARINO[2], K. TEAGUE[2,3,4], M. MISAKI[1], T. VICTOR[1], J. SAVITZ[1,5], B. MCKINNEY[6], P. BELLGOWAN[1,5], W. DREVETS[1,7], J. BODURKA[1,8]; [1]Laureate Inst. For Brain Res., TULSA, OK; [2]Surgery, Univ. of Oklahoma Sch. of Community Med., TULSA, OK; [3]Pharmaceut. Sci., Univ. of Oklahoma Col. of Pharm., Tulsa, OK; [4]Biochem. and Microbiology, Oklahoma State Univ. Ctr. for the Hlth. Sci., Tulsa, OK; [5]Community Med., The Univ. of Tulsa, Tulsa, OK; [6]Mathematical and Computer Sci., The Univ. of Tulsa, TULSA, OK; [7]Janssen Pharmaceuticals, LLC, Johnson & Johnson, Inc., Titusville, NJ; [8]Univ. of Oklahoma Col. of Engin., Norman, OK

Abstracts: Chronic stress dysregulates neuroimmune balance facilitating our stress adaptation and leaves brain structure vulnerable to disorders. In Major Depressive Disorder (MDD), anhedonia has been separately associated with structural changes in the subgenual anterior cingulate cortex (sgACC), mesolimbic dopaminergic system, and innate immune system. This study examined neuroimmune interactions between MDD status and expression of two genes previously implicated in MDD, the dopamine-neuron trophic gene, NR4A2, and neuroinflammation-related gene, CITED2, on sgACC thickness. Twenty unmedicated adults with MDD (14 females, mean age = 38) and 25 healthy adults (18 females, mean age = 31) underwent blood sampling and MR imaging (3T GE MR750 scanner, including a structural T1-weighted scan). Monocyte RNA was extracted and reverse transcribed into complementary DNA; quantitative real-time polymerase chain reactions were used to determine NR4A2 and CITED2 relative transcript abundance. FreeSurfer was used to segment the bilateral subcallosal cortex (posterior sgACC), and mean cortical thickness was measured. Hierarchical regressions were performed to predict sgACC thickness: MDD status, NR4A2 levels, and CITED2 levels in the first model, all possible two-way interactions in the second model, and a three-way interaction in the third model. Age, gender, and body mass index were covaried in all models. The first and second models did not indicate any main effect or two-way interaction. However, the third model showed that the three-way interaction predicted left sgACC thickness significantly ($\beta = .85$, $t(34) = 2.75$, $p < .01$) and right marginally ($\beta = .67$, $t(34) = 1.91$, $p = .064$). A follow-up regression within each group revealed that reduced left sgACC thickness was associated with decreased transcript abundance of NR4A2 and CITED2 in MDD ($\beta = 2.75$, $t(16) = 2.51$, $p < .05$), but not healthy controls (see Fig. 1). These findings provided insights into the neuroimmune interactions in MDD, such that CITED2 expression modulates the effect of NR4A2 on the sgACC in MDD.

Fig. 1: A 3-D scatter plot with a linear surface representing the relationship between left sgACC thickness, NR4A2, and CITED2 in MDD and healthy controls



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Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.07/X11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: the National Basic Research Program of China (No2011CB505101)

the key lab open project of Beijing University of Chinese Medicine (2011-SYSKFKT03)

the Shangshan funding

Title: The emotion and brain wave activity to stress for women with premenstrual syndrome

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Abstracts: The purpose of the present study was to investigate the relation between electroencephalogram (EEG) rhythm to stress and emotion for women with premenstrual syndrome (PMS). thirty women (18~30 years old, 22±2.19) took part in the study, among them, fifteen women were diagnosed to be PMS group, while the left formed the non-PMS group. The participants received the EEG stress evaluation test (eyes-open resting, eyes-closed resting, attention and cognition tests) and filled in the positive affect and negative affect scale (PANAS) in non-menstrual phase (luteal or follicular phase). The EEG brain wave rhythms (theta, alpha, sensorimotor and low beta rhythms) under stress test were recorded and analyzed. The results showed that compared to non-PMS group, the PMS group had higher negative affect and lower positive affect scores on PANAS. Moreover, the PMS group got stronger alpha rhythm than non-PMS group under the eyes-open resting and attention tests. Finally, under the stress evaluation test, the EEG rhythm was positively correlated to negative affect and negatively correlated to positive affect (Table 1). The results indicated that the PMS females had stronger EEG arousal under stress and this was correlated with their higher negative affect and lower positive affect. The emotion variation of PMS females may partly explain their sensitive EEG arousal to stress.
Key words: stress; premenstrual syndrome; EEG rhythm; positive affect; negative affect Table1 The correlations of the scores of positive affect and negative affect scale and EEG rhythms (theta, alpha, SMR and low beta) under different tasks (open eyes resting, closed eyes resting, attention test, and cognition test).

Tasks	**EEG Rhythms**	**Positive Affect**	**Negative Affect**
	Theta	-0.365 (0.048)	0.40 (0.028)
	Alpha	-0.40 (0.028)	
	SMR	-0.378 (0.039)	0.325 (0.080)
	Low Beta	-0.403 (0.027)	0.362 (0.050)
Open eyes resting	Theta	-0.467 (0.009)	
Closed eyes resting	SMR	-0.319 (0.085)	
Attention test	Low Beta	-0.485 (0.007)	0.339 (0.067)
	Theta	-0.464 (0.010)	
	Alpha	-0.358 (0.052)	0.562 (0.001)
	SMR	-0.396 (0.031)	0.440 (0.015)
	Low Beta	-0.373 (0.042)	0.450 (0.013)

Note: The unit of EEG rhythms (theta, alpha, sensorimotor and low beta rhythms) was Hz.

Disclosures: Q. Liu: None. **R. Zhou:** A. Employment/Salary (full or part-time):; Beijing Key Lab of Applied Experimental Psychology, School of Psychology, Beijing Normal University, Beijing 100875, China, State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Center for Collaboration and Innovation in Brain and Learning Sciences, Beijing Normal University,

Research Center of Emotion Regulation, Beijing Normal University, Beijing 100875, China. 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.; the National Basic Research Program of China (No2011CB505101), the key lab open project of Beijing University of Chinese Medicine (2011-SYSKFKT03), the Shangshan funding. **W. Chen:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.08/X12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH MH048153

NIH MH098554

Title: Increased Toll-like receptor 2 and 6 protein expression in the depressed suicide brain

Authors: *X. REN, H. S. RIZAVI, G. N. PANDEY;
Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstracts: Abnormalities of the immune function in depression and suicide are based in part on the observation of increased levels of proinflammatory cytokines in the serum and in postmortem brain of depressed and suicidal patients. Several studies suggest dysregulation of the immune system in suicide as increased microgliosis has been reported in postmortem brain of suicide subjects and increased levels of proinflammatory cytokines in the CSF of suicidal patients. This observed abnormality of cytokines in suicide may be related to altered innate immune receptors known as Toll-like receptors (TLRs). In a recent study we reported a significant increase in the protein and mRNA levels of TLR3 and TLR4. To further examine the role of TLR in suicide we have now studied the expression of TLR2, TLR5, and TLR6 in depressed suicide subjects. We determined the protein expression of TLR2, TLR5, and TLR6 in the PFC of 24 depressed suicide victim and 24 normal control subjects. The postmortem brain tissues were obtained from the Maryland Brain Collection and the psychological autopsies were performed for the diagnosis of the subjects using DSM-IV-SCID. Protein expression was determined using Western blot technique. When we compared the protein expression of TLR2, we found that the protein expression of TLR2 was significantly increased in depressed suicide victims compared with

normal control subjects, while there was no difference in TLR5 protein expression in depressed suicide victims compared with normal control subjects. The protein expression of TLR6 was also significantly increased in the PFC of depressed suicide subjects compared with normal controls. These results suggest that overexpression of TLR2 and TLR6 protein may be in part related to the abnormalities of proinflammatory cytokines in the brain of suicide victims and that abnormalities of innate immunity are associated with suicide.

Disclosures: X. Ren: None. **H.S. Rizavi:** None. **G.N. Pandey:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.09/X13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH MH56528

Title: Altered gene expression of proinflammatory cytokines and their receptors in the lymphocyte of depressed patients

Authors: *H. ZHANG[1], H. S. RIZAVI[2], X. REN[2], G. N. PANDEY[2];
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Abstracts: Abnormalities of the immune function have been implicated in the pathophysiology of depression. This is primarily based on the observation that treating cancer patients with a cytokine interferon(IFN)-α causes depression-like symptoms and that protein levels of proinflammatory cytokines and their soluble receptors are increased in the serum of depressed patients. The soluble receptors are derived from proteolytic degradation of membrane-bound cytokine receptors, which are involved in signal transduction and mediate the functional and biological effects of cytokines. Although soluble cytokine receptors have been studied in depression, to our knowledge membrane-bound cytokine receptors were not studied in depression or other psychiatric disorders. To examine if major depressive disorder (MDD) is also associated with abnormal gene expression of cytokines and their receptors, we determined mRNA expression of proinflammatory cytokines and their receptors in the lymphocytes of drug-free MDD patients and normal control subjects. We determined the protein and mRNA expression of proinflammatory cytokines, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, and mRNA expression of their receptors IL-1R1, IL-1R2, IL-1R antagonist (IL-1RA),

IL-6R, glycoprotein (Gp)130, TNFR1, and TNFR2 in the lymphocytes from 26 drug-free, hospitalized MDD patients and 29 drug-free normal control (NC) subjects. The subjects were diagnosed according to DSM-IV criteria. Protein levels of cytokines were determined by ELISA, and mRNA levels of cytokines and cytokine receptors in lymphocytes were determined by the qPCR method. We found that the mean mRNA levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α were significantly increased in the lymphocytes of MDD patients compared with normal controls. The protein levels of the cytokines IL-1β, IL-6, and TNF-α were also significantly increased in the plasma of MDD patients compared with normal controls. The mRNA levels of IL-1R1, IL-1RA, TNFR1, and TNFR2 were significantly increased in the lymphocytes of MDD patients compared with normal controls. No significant differences were observed in mRNA levels of IL-1R2, IL-6R, or Gp130 between MDD patients and normal controls. These studies suggest that the reported abnormalities of cytokines and their soluble receptors observed in the plasma of MDD patients may be related to an abnormal gene expression of these cytokines and their receptors in the lymphocytes of MDD patients, and that their mRNA expression levels in the lymphocytes could be a useful biomarker for depression. To our knowledge, this is the first study of cytokine receptors in depressed patients.

Disclosures: H. Zhang: None. **H.S. Rizavi:** None. **X. Ren:** None. **G.N. Pandey:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.10/X14

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Israel Ministry of Health Chief Scientist grant

Herman Dana Foundation.

Title: Genome scale mononuclear cell gene transcriptional reactivity among Methylphenidate responders

Authors: *T. GOLTSER[1], E. GALILI-WEISSTUB[2], G. BODENHEIMER[3], A. MELTZER[3], A. SHARON[3], R. GIESSER[3], L. KALMAN[3], A. SHALEV[3], L. CENETTI[4], R. SEGMAN[4]; [1]Hadassah Hebrew U, Jerusalem, Israel; [2]The Herman-Danna Div. of Pediatric Psychiatry, Dept. of Psychiatry, Hadassah - Hebrew Univ. Med. Center; Jerusalem Israel, J, Israel; [3]The Herman-Danna Div. of Pediatric Psychiatry, Dept. of Psychiatry, Hadassah - Hebrew Univ. Med. Center;

Jerusalem Israel, Jerusalem, Israel; [4]Mol. Psychiatry Lab. - Dept. of Psychiatry, Hadassah - Hebrew Univ. Med. center, Jerusalem, Israel, Jerusalem, Israel

Abstracts: Background: Application of genomes scale tools to study stimulant drug action is mostly limited to animal models due to lack of timely access to relevant neural cells in humans. Methods: Drug naive children with Attention Deficit Hyperactivity Disorder (ADHD) underwent a standardized prospective assessment of medication treatment response. Genome scale peripheral blood mononuclear cell (PBMC) expression reactivity was compared before and after acute (2 hours) and sub acute (2 weeks) MPH exposure, and correlated with prospectively documented response. Results: MPH induced PBMC expression changes among responders implicated several informative pathways, and previously described ADHD risk genes. Conclusions: Prospectively documented surrogate mononuclear cell genome scale transcriptional reactivity to MPH among previously drug naïve children with ADHD, may point to selective underlying molecular targets of MPH response.

Disclosures: **T. Goltser:** None. **E. Galili-Weisstub:** None. **G. Bodenheimer:** None. **A. Meltzer:** None. **A. Sharon:** None. **R. Giesser:** None. **L. Kalman:** None. **A. Shalev:** None. **L. Cenetti:** None. **R. Segman:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.11/X15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Serum levels of MMP-3 in mood disorders and schizophrenia: A pilot study

Authors: C. SHIBASAKI[1,2], H. ABE[1,3], M. OKADA-TSUCHIOKA[1], N. KAJITANI[1,3], K. ITAGAKI[1], K. HISAOKA-NAKASHIMA[3], *M. TAKEBAYASHI[1];
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Abstracts: [Background] Matrix metalloproteinases (MMPs) are implicated in remodeling synaptic circuits of neuron and glia in the brain as well as regulating to inflammation and immune system, which suggests a pathophysiology of psychiatric disorders. We have previously reported on two major gelatinases, MMP-9 and MMP-2, suggesting that serum levels of MMP-9

were treatment-specific markers for electroconvulsive therapy (ECT) and that serum levels of MMP-2 were disease-specific markers for mood disorders (Neuroscience 2013). [Purpose] We measured serum levels of MMP-3, a stromelysin, which is known to be involved in inflammatory and immune diseases, in patients with mood disorders and schizophrenia during ECT treatment. [Subject and Methods] This study was performed on depressed (N = 29) and schizophrenic patients (N = 19) following a course of ECT. We also compared the serum levels of these patients to those of healthy controls (N = 40). Serum MMP-3 concentrations were measured by enzyme-linked immunosorbent assay. The clinical severity was assessed using the 17-item Hamilton Rating Score for depression (HRSD) and the Brief Psychiatric Rating Scale (BPRS) for schizophrenia. The ethics Committee of NHO Kure Medical Center approved the study protocol, and all participants provided written consent. [Results] In healthy controls, the serum MMP-3 levels of male were significantly higher compared to those of female (24.4 [SD, 7.9] ng/ml vs 11.4 [SD, 3.7] ng/ml, $p < 0.05$). Serum MMP-3 levels were significantly lower in male patients with both mood disorders (16.8 [SD, 6.9] ng/ml) and schizophrenia (16.1 [SD, 9.4] ng/ml) at baseline before ECT compared to those of male control subjects. MMP-3 levels were not significantly associated with severity of symptoms and dose of psychotropic drugs. In contrast, there were no significant differences in serum levels of MMP-3 between the female patients groups and female control group at baseline. After ECT treatment, MMP-3 levels did not changed significantly across groups. [Conclusion] Our findings suggest that serum MMP-3 levels, without being influenced by ECT, were significantly lower only in males with both mood disorders and schizophrenia. Serum levels of MMP-3 might be a gender-specific marker for psychiatric disorders.

Disclosures: **C. Shibasaki:** None. **H. Abe:** None. **M. Okada-Tsuchioka:** None. **N. Kajitani:** None. **K. Itagaki:** None. **K. Hisaoka-Nakashima:** None. **M. Takebayashi:** None.

Poster

615. Depression Biology

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Program#/Poster: 615.12/X16

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NARSAD independent investigator award

Israel Science Foundation Grant 1563-08

Herman Dana Foundation

Title: Genome scale mononuclear cell expression differences implicate altered immune reactivity during the triggering of post partum depression

Authors: *R. SEGMAN[1], D. HOCHNER-CELNIKIER[2], L. CANETTI[3], E. GALILI-WEISSTUB[4], T. GOLTSER DUBNER[3];
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Abstracts: Background: A clinically significant depressive episode affects 14% of mothers, constituting the most common medical complication after delivery. The underlying mechanisms that trigger post partum depression (PD) among this susceptible minority have not been deciphered. Methods: We compared genome scale transcriptional profiles in peripheral blood mononuclear cells (PBMCs) sampled immediately after delivery, between drug naïve mothers prospectively diagnosed with a post partum onset depressive episode and resilient mothers who did not express depressive symptoms upon 6 months follow up. Results: Applying weighted gene correlation network analysis (WGCNA) and pathway analyses, we found replicated evidence for reduced mononuclear cell transcriptional engagement in cell proliferation and coupled histone synthesis among mothers entering a prolonged depressive episode. Pathway enrichment data suggest perturbed transcriptional reactivity to post partum hormonal changes, exert altered immune regulation among susceptible mothers. Discussion: Distinct mononuclear cell transcriptional changes during the triggering of a persisting PD episode suggest an altered immune reactivity, and point to relevant underlying molecular abnormalities

Disclosures: R. Segman: None. **D. Hochner-Celnikier:** None. **L. Canetti:** None. **E. Galili-Weisstub:** None. **T. Goltser Dubner:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.13/X17

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Brain nerve growth-related gene NTRK2,BDNF is associated with amygdala and orbitofrontal volumes in the human brain and mood: A voxel-based morphometry (VBM) study

Authors: *S. AIZAWA, J. AKIYOSHI, H. HIRAKAWA, K. MASUDA;
Oita Univ. Fac. of Med., Yufu-Shi, Japan

Abstracts: [Introduction] Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors that induce its actions through the neurotrophic tyrosine receptor kinase type 2 (NTRK2). Genetic variation in BDNF and NTRK2 has been associated with major depressive disorder (MDD) and anxiety disorders. These variations also have been correlated with brain variations including regions constantly associated with MDD and anxiety disorder. The aim of our study was to evaluate the association of regional gray matter (GM) volume within the amygdala and other unpredicted regions at the whole-brain level with the BDNF and NTRK2 polymorphism with depressive and anxiety symptoms. [Methods] We genotyped 112 healthy controls with respect to 6 at-risk reference SNPs (rs) of BDNF: rs6265, NTRK2はrs11140800,rs1187286,rs1867283,rs1147198,rs10868235. All participants underwent structural magnetic resonance imaging, and the data was statistically analyzed with voxel-based morphometry (VBM) using Statistical Parametric Mapping (SPM8). We examined the State-Trait Anxiety Inventory (STAI), Profile of Mood States (POMS) and Depression and Anxiety Cognition Scale (DACS), a Japanese psychological questionnaire, to assess automatic thoughts. We also examined Temperature Character Inventory (TCI) and Revised NEO Personality Inventory (NEO-PI-R). [Results] We found that there was a significant difference of Fatigue (F) of POMS, Neuroticism and Openness to experience and Agreeableness of NEO-PI-R, and Harm Avoidance of TCI between major-allele homozygote, heterozygote, and minor-allele homozygote in NTRK2. There was also Anger-Hostility (A-H), Fatigue (F) and Confusion (C) of POMS between major-allele homozygote, heterozygote, and minor-allele homozygote in BDNF. We found significant alterations in amygdala, orbitofrontal volumes with rs1187286 in NTRK2 and in insula with rs6265 in BDNF. [Discussion] The relationship between NTRK2,BDNF SNPs and psychological tests suggest that NTRK2,BDNF are associated with anxiety and depression. These effects of NTRK2,BDNF SNPs on brain morphology provide further evidence of its involvement in the neurobiology of depression and anxiety.

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Poster

615. Depression Biology

Location: Halls A-C

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Program#/Poster: 615.14/X18

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The result of "Integrated Research on Neuropsychiatric Disorders" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Title: Identical blood biomarkers in late-onset major depressive disorder patients and model mice

Authors: *S. MIYATA[1], M. KURACHI[2], N. SAKURAI[1], K. TAKAHASHI[1], H. YAMAGATA[3], K. MATSUO[3], K. NARITA[1], M. FUKUDA[1], Y. ISHIZAKI[2], M. MIKUNI[1]; [1]Gunma University, Psychiatry and Neurosci., Gunma, Japan; [2]Gunma University, Mol. and Cell. Neurobio., Gunma, Japan; [3]Yamaguchi University, Div. of Neuropsychiatry, Yamaguchi, Japan

Abstracts: The absence of objective biomarkers for major depressive disorder (MDD) is a central problem in the diagnosis and treatment. Additionally, the discovery of new antidepressants is desired, but the screening of antidepressant effects has largely relied on traditional behavioral tests in rodents, which have weak validation in human MDD. Here we identified objective and state-dependent biomarkers in blood cells of late-onset MDD patients (i.e., age at onset of MDD over 50 years) and its model mice by cross-matching with their gene expression profiles. Ten patients with MDD (DP), 10 patients with MDD in a remitted state (RM) and 12 healthy controls (HC) were enrolled. Mean ages, sex composition and Mini Mental State Examination score did not differ between the three groups, but the Structured Interview Guide for the Hamilton Depression Rating Scale scores were significantly higher in DP than HC and RM. The age at onset was not different between DP and RM. By the microarray analysis, the expression of 3,066 probes (2,207 up/859 down) was significantly changed in DP vs. HC and RM vs. DP, but not RM vs. HC, indicating that the expression of these probes was state-dependently changed in the blood cells. Next, we generated the animal model of late-onset MDD; ovariectomized (OVX) mice with chronic exposure to unpredictable mild stress (CUMS), which have a pathophysiological similarity to late-onset MDD. OVX+CUMS mice exhibited the depression-like behavior in the forced swimming test and the anxiety-like behavior in the open-field test. The expression of 637 probes (338 up/299 down) was the specific change in the blood cells of OVX+CUMS mice in the microarray analysis. The differentially expressed genes from patients were cross-matched with those in the animal model by reference to each gene symbol, and we found the identical 14 genes. We suggest that these genes in blood cells will be helpful for properly diagnosing late-onset MDD and bridging the gap between animal studies and human clinical trials.

Disclosures: S. Miyata: None. **M. Kurachi:** None. **N. Sakurai:** None. **K. Takahashi:** None. **H. Yamagata:** None. **K. Matsuo:** None. **K. Narita:** None. **M. Fukuda:** None. **Y. Ishizaki:** None. **M. Mikuni:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.15/X19

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NRF Grant 20110018358

BK21+ program of Ministry of Education of Korea

Title: Genetic ablation of a susceptibility gene for bipolar disorder, Trpm2 exhibits altered stress-related innate behaviors and social behaviors

Authors: G. HONG[1], H. CHUN[1], J. WEE[1], S. LEE[1], D. YANG[1], Y. JANG[2], D. JEON[1], *U. OH[3,4];
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Abstracts: Bipolar disorder (BD) is a psychotropic disorder that causes unusual shifts in mood from the manic to the depressive state. Genetic linkage studies have shown that TRPM2, a Ca2+-permeable cation channel is associated with BD, but the nature of this linkage is not known. Here we show that the genetic ablation of Trpm2 elicits altered stress-related innate behaviors and social behaviors. In addition, Trpm2-/- mice exhibited abnormal oscillation and synchronization of electroencephalogram, such as reduced value of entropy, decreased correlation coefficient values, lower coherence between the two regions. These EEG traits are frequently emerged in BD patients. Interestingly, amphetamine administration to wild-type mice evoked a large increase in open-field activity, which was reversed by lithium. However, the anti-manic action of lithium was not observed in the Trpm2-/- mice. Because TRPM2's dysfunction in mice provides several symptoms frequently emerged in BD patients, Trpm2-/- mice may be useful for animal model for BD as well as mechanistic study for its etiology in future. Supported by a grant from the National Research Foundation of Korea (No. 20110018358) and a grant from BK21+ program of Ministry of Education of Korea.

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Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.16/X20

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Neural correlates of successful psychotherapy of depression in adolescents

Authors: J. STRAUB[1], P. L. PLENER[1], N. SPRÖBER[1], L. SPRENGER[4], M. G. KOELCH[1], *T. KAMMER[2], G. GRÖN[3], B. ABLER[3];
[1]Dept. of Child and Adolescent Psychiatry and Psychotherapy, [2]Section for Neurostimulation, Dept. of Psychiatry, [3]Dept. of Psychiatry, Univ. of Ulm, Ulm, Germany; [4]Dept. of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Philipps-University of Marburg, Marburg, Germany

Abstracts: Introduction: While major effort has been invested in investigating the neural correlates of depression and their modulation by treatment in adults, less is known about the effects of psychotherapy in adolescents. Given the concordance of the amygdala, hippocampus and the subgenual anterior cingulate cortex (sgACC) as correlates of depression and their involvement in reward processing, we used functional magnetic resonance imaging during performance of a monetary reward task in an intervention vs. waitlist-control design to investigate the clinical and neural effects of cognitive behavioural group therapy (CBT-G). Methods: We investigated 22 medication naïve adolescents with major depressive disorder. Participants were scanned before and after five sessions of 90 minutes of CBT-G (PAT-I), or before and after a five weeks of waiting (PAT-W) with treatment as usual. Symptoms were assessed using the Children's Depression Rating Scale and the Beck Depression Inventory Revision. Changes in symptom scales were analyzed along with neural activation changes within the three independent regions of interest (ROI). Results: Pre-post-test psychometric assessments were insignificant in PAT-W but significant in PAT-I for BDI-II and CDRS-R. Time by group interaction effects were significant with respect to BDI-II ($t(20) = -1.82$, $p = .042$) and CDRS-R ($t(20) = -2.05$, $p = .027$). Pre-post-test ROI activation also stayed constant in PAT-W in the sgACC, Hippocampus und Amygdala. In PAT-I, pre-to-post-test brain activation was significantly altered in the left amgygdala, left hippocampus and bilateral sgACC. Time by group interaction effects were significant for the right sgACC ($t(20) = -1.89$, $p = .037$), left Amygdala ($t(20) = -2.49$, $p = .011$) and left Hippocampus ($t(20) = -1.74$, $p = .049$). In line with previous findings in adults, pre-to-post symptom improvement in the BDI-II correlated with pre-to-post activity change in the bilateral sgACC (left sgACC: $r = .57$, $p = .01$; right sgACC: $r = .54$, $p =$

.02). Correlation coefficients further increased with pre-to-follow-up difference scores in the BDI-II (left sgACC: r= .69, p = .002; right sgACC: r = .73, p = .001). Furthermore individual pre-to-follow-up symptom change, assessed by means of BDI-II, was significantly related to baseline sgACC activation (left sgACC: r = -.56, p = .02, right sgACC: r = -.59, p = .01). Conclusions: Successful group psychotherapy of depression in adolescents was related to signal changes in brain regions previously demonstrated to be reliably linked with successful, particularly pharmacological treatment in adults.

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Poster

615. Depression Biology

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Title: High-resolution functional mapping of the human habenula in depression

Authors: *B. A. ELY[1], K. A. B. LAPIDUS[2], D. L. ROSENTHAL[3], K. E. SIP[3], J. XU[4], E. R. STERN[2];
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Abstracts: Background: Recent studies have suggested that the habenula (Hb), a pair of small grey matter nuclei bordering the dorsomedial thalamus, may play an important role in depression. Animal studies have indicated that projections from the Hb act to inhibit the reward system through modulation of midbrain dopaminergic and serotonergic signaling. In a case study following Hb deep brain stimulation, a patient with treatment-resistant depression reported remission. However, the small size of the human Hb has limited *in vivo* investigation via standard neuroimaging methods. Using novel high-resolution resting-state fMRI (rfMRI) approaches, we aim to address this hurdle and examine Hb connectivity in a group of subjects with major depression (MDD), treatment-resistant depression (TRD), and healthy controls (HC). **Methods:** Subjects (two HC and one MDD) underwent 15-minute rfMRI scans at both 3T and 7T using 32-channel head-coils. Isotropic high-resolution multiband accelerated gradient echo EPI images of 2.1mm^3 at 3T (one run) and both 1.6mm^3 and 1.28mm^3 (two runs each) at 7T were acquired. Left and right 2mm-radius spherical Hb regions-of-interests (ROIs) were identified based on anatomical landmarks using FSL software. Spatial and temporal preprocessing of the time series were conducted using SPM12/FSL, followed by functional connectivity analysis via the CONN toolbox. **Results:** A consistent pattern of bilateral association of Hb connectivity was seen for all three subjects at 3T, with each ROI positively correlating with the contralateral Hb as well as the bilateral thalamus and insula. Hb connectivity in the two controls appeared more widespread, including the midbrain and prefrontal cortex. In the MDD subject, stronger connectivity between the left and right Hb was observed. These results from 3T were corroborated by ultra-high-resolution rfMRI data acquired in the same subjects at 7T (both 1.6mm^3 and 1.28mm^3) with exquisite spatial specificity. **Conclusions:** These preliminary findings demonstrate the ability to detect expected patterns of Hb connectivity using high-resolution 3T rfMRI and the added benefits of spatial specificity using ultra-high-resolution 7T rfMRI. The higher bilateral Hb functional association in the MDD subject is consistent with the literature and will be verified in this pilot case-control study (MDD, TRD, and HC, 10 subjects each).

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Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.18/X22

Topic: C.19. Drug Discovery and Development

Support: This work was funded by the Intramural Research Program at the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH; grant number 04-M-0222).

Title: Glutamatergic levels in the healthy and depressed brain: An ultra-high field 1h-mrs ketamine treatment study

Authors: *N. LALLY[1,2], L. AN[3], A. NUGENT[1], D. BANNERJEE[1], J. SHEN[3], C. ZARATE[1]; [1]NIH, Bethesda, MD; [2]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom; [3]Magnetic Resonance Spectroscopy Core, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstracts: Recent evidence has highlighted aberrant glutamatergic-signalling in patients diagnosed with depression. Additionally, evidence suggests that ketamine, a non-competitive N-Methyl-D-Aspartate antagonist, may possess rapid acting antidepressant efficacy mediated through the enhancement of glutamatergic transmission. However, due to technical limitations, it has not been possible to reliably determine whether such alterations in the glutamatergic system occur in the in-vivo in the human patients and whether successful treatment with ketamine resolves any dysfunction. Here, using a 7 Tesla Siemens MRI, we acquired baseline 1H-MRS data, using an adapted point resolved spectroscopy sequence, from the pregenual anterior cingulate in healthy volunteers (N=18) and also at baseline and 24 hours post-ketamine and -placebo infusions in medication free treatment-resistant patients with major depressive (N=7) and bipolar disorder (N=2) in a double-blind, randomized, placebo controlled investigation. A water linewidth of < 16Hz was required for data points to be included in the analyses and 1H-MRS metabolites were referenced to creatine. In contrast to previous reports, we found no statistical difference in pregenual glutamate levels between patients with depression (M=1.33) and healthy volunteers (M=1.43; $t(25)=1.64$, $p=0.11$, $d=0.69$). In congruency with previous investigations, there was no significant difference between glutamate levels post-ketamine (M=1.38) and post-placebo (M=1.33; $t(7)=0.77$, $p=0.47$, $d=0.27$). These results attenuate the emphasis of the glutamatergic system, as measured by 1H-MRS, in the neurobiology of depression. However, the current sample size precludes any firm deductions surrounding the pathophysiology and treatment mechanisms of depression; effect sizes suggest that increasing the sample size may yield statistically significant differences between healthy and depressed subjects.

Disclosures: **N. Lally:** None. **L. An:** None. **A. Nugent:** None. **D. Bannerjee:** None. **J. Shen:** None. **C. Zarate:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A patent application for the use of ketamine in depression has been submitted listing Dr. Carlos A. Zarate among the inventors; he has assigned his rights on the patent to the U.S. government, but wil.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.19/X23

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Grant-in-Aid for Scientific Research (Number 20591371) from the Japanese Society for the Promotion of Science (JSPS)

Title: FKBP5 is associated with amygdala volumes in the human brain and mood: A voxel-based morphometry (VBM) study

Authors: *J. AKIYOSHI, H. HIRAKAWA, K. MASUDA, S. AIZAWA;
Oita Uiniversity Fac. of Medicine, Dept. of Neuropsychiatry, Oita, Japan

Abstracts: Introduction: The FK506 binding protein 51 (FKBP5) genes control the stress hormone system and are associated with mental illness due to stress. Genetic studies suggest that FKBP5 plays an important role in depression, post-traumatic stress disorder, and anxiety disorder. The aim of the present study was to investigate the effects of 6 FKBP5 single nucleotide polymorphisms (SNPs) on human amygdala volumes, using voxel-based morphometry (VBM). Methods: We genotyped 112 healthy controls with respect to 6 at-risk reference SNPs (rs) of FKBP5: rs3800373, rs992105, rs9296158, rs1360780, rs9470080, and rs2766534. All participants underwent structural magnetic resonance imaging, and the data was statistically analyzed usingVBM. We analyzed the association between VBM, mood and anxiety. Results: We found significant alterations in amygdala volumes with rs 3800373, rs992105, rs1360780, and rs9470080. According to the Profile of Mood States (POMS), rs992105 and rs9470080 were associated with tension-anxiety, depression-dejection, anger-hostility and Vigor. Discussion: These effects of FKBP5 SNPs on brain morphology provide further evidence of its involvement in the neurobiology of depression and anxiety.

Disclosures: J. Akiyoshi: None. **H. Hirakawa:** None. **K. Masuda:** None. **S. Aizawa:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.20/X24

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Selective serotonin reuptake inhibitor, electro-convulsive therapy, and repetitive transcranial magnetic stimulation treatments affect distinct neural systems in Major Depressive Disorder: A comprehensive meta-analytic investigation

Authors: *D. T. CHAU[1], P. FOGELMAN[1], W. C. DREVETS[1,2], P. J. HAMILTON[1];
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Abstracts: A large number of functional neuroimaging studies have investigated the neural mechanisms of conventional interventions for Major Depressive Disorder (MDD). Unfortunately, the results of these studies have been mixed and, correspondingly, have been equivocal with respect to the primary mechanisms underlying the specific modes of treatment for MDD. In the present study, we conducted voxel-wise, whole-brain meta-analytic syntheses of studies reporting changes in resting-state brain activity, as indexed by regional cerebral blood flow or regional glucose usage, in MDD. Studies included in the present meta-analysis employed one of three distinct classes of intervention: selective serotonin reuptake inhibitor (SSRI)-based pharmacotherapy, electro-convulsive therapy (ECT) or repetitive transcranial magnetic stimulation (rTMS). In addition to investigating concomitant changes associated with each modality, we examined these results relative to each other and to reliable resting-state baseline activation abnormalities in MDD. Our meta-analytic study revealed the following: 1) SSRIs decrease activity in the left mid and posterior insula in MDD; 2) ECT decreases activity in MDD in the right posterior cingulate gyrus and left medial frontal gyrus (primary nodes of the brain's default network); and 3) rTMS decreases activity in the left ventral anterior cingulate and right thalamus. Interestingly, there was no overlap across treatment modalities in brain regions exhibiting decreased activity as a result of treatment. Moreover, regions exhibiting decreased activity following SSRI, ECT, and rTMS treatments did not overlap with regions showing abnormal baseline activity in MDD _ increased activity in the right subcallosal gyrus and right and left pulvinar and decreased activity in the right and left fronto-insular, right dorsal anterior cingulate, and left dorsolateral prefrontal cortex. The current data suggest that SSRIs, rTMS, and ECT work by targeting neural mechanisms that do not exhibit abnormal resting activity in MDD. Specifically, SSRIs exert their effects via the mid and posterior insula, which receive major afferents from the vagus nerve, possibly reducing the flow of viscero-motor information to the cerebral cortex in MDD; alternatively, ECT may alleviate persistent and treatment-resistant negative mood by dampening activity in default network nodes postulated to subserve ruminative, self-referential processing in MDD. Finally rTMS may, through indirect pathways,

normalize dysfunctional activity increases in limbic cortico-striatal-pallido-thalamic circuitry that contributes to motivational deficits and anhedonia in MDD.

Disclosures: D.T. Chau: None. **P. Fogelman:** None. **W.C. Drevets:** None. **P.J. Hamilton:** None.

Poster

615. Depression Biology

Location: Halls A-C

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Program#/Poster: 615.21/Y1

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Associations between oxytocin-related genes, anxiety, depressive, cognitive symptoms and neuroimaging

Authors: *H. HIRAKAWA, J. AKIYOSHI, K. MASUDA, S. AIZAWA;
Hasama-Machi, Yufu-Shi, Japan

Abstracts: [Introduction] Oxytocin (OT) has been studied reported widely in the neuroendocrine system. Some reports have indicated extensive behavioral influences and various possible effects. Association between OT and social functioning is very important in this system. Animal studies showed that central OT receptor distributes the critical regions in relation to couple attachment and maternal maintenance. We examined the association between the associations between oxytocin-related genes, anxiety, depressive, cognitive symptoms and neuroimaging such as fMRI and VBM. [Methods] The study included 610 healthy participants. We examined the State-Trait Anxiety Inventory (STAI), Profile of Mood States (POMS) and Depression and Anxiety Cognition Scale (DACS), a Japanese psychological questionnaire, to assess automatic thoughts. We also examined Temperature Character Inventory (TCI) and Revised NEO Personality Inventory (NEO-PI-R). OT gene SNP rs53576 was selected according to their minor allele frequency. Linear regression models were used to test association of mean psychological scores with each allele (major-allele homozygote, heterozygote, and minor-allele homozygote). All participants underwent structural magnetic resonance imaging, and the data was statistically analyzed using SPM8 and VBM. We analyzed the association between fMRI, VBM, mood, anxiety and cognition. [Results] We found that there was a significant differences of Tension-Anxiety (T-A), Anger-Hostility (A-H), and Vigor (V) of POMS between major-allele homozygote, heterozygote, and minor-allele homozygote. There was a significant differences of Novelty seeking (NS), Harm avoidance (HA) and Reward dependence (RD) in TCI between

major-allele homozygote, heterozygote, and minor-allele homozygote. We also found significant alterations in hippocampus, cingulate, insula, orbitofrontal, thalamus, caudate, pallidum volumes between major-allele homozygote, heterozygote, and minor-allele homozygote. [Discussion] We found significant differences of VBM, psychological, and personality test between major-allele homozygote, heterozygote, and minor-allele homozygote. These results suggest that OT gene SNP rs53576 has influenced anxiety and depressive symptoms in conjunction with brain volume change.

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Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.22/Y2

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: MGAT4A gene is associated with anxiety, depressive symptoms and self-denial

Authors: *K. MASUDA, J. AKIYOSHI, H. HIRAKAWA, S. AIZAWA;
Oita Univ. Fac. of Med., Yufu-Shi, Japan

Abstracts: [Introduction] Epigenetic alterations suggest explaining the mechanism of disease and describing the pathophysiology of epigenetic disruptions. Single-gene interruptions of the epigenetic organization have long been identified to induce bipolar disorders. Relationship between MGAT4A gene and bipolar disorder has been reported recently (Gamazon et al., 2013). The SNP regulates the methylation of inositol polyphosphate phosphatase 4A (INPP4A) in cis. The gene INPP4A encodes one of the enzymes comprised in the phosphatidylinositol signaling pathway, which is a target for the effects of lithium. We examine the relationship between MGAT4A gene, salivary amylase response, salivary cortisol response, personality and psychological tests (include self-denial) for depressive and anxiety symptoms. [Methods] The study included 593 healthy participants. We collected saliva samples from participants before after, electrical stimulation and TSST to measure the concentrations of salivary alpha-amylase (sAA) and salivary cortisol. Profile of Mood State (POMS) and State-Trait Anxiety Inventory (STAI) scores and Heart Rate Variability (HRV) were also determined following stimulation. MGAT4A gene (rs12618769) was selected according to their minor allele frequency. Linear regression models were used to test association of mean psychological scores with each allele (major-allele homozygote, heterozygote, and minor-allele homozygote). The significant α-value

was set at $\alpha < 0.0025$. Statistical analysis was conducted using SNPStats. Call rates for all genotypes were > 98%. [Results]There was a significant differences of state and trait anxiety in each allele in MGAT4A gene (rs12618769). Tension-Anxiety (T-A), Depression-Dejection (D) and Fatigue (F) scores of POMS in heterozygote and minor-allele homozygote were more than that in major-allele homozygote. Vigor (V) scores of POMS in minor-allele homozygote were less than that in heterozygote and major-allele homozygote. Future denial (FD), threat prediction (TP), self-denial (SD), and past denial (PD) in minor-allele homozygote were more than that in heterozygote and major-allele homozygote. [Discussion]Our results demonstrated that DACS scores showing significant interaction with the MGAT4A gene SNPs may be regarded as appropriate traits to detect the diathesis of automatic thoughts. The MGAT4A gene SNPs may be important loci in research on cognitive vulnerability to depression and anxiety.

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Poster

615. Depression Biology

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Title: A delayed brain-derived neurotrophic factor response to ketamine infusion: Implications for rapid symptom improvements in treatment-resistant depression

Authors: *A. P. ALLEN[1], M. NAUGHTON[2], G. CLARKE[1], J. DOWLING[3], A. WALSH[3], F. ISMAIL[2], G. SHORTEN[3], L. SCOTT[2], J. F. CRYAN[4], T. G. DINAN[1];

[1]Psychiatry/Alimentary Pharmabiotic Ctr., [2]Psychiatry, [3]Anaesthesia and Intensive Care Med., [4]Anat. & Neuroscience/Alimentary Pharmabiotic Ctr., Univ. Col. Cork, Cork, Ireland

Abstracts: Introduction: Ketamine is associated with rapid antidepressant efficacy, but research is required to unearth the underlying biological mechanisms. Brain derived neurotrophic factor (BDNF), a neurotrophin associated with hippocampal neurogenesis, is reduced in depression and can be normalised following antidepressant treatment. Previous research has shown that ketamine enhancement of plasma BDNF four hours post-infusion in patients with treatment-resistant depression (TRD) was associated with symptomatic response to ketamine treatment . However, although the clinical response to ketamine can persist for one week or longer, it is unknown if BDNF increases are stable over this time period. It is also unknown if multiple infusions are additive or lead to sustained effects on BDNF levels. This study aimed to examine the effect of multiple ketamine infusions on serum BDNF and severity of depressive symptoms. Methods: Patients with TRD (N = 17) and healthy controls (N = 20) were recruited. Between 1 and 3 infusions of ketamine (0.5mg/kg) were administered to TRD patients at visits one week apart. Blood samples were collected at baseline in all participants, and within the TRD cohort at 24 hours following the first infusion and at 2 hours and 1 week following each infusion. Symptomatic response to ketamine was assessed using the Hamilton Depression Rating Scale (HDRS). Patients who showed a 50% or greater reduction in HDRS score were classified as responders. BDNF levels were assessed in serum using MesoScale Discovery custom assays. For each sampling time, to assess the effect of ketamine treatment on BDNF, post-ketamine BDNF data were compared to corresponding baseline data for patients who responded symptomatically. Results: Ketamine was associated with a significant clinical response; a majority of patients showed a clinical response at all sampling time points. At baseline the TRD group had significantly lower BDNF (M = 13.3 ng/ml, SD = 6.6) compared to the healthy controls (Mean = 19.6, SD = 8.9, p = 0.03). Those who responded clinically at 2 hours post-infusion did not have higher BDNF at 2 hours following any infusion. However, patients who responded clinically to ketamine at 1 week post-infusion showed enhanced BDNF at 1 week post-infusion compared to baseline (Mean increase = 4.6, SD = 4.3, p = .03), but only for the first infusion. Conclusions: We found a delayed rather than immediate effect of ketamine treatment on circulating BDNF levels. Ketamine infusion may be associated with enhanced serum BDNF, although this may not occur as rapidly as the clinical response, and may not persist over multiple infusions of ketamine.

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Poster

615. Depression Biology

Location: Halls A-C

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Program#/Poster: 615.24/Y4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: HRB Grant R14587

Title: Whole blood microRNAs as biomarkers of treatment-resistant depression? Effects of ketamine and electroconvulsive therapy

Authors: *K. A. SCOTT[1], G. M. MOLONEY[1], M. NAUGHTON[2], R. M. O'CONNOR[1], D. M. MCLOUGHLIN[5], J. DOWLING[3], G. SHORTEN[3], A. WALSH[3], L. SCOTT[2], F. ISMAIL[2], G. CLARKE[2,4], T. G. DINAN[2,4], J. F. CRYAN[1,4];
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Abstracts: Major depressive disorder (MDD) is a debilitating and potentially life-threatening illness that affects nearly 1 in 5 individuals during the course of a lifetime. A significant number of patients fail to experience remission of symptoms in response to traditional antidepressant therapies such as those targeting the serotonergic system. Two therapies that have shown much promise for treatment resistant depression (TRD) are ketamine (KET) and electroconvulsive therapy (ECT), although their exact mechanisms of action are not clear. Understanding the molecular mechanisms underlying their effects may provide insight into the development of novel therapeutic strategies for depressive illness while minimizing adverse side effects often associated with antidepressant treatments. There has been an increasing emphasis on the role of microRNAs in brain function and behavior and their potential as biomarkers in addition to mediators of pathology. MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs that affect gene expression, typically by targeting mRNAs for degradation or by preventing their translation to protein. In the current study we have assessed miRNA expression in whole blood from healthy controls and compared it with miRNA of patients with TRD prior to and following KET or ECT treatment. Whole blood was collected in PAXgene blood RNA tubes and total RNA was isolated using the PreAnalytiX Blood miRNA Kit. RNA quantity and quality were assessed using a Nanodrop 2000 spectrophotometer and Agilent Bioanalyser. A microRNA microarray analysis was then performed to identify miRNAs that differed between groups. We have identified a number of potential miRNA biomarkers in the whole blood of TRD patients that differ from those of controls. Furthermore, we have identified miRNAs that change in response to successful treatment with ECT or KET. Further validation of these findings using real time qPCR is now warranted. Our preliminary findings suggest that circulating miRNAs differ between healthy controls and patients with TRD, suggesting that miRNAs may be an

effective biomarker of depressive illness. Furthermore, successful treatment of depression also alters blood miRNAs. Understanding the roles of these miRNAs may in turn inform novel and more efficacious treatments for stress-related disorders such as depression and anxiety.

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Poster

615. Depression Biology

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

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Title: Cingulo-frontal network dynamics explains the correlation between EEG theta and treatment outcome in depression

Authors: J. RAMIREZ-MAHALUF[1], A. ROXIN[2], H. S. MAYBERG[3], *A. COMPTE[1]; [1]IDIBAPS, Barcelona, Spain; [2]Ctr. de Recerca Matemàtica, Bellaterra, Spain; [3]Emory Univ., Atlanta, GA

Abstracts: Major depression disease (MDD) has been associated with a dysfunction of cingulo-frontal networks following glutamate metabolism dysfunction in the ventral anterior cingulate cortex (vACC), but theoretical frameworks do not yet provide a principled approach to MDD treatment. Recently, electroencephalography (EEG) is increasingly viewed as a promising

neurophysiological biomarker of treatment outcome: resting ACC activity in the θ band (4-8 Hz) predicts treatment response (Pizzagalli, Neuropsychopharmacol 2011). We provide here a physiological interpretation in terms of the dynamics of the cingulo-frontal network. We built a biophysical computational model of 2 cortical areas (vACC, and dorso-lateral prefrontal cortex, dlPFC) that acts as a bistable switch between emotional and cognitive processing: the two areas cannot be co-active due to effective mutual inhibition. dlPFC activates persistently in response to brief external cognitive signals, and deactivates vACC by means of dlPFC-vACC disynaptic inhibition. Reciprocally, emotional signals activate vACC directly and deactivate dlPFC. In such networks of coupled excitatory and inhibitory neurons, we found a regime characterized by θ oscillations when the system operated as a switch, in line with midline θ characterizing executive processes in healthy humans. We simulated MDD by slowing down glutamate re-uptake in vACC. Excessive excitation led to non-switchable, constant vACC activation, and this prevented dlPFC from responding to cognitive signals, mimicking sustained sadness and cognitive dysfunction in MDD. Interestingly, these dynamics were reflected in the model-derived EEG: MDD models had reduced θ and increased β oscillations, similar to MDD patients (Knott et al., Psychiat Res 2001). We modeled serotonergic treatments (SSRI) through hyperpolarization of vACC neurons, based on the high incidence of 5-HT_{1A} receptors in vACC. SSRI counteracted aberrant vACC activity and impacted the EEG: oscillations at β (θ) decreased (increased) in amplitude, mirroring EEG β in patients after treatment (Tarn et al. J Affect Disorders 1993). Importantly, because the strength of θ oscillations indicated in our network the proximity of the system to the switch mode, there was a correlation between the amplitude of θ oscillations and the effective deactivation of vACC after a given dose of SSRI. This provides a biophysical explanation for the experimentally observed correlation between θ activity before treatment and treatment outcome (Pizzagalli, 2011). In this view, EEG theta measures how close the cingulo-frontal network is to the switch regime that underlies healthy function.

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Poster

615. Depression Biology

Location: Halls A-C

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Program#/Poster: 615.26/Y6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Brain responses to unpredictable threat in individuals at high risk for depression

Authors: *N. KIRLIC[1,3], M. MISAKI[3], J. BODURKA[3], W. C. DREVETS[4], R. P. ALVAREZ[3,2];
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Abstracts: First-degree relatives of individuals with mood disorders are at increased biopsychosocial risk for psychopathology. Exaggerated emotional reactivity and dysregulation to aversive stimuli pose as risk factors for depression. Using an instructed threat paradigm and high resolution fMRI, we investigated the neurocircuitry underlying responses to unpredictable threat in 21 psychiatrically-healthy individuals at risk for depression by virtue of family history (HR) and 21 age- and gender-matched controls with no family history of mood disorders (LR). Subjects virtually navigated two computer-generated contexts, one where unsignaled shocks on the ankle could occur at any time (Threat), and one where no shock was ever delivered (Safe). Subjects were presented with 20 Threat and 20 Safe contexts over 4 scans lasting 6 min each. Context presentations lasted 18 sec and were followed by a 14-18 sec inter-stimulus interval during which subjects performed a low-level vigilance task. Subjects received 1-2 shocks per scan for a total of 5. Fear ratings and skin conductance responses (SCRs) indexed anxiety reactivity to Threat compared to the Safe condition. Scanning was performed using a GE MR750 3T scanner, a 32-channel phased-array coil, and parallel imaging. A gradient echo EPI sequence with Sensitivity Encoding and FOV/slice thickness=24/2.9mm was used (TR=2000ms, TE=25ms, acceleration=2, image matrix=96x96, flip=40°, and 35 axial slices). Advanced normalization tools with symmetric diffeomorphic image registration was utilized for spatial normalization. Fear ratings and SCRs indicated that anticipation of unpredictable shock elicited sustained apprehension in both HR subjects and LR controls. Correspondingly, whole brain analyses revealed that anxious reactivity to Threat compared to Safe elicited activation in a broad neural network associated with emotion and pain processing, including anterior cingulate, insula, bed nucleus of the stria terminalis, and periaqueductal gray. Compared with the LR group, the HR group displayed significantly increased activity in subgenual anterior cingulate (sgACC) and orbitomedial prefrontal cortex (omPFC) to Threat compared to the Safe condition. These data suggest that sustained anxiety in HR and LR individuals elicits activity in common and distinct neurocircuitry. The sgACC and omPFC have been implicated in emotion regulation and commonly show hyperactivity in depressed patients during emotional processing. Dysfunction in these prefrontal regions in high-risk individuals may be a marker of vulnerability for subsequent development of depression particularly when facing adverse life experiences.

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Poster

615. Depression Biology

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Effects of vagus nerve stimulation on pupil function

Authors: V. DESBEAUMES[1], D. K. NGUYEN[2], M. PHILIBERT[1], M.-P. FOURNIER-GOSSELIN[3], P. LESPÉRANCE[4], *F. RICHER[5];
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Abstracts: Background: Vagus nerve stimulation is a recognized treatment for refractory epilepsy and depression. The main site of innervation of the vagus nerve is the nucleus of the tractus solitarius (NTS). NTS is connected to the locus coeruleus (LC), which in turn, projects to preganglionary parasympathetic nuclei of the brain stem, including the Edinger-Wesphal (EW) nucleus controlling the pupil. Pupillary effects of vagus nerve stimulation have not yet been examined in humans. The objective of this study is to evaluate the acute effects of VNS on baseline pupil size and the pupillary light reflex. Method: We studied 25 patients (10 with major depression, 15 with partial epilepsy) treated with chronic VNS (30 sec ON stim, 5 min OFF stim). Patients fixated a dark screen where a light disc appeared during 500ms. Pupil recordings (3.4 s, infrared head-mounted video camera) started 1.5 s before visual stimulation. Six trials were presented ON VNS stimulation and 6 trials OFF. Results: VNS stimulation was associated with a significant increase in baseline pupil diameter ($t(24)=-4.3$; $p<0.01$) and a smaller amplitude of peak constriction ($t(24)=-5.0$;$p<0.001$) compared to unstimulated periods. There was no difference between the patient groups on any of the pupil measures. Conclusion: Trains of stimulation of the vagus nerve produced a significant increased in pupil diameter during resting state and reduced the amplitude of the pupillary light reflex. These effects are compatible with the known connectivity between LC and EW nuclei and could serve as markers of autonomic effects of VNS.

Disclosures: V. Desbeaumes: None. **F. Richer:** None. **D.K. Nguyen:** None. **M. Philibert:** None. **M. Fournier-Gosselin:** None. **P. Lespérance:** None.

Poster

615. Depression Biology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 615.28/Y8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Depression and Alzheimer's Disease: Novel postmortem brain studies reveal a possible common mechanism

Authors: *Y. TATEBAYASHI, N. NIHONMATSU-KIKUCHI, Y. MATSUDA;
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Abstracts: The relationship between depression and Alzheimer's disease (AD) has always been relevant and controversial. Recent large-scale epidemiological studies, however, have supported the possibilities that major depressive disorders (MDD) that occur during adolescence or middle age represent a risk factor for AD and/or diagnoses of MDD in the elderly often constitute a prodrome of AD. Here, we introduce our recent research about postmortem brains from patients with MDD. Our novel methodological approaches have revealed that MDD may be associated with an unknown type of myelin/myelination abnormalities in the frontopolar cortex. Based mainly on our findings, as well as on neuropathological observations by Braak & Braak (Acta Neuropathol 9, 197-201, 1996), we discuss the possible existence of an as yet unknown common mechanism linking the pathophysiologies underlying both depression and AD.

Disclosures: Y. Tatebayashi: None. **N. Nihonmatsu-Kikuchi:** None. **Y. Matsuda:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.01/Y9

Topic: C.17. Drugs of Abuse and Addiction

Support: Korea Institute of Oriental Medicine (K13290)

National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2011-0030124)

National Research Foundation of Korea (NRF) grant funded by the Korea government MEST (2011-0016038)

Title: Involvement of reactive oxygen species in cocaine taking-behaviors in rats

Authors: *S. CHANG[1], E. JANG[2], Y.-H. RYU[3], B. LEE[1], R. J. FOLSOM[2], N. D. SCHILATY[2], K. KIM[1], C. YANG[1], S. C. STEFFENSEN[2], H. KIM[1];
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Abstracts: Reactive oxygen species (ROS) have been implicated in the development of behavioral sensitization following repeated cocaine exposure. We hypothesized that increased ROS following cocaine exposure would act as signaling molecules in the mesolimbic dopamine (DA) system, which might play an important role in mediating the reinforcing effects of cocaine. The aim of this study was to evaluate cocaine enhancement of brain metabolic activity and the effects of ROS scavengers on cocaine self-administration behavior, cocaine-induced ROS production in the nucleus accumbens (NAc) and cocaine enhancement of DA release in the NAc. Metabolic neural activity monitored by temperature and oxidative stress were increased in NAc following cocaine exposure. Systemic administration of the ROS scavenger PBN or TEMPOL, either pre- or post-treatment, significantly decreased cocaine self-administration without affecting food intake. Infusion of TEMPOL into the NAc inhibited cocaine self-administration. Increased oxidative stress was found mainly on neurons, but not astrocytes, microglia or oligodendrocytes, in NAc of rats self-administering cocaine. TEMPOL significantly attenuated cocaine-induced enhancement of DA release in the NAc, compared to saline control. TEMPOL had no effect on the enhancement of DA release produced by the DA transporter (DAT) inhibitor GBR12909. Taken together, these findings suggest that enhancement of ROS production in NAc neurons contributes to the reinforcing effect of cocaine.

Disclosures: S. Chang: None. **E. Jang:** None. **Y. Ryu:** None. **B. Lee:** None. **R.J. Folsom:** None. **N.D. Schilaty:** None. **K. Kim:** None. **C. Yang:** None. **S.C. Steffensen:** None. **H. Kim:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.02/Y10

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA 06886

NIDA Grant DA 029873

Title: Evidence for tuning to preferred drug levels during cocaine self-administration by nucleus accumbens neurons

Authors: *O. A. KIM[1,2], K. R. COFFEY[1], D. J. BARKER[1], S. MA[1], A. P. PAWLAK[1], A. T. FABBRICATORE[1], M. O. WEST[1];
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Abstracts: Drug consumption can transition from recreational use to abuse when individuals repeatedly consume the substance in bouts or "binges," leading to severe negative consequences for the user. However, there is evidence that drug abusers remain sensitive to the immediate consequences of drug consumption. During a binge, animals work to maintain stable, "preferred" brain concentrations of cocaine. Each injection leads to a rapid rise in drug level followed by a gradual pharmacokinetic decay, allowing animals to "titrate" their cocaine levels by spacing self-infusions according to dose. A binging animal responds each time drug level decays to a level termed the "maintenance threshold." The present report sought to characterize the relationship of drug level to firing rates in the Nucleus Accumbens (NAcc), a component of dopaminergic pathways implicated in drug self-administration (SA) and motivated behaviors. Following two weeks of cocaine SA training, single-unit recordings of NAcc neurons in male, Long-Evans rats were taken and analyzed in relation to calculated drug levels and observed drug consumption. A subset of neurons (15%) exhibited tuning to the maintenance threshold. These data suggest that the NAcc, via its downstream connections to motor areas, is involved in synthesizing information regarding proximity to preferred drug levels and influencing the occurrence of drug consumption.

Disclosures: O.A. Kim: None. **K.R. Coffey:** None. **D.J. Barker:** None. **S. Ma:** None. **A.P. Pawlak:** None. **A.T. Fabbricatore:** None. **M.O. West:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.03/Y11

Topic: C.17. Drugs of Abuse and Addiction

Support: DA006886

Title: Exploring individual differences in rats' addiction susceptibility: drug level titration ability is inversely related to cue induced responding

Authors: *K. COFFEY[1], D. SHOLLER[2], M. WEST[2];
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Abstracts: While only a sub-population of humans show substance abuse or dependence potential, methods for differentiating sub-populations of addiction-prone animals are lacking. In the present study, we sought to effectively characterize subjects who were susceptible to cue induced responding, a maladaptive trait which promotes substance dependence and relapse in humans. Animals were trained on a variable interval schedule of cocaine self-administration with a discriminative stimulus (SD) tone signaling drug availability. Animals' response variance was modeled with respect to calculated brain drug concentration and SD latency. Results demonstrate that animals' abilities to effectively titrate drug are learned and inversely related to responsiveness to the SD. Specifically, poor titration of drug levels is linked to higher cue-reactivity while animals that are better able to titrate their drug levels show little SD-driven responding. Moreover, subjects failed to respond during roughly 50% of signaled availability periods, with drug levels at missed availability periods consistently greater than drug levels at hit availability periods. A custom normalized titration metric (NTM) was calculated from the distribution of differences between drug levels during missed and hit trials for each animal. Low titration ability using this NTM also predicts SD-induced responding. Animals' titration abilities and SD-responsivity exist along a spectrum that is captured by our response variance calculation and NTM. These metrics may be adapted for use with many self-administration paradigms to determine addiction susceptibility, potentially facilitating future studies of behavioral, psychological, or genetic differences among individuals. Such endeavors could be used to characterize addiction susceptibility in humans or to develop more effective genetic or behavioral therapies.

Disclosures: K. Coffey: None. **D. Sholler:** None. **M. West:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.04/Y12

Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC

CIHR doctoral award

Title: Potentiation of the expression of cocaine-induced sensitization by a conditioned stressor

Authors: *A. SASAKI[1], A. HUSSAIN[2], P. SHANMUGANATHAN[2], A. SIVAKUMARAN[2], S. SIVANATHAN[2], S. ERB[1,2];
[1]Dept. of Cell and Systems Biol., [2]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstracts: Stress is a major factor contributing to drug craving and relapse in humans, and in rodent models of drug seeking. Stress has also been found to cross-sensitize to the locomotor activating effects of psychostimulants in rodent models. In the present study, we examined the effect of a conditioned stressor on the expression of cocaine-induced sensitization in rats. More specifically, we determined whether a mint odor cue previously paired with footshock stress would elicit a sensitized locomotor response in rats previously given repeated exposures to cocaine. To this end, male Wistar rats were given once daily injections of cocaine (30mg/kg, i.p.) or saline for 6 days in locomotor monitoring chambers. Subsequently, equal numbers of rats in each drug condition were exposed to 15 min of brief, intermittent footshocks or no footshocks, either in the presence or absence of a mint odor cue. Odor-footshock pairing sessions occurred once daily sessions for 3 days. Approximately 5 days after the last of these sessions, all rats were given 3 locomotor tests for sensitization. The first was a test for conditioned locomotion, in which all rats were returned to the locomotor chambers in which cocaine or saline injections had been administered, and activity was monitored for 30 min; no injections or stress manipulations were given during this test. In the second test, all rats were exposed to the mint odor in the context of the locomotor chambers, in order to test for the cross-sensitizing effects of a conditioned stressor. In the third test, all rats were given a challenge injection of cocaine (10mg/kg, i.p.), either in the presence or absence of the mint odor. Irrespective of the prior stress condition, cocaine pre-exposed rats showed a robust conditioned locomotion effect upon re-exposure to the activity chambers in Test 1, and a sensitized locomotor effect in response to a challenge injection of cocaine in Test 3. Although the conditioned stressor itself did not elicit a cross-sensitized locomotor response in Test 2, it strongly potentiated the effect of a cocaine challenge in cocaine-sensitized rats in Test 3. That is, rats that had been given repeated cocaine injections followed by odor-footshock pairings showed a much higher level of activity in response to a cocaine challenge at test than did any other group. To our knowledge, this is the first report of a facilitation of cocaine-induced locomotor sensitization by a conditioned stressor.

Disclosures: **A. Sasaki:** None. **A. Hussain:** None. **P. Shanmuganathan:** None. **A. Sivakumaran:** None. **S. Sivanathan:** None. **S. Erb:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.05/Y13

Topic: C.17. Drugs of Abuse and Addiction

Support: DA009815

Title: Aversive taste reactivity predicts escalation of cocaine self-administration behavior and preference in rats

Authors: *E. M. COLECHIO[1], D. N. ALEXANDER[2], C. G. IMPERIO[1], K. JACKSON[3], P. S. GRIGSON[2];
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Abstracts: Addiction is a disease of chronic relapse, often elicited by drug-associated cues even after prolonged periods of abstinence. Therefore, understanding the link between cues and drugs of abuse is of great interest. Our central hypothesis focuses on elucidating the predictive value of early cue reactivity for later cocaine intake and addiction-like behaviors in a rodent model. Specifically, we tested whether taste reactivity (TR) to a drug-paired cue relatively early in acquisition can predict later escalation of cocaine self-administration behavior. Phase I. Rats received 6 TR-self-administration sessions. During each session, a Kool-Aid flavored saccharin solution was intraorally infused at a rate of 1 infusion/min for 30 min followed by a 2 h opportunity to self-administer 0.33 mg/infusion cocaine (n=17) or saline (n=5). Phase II. Thereafter, the rats were given 15 daily trials of 6 h access to cocaine (0.66 mg/infusion), after which the willingness to work for cocaine was assessed using progressive ratio (PR). Phase III. The relative value of the Kool-Aid-flavored saccharin cue vs. cocaine was assessed in a single 6-h two-choice test. Results. The results showed that greater aversive TR during Phase I was associated with escalated cocaine self-administration during the extended-access phase, a greater willingness to work for cocaine (PR), and an increased preference for cocaine over Kool-Aid-flavored saccharin when both were available. Thus, TR to the drug-paired cue early in training predicts later drug-taking behavior: eg., how fast a rat will take cocaine, how much cocaine the rat will take, whether drug-taking will escalate over time, how hard a rat will work for drug, and

whether a subject will prefer drug or the Kool-Aid flavored natural reward cue. This work was supported by DA009815.

Disclosures: **E.M. Colechio:** None. **D.N. Alexander:** None. **C.G. Imperio:** None. **K. Jackson:** None. **P.S. Grigson:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.06/Y14

Topic: C.17. Drugs of Abuse and Addiction

Support: 101-2410-H-006-045-MY2

Title: The modulating effects of rottlerin on psychostimulant-induced conditioned place preference

Authors: *T. LIAO, L. YU;
Inst. of Behavioral Medicine, Natl. Cheng K, Tainan, Taiwan

Abstracts: Brain-derived neurotrophic factor (BDNF) has been known to regulate cocaine-seeking behavior. Rottlerin can increase brain BDNF levels in a long-lasting manner. We hypothesized that rottlerin can decrease psychostimulant-induced conditioned place preference (CPP). To test this hypothesis, C57BL/6 mice were pretreated with single intraperitoneal rottlerin injection (5 mg/kg) immediately after the unconditioned place preference pretest. We found that such a treatment effectively decreased methamphetamine (MA)- (1 mg/kg/conditioning) and cocaine (10 mg/kg/conditioning)-induced CPP as three drug-place and vehicle-place conditionings were employed. Likewise, pretreatment with 7,8-dihydroxyflavone (10 mg/kg, i.p.), a selective TrkB agonist, prior to each cocaine-place conditioning was found to decrease cocaine-induced CPP. In an attempt to characterize the modulating effects of rottlerin on the maintenance of psychostimulant memory, single rottlerin injection was given approximately 24 hours before the 3-day forced extinction trainings. Rottlerin (5 mg/kg) did not affect the extinction of the psychostimulant-induced CPP but to significantly decrease the drug-primed reinstatement of psychostimulant-induced CPP. These results, taken together, suggest that systemic rottlerin administration may be beneficial in decreasing the hedonic value of psychostimulants and facilitating the forgetting of the psychostimulant-associated memory.

Disclosures: **T. Liao:** None. **L. Yu:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.07/Y15

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA-IRP (AHN)

DA012460 (MAN)

DA022413 (JAJ)

MH54137 (JAJ)

Title: The development of novel dopamine D3 receptor-selective partial agonists as potential medications to treat psychostimulant abuse

Authors: *C. A. BOATENG[1], O. M. OKUNOLA-BAKARE[1], T. M. KECK[1], C. BURZYNSKI[1], C. SCHWEPPE[1], R. RAIS[2], B. SLUSHER[2], P. DONTHAMSETTI[3], J. A. JAVITCH[3], W. JOHN[4], P. CZOTY[4], M. A. NADER[4], A. H. NEWMAN[1];
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Abstracts: The dopamine D3 receptor (D3R) is involved in brain reward pathways and is a promising therapeutic target for treatment of substance abuse and other neuropsychiatric disorders. Several highly selective D3R antagonists and partial agonists based on the 4-phenylpiperazine scaffold have been discovered. One of the most selective and high affinity D3R partial agonists, (±)PG648 (N-(4-(4-(2,3-dichloro-phenyl)piperazin-1-yl)-3-hydroxybutyl)-1H-indole-2-carboxamide; Newman et al. 2009) and its enantiomers were selected as leads for behavioral evaluation, first in a quinpirole-elicited yawning model of D3R *in vivo* activity and later in both rodent and nonhuman primate models of psychostimulant abuse. Although microsomal stability, pharmacokinetic and behavioral data in rodents looked promising, PG648 was not active in nonhuman primates. We hypothesized that either bioavailability/metabolism in the rhesus monkeys was different from rodents or its very high D3R selectivity was problematic. In order to address these questions, we have prepared analogues in which the 2,3-diCl-phenyl

piperazine was replaced with either a 2-OMe, -3-Cl- or a 2,3-naphthyl-substituent. In addition, bioisosteric replacement of the indole in PG648 with quinoline was also explored. We also prepared analogues with either the unsubstituted or the 3-OH substituted 4-carbon linker between the arylpiperazine and aryl amide to determine its effects on D3R affinity, selectivity, efficacy and metabolic stability. Thirty-two novel ligands were synthesized by conjugating 4-bromobutyl- or epoxideethylphthalimide with the substituted arylpiperazines. The resulting intermediates were deprotected to give the primary amines, which were coupled with the arylcarboxylic acids to give the desired 3-OH-substituted or unsubstituted carboxamides. Binding affinities were determined using [3H]NMS radioligand binding competition assays in membranes prepared from HEK293 cells expressing human dopamine D2-like receptors. By varying the arylpiperazine substitution, we found several ligands displayed high affinity (Ki=0.1-3.6 nM) and (20-100-fold) selectivity profiles at the D3 versus D2 receptors. Based on binding profiles, a subset of analogues was evaluated in a D3R BRET1-based GαoA activation functional assay and for mouse microsomal stability. These new lead compounds are currently being evaluated in models of psychostimulant abuse and preliminary data suggest that chemical modification of PG648 has resulted in promising behavioral activity in nonhuman primates.

Disclosures: **C.A. Boateng:** None. **O.M. Okunola-Bakare:** None. **T.M. Keck:** None. **C. Burzynski:** None. **C. Schweppe:** None. **R. Rais:** None. **B. Slusher:** None. **P. Donthamsetti:** None. **J.A. Javitch:** None. **W. John:** None. **P. Czoty:** None. **M.A. Nader:** None. **A.H. Newman:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.08/Y16

Topic: C.17. Drugs of Abuse and Addiction

Title: The use of 6Beta Naltrexol to treat anxiety and depression during acute cocaine withdrawal

Authors: *M. J. MUELLER[1], T. DEYOUNG[2], J. BOYETTE-DAVIS[2];
[1]York Col. of Pennsylvania, New Park, PA; [2]York Col. of Pennsylvania, York, PA

Abstracts: Cocaine addiction has been and continues to be an issue in today's society with 16.5% of those aged 26 and older using cocaine at some point in their lifetime. Withdrawal symptoms are unpleasant and include an overwhelming craving for the drug as well as fatigue,

anxiety, sleeplessness, irritability and depression. Naltrexone has been used to decrease withdrawal from various addictive substances, including alcohol and narcotics. Unfortunately, various reports have found that withdrawal symptoms may temporarily increase with the use of Naltrexone. More recent reports have found that 6Beta Naltrexol, a novel neutral receptor antagonist, lessens withdrawal symptoms for alcohol cessation. This study investigated the effects of Naltrexone and 6Beta Naltrexol on cocaine withdrawal. Twenty-seven male Sprague Dawley rats received injections of 15mg/kg of cocaine for 14 consecutive days. The rats were withdrawn from the drug for 24 hours prior to the beginning of behavioral testing. Anxiety and depression were assessed in the rats using the elevated plus maze (EPM) and the forced swim test (FST), respectively. The results showed that half of the rats responded to the drugs while the other half did not. When the rats were split into high-responders and low-responders, it was found that those who were high-responders to the drugs experienced significantly less anxiety and depression. This study may have implications for the medications given to an addict when they are experiencing cocaine withdrawal.

Disclosures: **M.J. Mueller:** None. **T. DeYoung:** None. **J. Boyette-Davis:** None.

Poster

616. Cocaine: Behavioral Studies

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Program#/Poster: 616.09/Y17

Topic: C.17. Drugs of Abuse and Addiction

Support: LABEX BRAIN ANR-10-LABX-43

ANR-2010-BLAN-1404-01

CRA11004375/11004699

CNRS

Université de Bordeaux

Title: Neuronal correlates of resilience to cocaine addiction in rats

Authors: ***Y. VANDAELE**, S. NAVAILLES, A. DURAND, K. GUILLEM, S. AHMED; Gironde, IMN UMR 5293, Bordeaux, France

Abstracts: When faced with a choice between two competing actions, taking cocaine or drinking sweet water, most rats prefer the nondrug activity, even after prolonged drug use. Only few rats prefer the drug. These findings suggest that, like in people, most rats would be resilient to addiction while a minority would be vulnerable. Here we sought to unravel the neuronal correlates of resilience to addiction. We imaged brain activity in the majority of nondrug-preferring rats in response to cocaine versus sweet water (the preferred activity), using large-scale Fos mapping. Rather surprisingly, we found that the preferred activity induced little specific brain activity, except in the thalamocortical gustatory pathway. In contrast, cocaine taking elicited more brain activity; particularly in brain regions involved in reward omission and frustration (e.g., the lateral habenula and the tail of the VTA). We hypothesize that this result likely reflects the frustration of not having access to the preferred reward during testing. We are currently testing this hypothesis by imaging brain activity in nondrug-preferring rats in experimental conditions that rule out the involvement of preferred reward omission and frustration. We will also compare and contrast the obtained pattern of brain activity to that observed in drug-preferring rats. Ultimately, this work will identify specific neuronal correlates of resilience and vulnerability to cocaine addiction.

Disclosures: **Y. Vandaele:** None. **S. Navailles:** None. **A. Durand:** None. **K. Guillem:** None. **S. Ahmed:** None.

Poster

616. Cocaine: Behavioral Studies

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.10/Y18

Topic: C.17. Drugs of Abuse and Addiction

Support: 101-2410-H-006-045-MY2

Title: The modulating effects of companions on cocaine-induced conditioned place preference

Authors: *W.-Y. TZENG[1], L. YU[2];
[1]Inst. of Basic Med. Sci. of NCKU, Tainan, Taiwan; [2]Inst. of Behavioral Med. of NCKU, Tainan, Taiwan

Abstracts: A number of social factors have been known to affect drug use and the development of drug dependence. Lately, we found that the presence of three cocaine-free or -treated companions in cocaine conditionings reliably decreased the magnitude of cocaine-induced

conditioned place preference (CPP) especially as moderate doses (10 and 20 mg/kg/conditioning) of cocaine were used. We hereby further showed that the presence of three methamphetamine (MA)-treated (1 mg/kg) companions also decreased cocaine (20 mg/kg/conditioning)-induced CPP. Likewise, the presence of three saline-treated companions during the MA (1 mg/kg/conditioning) conditionings significantly decreased the magnitude of MA-induced CPP. Finally, ibotenic acid (5 μg/side) infusion-produced lesion was performed in bilateral dorsal hippocampus (DH) and amygdala (Amg) three days prior to the unconditioned preference pretest. The results showed that bilateral amygdalar or hippocampal lesion per se did not affect the magnitude of cocaine-induced CPP. Nonetheless, bilateral amygdalar, not hippocampal, lesion abolished the companion-produced decreasing effects on cocaine-induced CPP. The results, taken together, suggest that the presence of companions during psycho-stimulant conditionings can diminish the psycho-stimulant-induced CPP. Moreover, Amg is necessary for mediating the companions' decreasing effects on cocaine-induced CPP.

Disclosures: W. Tzeng: None. **L. Yu:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.11/Y19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Effects of Δ^8-Tetrahydrocannabivarin (Δ^8-THCV) on appetitive effects of cocaine and nicotine in rodents

Authors: *E. L. GARDNER[1], P. MULDOON[2], X.-F. WANG[1], G.-H. BI[1], M. DAMAJ[2], A. H. LICHTMAN[2], R. G. PERTWEE[3], Z.-X. XI[1];
[1]Chem Biol Res. Br., NIDA/IRP, BALTIMORE, MD; [2]Dept. of Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA; [3]Sch. of Med. Sciences, Inst. of Med. Sci., Univ. of Aberdeen, Aberdeen, United Kingdom

Abstracts: Growing evidence suggests that blockade of brain cannabinoid CB_1 receptors or activation of brain cannabinoid CB_2 receptors attenuates the rewarding effects of cocaine or other addictive drugs such as nicotine. Δ^8-Tetrahydrocannabivarin (Δ^8-THCV) is a synthetic analogue of the plant cannabinoid Δ^9-tetrahydrocannabivarin (Δ^9-THCV), which exhibits CB_1

receptor antagonist and CB_2 receptor agonist profiles. Thus, dual CB_1 receptor blockade and CB_2 receptor activation might produce an additive or synergistic therapeutic anti-reward effect. To test this hypothesis, we observed the effects of Δ^8-THCV on cocaine and on nicotine self-administration and other addiction-related behavior. We found that systemic administration of Δ^8-THCV (3, 10, 20 mg/kg, i.p.) failed to alter intravenous cocaine self-administration in wild-type or CB_2 receptor-knockout mice, but dose-dependently inhibited intravenous nicotine self-administration in alcohol-preferring rats and wild-type mice. Co-administration of Δ^8-THCV and AM630, a selective CB_2 receptor antagonist, blocked Δ^8-THCV's action in alcohol-preferring rats, and genetic deletion of CB_2 receptors (in CB_2 receptor-knockout mice) partially attenuated Δ^8-THCV's action on nicotine self-administration. Also, systemic administration of Δ^8-THCV (0.3-3 mg/kg, i.p.) inhibited nicotine-induced conditioned place preference and nicotine-seeking behavior in wild-type mice during extinction in the absence of nicotine and nicotine-associated cues. Further, CB_2 receptor-knockout mice show significantly lower levels of nicotine self-administration with longer inter-infusion intervals than their wild-type littermates, an effect similar to drug-taking behavior maintained by a higher dose of nicotine. Taken together, these findings suggest that: 1) Δ^8-THCV may have anti-nicotine, but not anti-cocaine, therapeutic effects - partially mediated by activation of CB_2 receptors; and 2) deletion of CB_2 receptors appears to enhance nicotine's rewarding effects. Further studies are required to confirm these findings.

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Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

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Program#/Poster: 616.12/Y20

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA PO1 DA031656

CIHR 201311MFE

Title: Taking cocaine versus staying on task: Drug cue-evoked competition for attention and individual differences in vulnerability to cue-evoked task shifts

Authors: *K. PITCHERS, C. J. SKRZYNSKI, T. E. ROBINSON, M. SARTER;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstracts: Subjects vulnerable for addiction-like behavior have a propensity for transforming conditioned reward cues to cues that capture their attention and instigate cue-directed behavior. The goals of this research are to develop a behavioral paradigm for determining the power of drug cues to shift subjects from performing a food-rewarded attention task to taking drug, to determine individual variation in the power of drug cues to do so, the neurobiological mediation of such variation, and to find treatments that increase the resistance of subjects to disengage from the task. Rats acquired an operant sustained attention task (SAT), which involved the reporting of hits and correct rejections (water-rewarded), and misses and false alarms (not rewarded) via retractable levers. Next, they were trained to self-administer cocaine, and after the acquisition of stable self-administration behavior, were either maintained on a FR schedule (nose-pokes; infusion criterion 40; IC40) or shifted to a second self-administration procedure: intermittent access (IntA). For IntA training, cocaine access was limited to 5-min periods cued by a tone (DS+), alternating with 25-min timeout periods (DS-, white noise). After stable behavior the propensity to shift from one task (SAT) to self-administration was assessed in chambers equipped with nose-poke ports placed at the opposite wall of the SAT intelligence panel. In IC40 animals, the presence of the nose-poke ports and concomitant availability of cocaine did not immediately disrupt SAT performance as rats only gradually switched to drug-taking late during SAT session. In contrast, in IntA rats, the presentation of the DS+ at the 10 min mark evoked an immediate switch away from SAT to drug-taking. Ongoing research will determine whether rats classified as sign- and goal-trackers differ in their vulnerability to DS+-evoked task shifts and whether this vulnerability can be improved with enhancement of the cholinergic system. Our paradigm models the power of drug cues, as opposed to unrestricted access to drug-taking, to deprioritize SAT performance and thus, is suitable for studying the cognitive and neurobiological mechanisms via which drug cues trigger drug-taking and relapse.

Disclosures: K. Pitchers: None. **C.J. Skrzynski:** None. **T.E. Robinson:** None. **M. Sarter:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.13/Y21

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of sensory overstimulation in early life on vulnerability to cocaine addiction

Authors: *S. RAVINDER, D. A. CHRISTAKIS, J. M. RAMIREZ, S. M. FERGUSON; Seattle Children's Res. Inst., Seattle, WA

Abstracts: We have previously developed a rodent model of "excessive non-normative stimulation" which mimics excessive media use in infants and toddlers whereby mice are exposed to audio stimuli (clips from the cartoon network channel) paired with visual stimulation (flashing lights) for six hours per day, starting at P10, for a total of 42 days. When tested subsequently, overstimulated mice demonstrate increased risk taking, decreased anxiety, poorer short-term memory and impaired learning relative to controls. In this study, we investigated the relationship between excessive non-normative stimulation early in life and vulnerability to drugs of abuse in adulthood by studying cocaine-induced psychomotor sensitization. Following sensory overstimulation, male CD-1 mice received 10 daily injections of cocaine (15mg/kg) or saline over a 2-week treatment period. After a 2-week withdrawal, all mice received an escalating dose challenge of cocaine (saline, 10mg/kg, 20mg/kg). Locomotor activity, monitored for 60 mins after each injection, was used as an index of psychomotor sensitization. We found that saline-treated overstimulated mice were hyperactive compared to controls, yet there did not appear to be differences between groups in locomotor responses following cocaine treatment. However, when normalizing to the hyperactive baseline, overstimulated mice displayed blunted locomotor sensitization to cocaine. Nonetheless, because of the differences in locomotor baselines we are currently conducting conditioned place preference experiments to further assess whether overstimulated mice show differences in drug abuse vulnerability. Our results suggest that non-normative overstimulation modifies the dopaminergic system leading to a blunted cocaine response and studies are underway to assess physiological changes in the VTA dopaminergic neurons of overstimulated mice to examine this. These findings are interesting in the context of ADHD, as Ritalin treatment blunts hyperactive responses in patients but has the opposite effect in controls. In addition, our observations are consistent with a large body of literature which suggests that exposure to positive as well as negative experiences early in life can have a profound impact on behavioral traits and can modulate vulnerability to developing neuropsychiatric illnesses.

Disclosures: S. Ravinder: None. **D.A. Christakis:** None. **J.M. Ramirez:** None. **S.M. Ferguson:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

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Program#/Poster: 616.14/Y22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant MH102930

NIH Grant DA025319

NIH Grant DA033877

Title: Psychopharmacological responsiveness to cocaine across early ontogeny: possible role of D2High receptors

Authors: *S. E. EATON, A. MOHD-YUSOF, C. A. CRAWFORD, S. A. MCDOUGALL; Dept. of Psychology, California State Univ., San Bernardino, CA

Abstracts: Research suggests that sensitivity to dopaminergic drugs varies across ontogeny, with behavioral responsiveness to psychostimulants being greater during the preweanling period than in adulthood. Adolescence, which is a developmental epoch that falls between the preweanling period and adulthood, is a time of unique behavioral responsiveness to psychostimulant drugs. It is most frequently reported that cocaine and amphetamine produce a hypoactive behavioral response in adolescent animals when compared to younger and older age groups. Curiously, other researchers report a nearly opposite pattern of effects, with acute and repeated cocaine treatment causing hyperresponsiveness during the adolescent period. The neural mechanisms responsible for these age-dependent differences in behavioral responsiveness are uncertain, but they cannot be explained in terms of ontogenetic changes in dopamine receptor numbers (i.e., maturational changes in D1 and D2 receptor densities do not correspond to alterations in drug sensitivity). Instead, it is possible that age-dependent differences in the percentage of high affinity D2 receptors, rather than overall receptor numbers, might account for ontogenetic changes in drug sensitivity. To test this hypothesis, $D2^{High}$ receptors in the dorsal striatum of male and female rats were measured during the preweanling period (i.e., PD 5, PD 10, PD 15, and PD 20), adolescence (PD 40), and adulthood (PD 80). Behavioral responsiveness to cocaine was assessed at PD 20, PD 40, and PD 80. D2 specific binding and the ratio of $D2^{High}$ receptors was measured in the dorsal striatum using [^{3}H]-domperidone; whereas, behavioral responsiveness to cocaine (0, 2.5, 5, 10, or 20 mg/kg) was assessed in automated activity chambers during a 120 min testing session. On the two days prior to cocaine testing, rats were injected with saline and habituated to the activity chambers for 60 min. Results showed that the percentage of dorsal striatal $D2^{High}$ receptors was significantly greater in preweanling rats (48.78%) than in adolescent (24.06%) or adult (24.39%) rats. Among the various preweanling age groups (PD 5, PD 10, PD 15, and PD 20) there was no difference in the percentage of $D2^{High}$ receptors. In terms of the behavioral data, rats tested with cocaine on PD 20 exhibited greater

behavioral responsiveness than the two older age groups. This pattern of results is consistent with the hypothesis that age-dependent changes in the percentage of $D2^{High}$ receptors are responsible for ontogenetic differences in sensitivity to psychostimulant drugs.

Disclosures: S.E. Eaton: None. **A. Mohd-Yusof:** None. **C.A. Crawford:** None. **S.A. McDougall:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

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Program#/Poster: 616.15/Y23

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant P30DK079638

NIH Grant P30DA028821

NIH Grant T32DA07287

Title: A novel neuropeptide regulator of behavioral responses to cocaine

Authors: *J. KASPER[1], C. R. BENZON[2], D. L. MCCUE[3], J. D. HOMMEL[2];
[1]Univ. Texas Med. Br., Galveston, ; [2]Pharmacol. and Toxicology, [3]Neurosci., Univ. of Texas - Med. Br., Galveston, TX

Abstracts: Neuromedin U (NMU) is a neuropeptide expressed in the brain, including the mesolimbic pathway. While NMU has been studied for its ability to regulate food reward, NMU has not been studied in the context of drugs of abuse (e.g., cocaine). Therefore, we evaluated the effects of NMU on behavioral sensitization to cocaine, a behavior that is dependent on neural plasticity in the nucleus accumbens shell (NAcSh). In this study, NMU was microinjected directly to the NAcSh of cocaine sensitized and non-sensitized rats shortly before a cocaine challenge. NMU was found to decrease acute, but not sensitized, cocaine-evoked locomotion. This suggests that NMU signaling regulates the acute locomotor response to cocaine. In a separate experiment, NMU blocked cocaine-evoked locomotion on challenge day when administered throughout sensitization. Taken together, these data indicate that NMU signaling in the NAcSh modulates the development of behavioral sensitization to cocaine. The neural pathways that might underlie the change in locomotor activity were investigated using confocal microscopy with immunofluorescence. The primary receptor for NMU in the brain, NMU

receptor 2 (NMUR2) was found in the NAcSh and colocalizes with synapsin I and GAD67, markers of synapses and GABAergic neurons respectively. Furthermore, anterograde viral tracers injected into the dorsal raphe nucleus suggest that synaptic NMUR2 in the nucleus accumbens is associated with dorsal raphe neurons. This work suggests a novel NMU pathway from the dorsal raphe to the nucleus accumbens which is capable of regulating behavioral responses to cocaine.

Disclosures: J. Kasper: None. **C.R. Benzon:** None. **D.L. McCue:** None. **J.D. Hommel:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

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Program#/Poster: 616.16/Y24

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R21-DA029787

VA Grant 589-KG-0012

Title: Synergistic effects of pilocarpine and tacrine on cocaine-reinforced behavior

Authors: *F.-C. YANG, H. XU, K. GRASING;
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Abstracts: Muscarinic agonists cause pronounced reductions in drug-reinforced responding, especially if they exhibit selectivity for the M1 muscarinic receptor subtype. Pilocarpine is a muscarinic agonist which shows good M1 selectivity in transformed cells, and has an acceptable safety profile in humans. Tacrine is an additional agent that strongly attenuates drug-reinforced behavior. To compare their respective mechanisms, we evaluated intravenous self-administration of cocaine after separate or combined treatment with pilocarpine and tacrine. METHODS: Rats were trained to self-administer cocaine under a fixed-ratio-5 (FR-5) schedule during two-hour multiple-component sessions in which 0.1, 0.2, and 0.4 mg/kg per injection of cocaine were each available for 40 minutes (low, intermediate, or high doses, respectively). Following intraperitoneal treatment with test compounds at low, intermediate, or high doses (1.0, 3.2, or 10 mg/kg-injection), rats were allowed to self-administer intravenous cocaine. RESULTS: Pretreatment with either pilocarpine or tacrine caused linear reductions in cocaine-reinforced responding, with high doses attenuating responding by more than 80% for low-dose cocaine.

After receiving combined treatment attenuation of responding for intermediate-dose cocaine was greater than additive effects of either agent. CONCLUSION: Based on this, we propose that the different mechanism underlying pilocarpine and tacrine effects on cocaine-reinforced responding exhibit synergism. Future studies should investigate the neurotransmitter changes underlying these actions. Because they are clinically available, pretreatment with pilocarpine and an monoamine oxidase inhibit may be a productive avenue for therapy of substance abuse disorders.

Disclosures: **F. Yang:** None. **H. Xu:** None. **K. Grasing:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

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Program#/Poster: 616.17/Y25

Topic: C.17. Drugs of Abuse and Addiction

Support: CNPq (303331/2012-7, 478930/2012-7)

FAP -DF (193.000.408/2010)

Title: Cocaine induces a persistent dose-dependent place-preference response in marmoset monkeys

Authors: ***A. C. BORGES**[1], R. B. M. DUARTE[2], L. L. NOGUEIRA[2], A. BORGES[2], M. BARROS[3];
[1]Univ. De Brasília, Brasilia, Brazil; [3]Dept. of Pharmaceut. Sciences, Sch. of Hlth. Sci., [2]Univ. de Brasilia, Brasília, Brazil

Abstracts: Place conditioning (CPP) using psychostimulant drugs has been extensively used in rodents, but few studies have analyzed drug-induced CPP in non-human primates. A previous study in our lab showed that a daily 5mg/kg i.pinjection of cocaine induced CPP in marmosets after nine consecutive sessions. Here we tested the influence of doses and the number of drug-context pairings on the magnitude and the duration of retention of cocaine-induced CPP in ten adult male marmoset monkeys (Callithrix penicillata). The CPP box consisted of a rectangular box with two compartments with different tactile and visual cues. A central door allowed the subjects entered to the apparatus. Tests were held from 13:00 to 17:00 h. The procedure was divided into three phases: Habituation: consisting of two consecutive 20-min sessions (days 1 and 2) with free access to both compartments. Conditioning: training was conducted for twelve

days (six drug sessions and six saline sessions) in which subjects received cocaine (3 or 7 mg/kg/i.p. according to their group) or saline (1ml/kg/i.p.) injections and were immediately confined in the drug- or the saline-paired compartments for 20 min. Tests: conducted on days 9 and 16. Animals were allowed to explore both compartments of the apparatus for 20 min. The preference was assigned by comparing the time spent in the drug-paired compartment in the habituation with total time spent during tests sessions. Results from habituation showed that the subjects had no inherent preferences for one specific compartment. We found that, after three cocaine-pairing sessions, a CPP response was observed only for the group that received the lower dose. The higher dose tended to increase time in the conditioned compartment but it did not reach statistical relevance. On the other hand, after the 6th drug-pairing session, there was a CPP of similar magnitude for both doses of cocaine tested. Fifteen days after the last drug-conditioning training, CPP remained only for the higher dose of cocaine tested. It is possible that the higher dose of cocaine initially caused aversion and CPP was only established when the aversive effect of the higher doses has been decreasing or changes in the brain occurred. The lower dose, although initially creating CPP, might not have long-lasting effects on the brain's circuitry.

Disclosures: A.C. Borges: None. **R.B.M. Duarte:** None. **L.L. Nogueira:** None. **A. Borges:** None. **M. Barros:** None.

Poster

616. Cocaine: Behavioral Studies

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.18/Y26

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA09815

Title: Ceftriaxone attenuates acquisition and facilitates extinction of cocaine-induced suppression in C57BL/6J mice

Authors: *C. S. FREET[1], A. L. LAWRENCE[2];
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Abstracts: Rodents avoid intake of a palatable natural reward when paired with a drug of abuse such as cocaine (Cappell, LeBlanc, & Endrenyi, 1973; Carey & Goodall, 1974; Goudie, Dickins, & Thornton, 1978). As such, cocaine-induced suppression models an important criterion for

substance use disorder found in the DSM-5 (i.e., loss of important or desired activities due to drug use). Growing evidence implicates glutamate homeostasis in a number of behaviors observed in addiction such as acquisition of drug taking, motivation and reinstatement (Ward et al., 2011; Knackstedt et al., 2010). The aim of the current study was to evaluate the beta-lactam antibiotic, ceftriaxone, which has been shown to normalize disrupted glutamate homeostasis associated with exposure to drugs of abuse, in the acquisition and extinction of cocaine-induced suppression in C57BL/6J mice. Individual differences in cocaine-induced suppression were observed (i.e., low and high suppressors) with differential effects of ceftriaxone. Ceftriaxone delayed suppression of saccharin intake in high suppressors but eliminated suppression in low suppressors. In addition, ceftriaxone history facilitated extinction in the high suppressors. These data suggest that changes in glutamate homeostasis may be involved in the development of cocaine-induced suppression of saccharin intake and that individual differences significantly influence the response of this system to cocaine.

Disclosures: C.S. Freet: None. **A.L. Lawrence:** None.

Poster

616. Cocaine: Behavioral Studies

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA007348

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NIH Grant DA033344

NIH Grant DA032898

Title: Rats with a cocaine history develop compulsive appetite due to disruption in non-homeostatic control of food intake

Authors: *F. WEISS[1], Y. HAO[1], A. MATZEU[1], G. DE GUGLIELMO[1], P. PANDAY[1], T. KERR[1], R. MARTIN-FARDON[1], T. C. JHOU[2], R. C. RITTER[3], N. SUTO[1];

[1]The Scripps Res. Inst., La Jolla, CA; [2]Med. Univ. of South Carolina, Charleston, SC; [3]Washington State Univ., Pullman, WA

Abstracts: Obesity and pathological overeating have received increasing recognition as disorders of 'food addiction' because of their similarities to substance dependence in behavioral manifestations and neurobiological underpinnings. However, the applicability of this nosology remains a matter of debate. We hypothesized that a cocaine history known to result in addiction-like drug motivation and corresponding brain changes would result in similar addiction-like motivation for food. Separate groups of rats were allowed to self-administer saccharine or cocaine. Extended daily access to cocaine (6h/day or 'long access' [LgA]) resulted in escalated cocaine intake, whereas limited access (1hr/day or 'short access' [ShA]) did not, as previously reported. All rats then were given the opportunity to self-administer a food reward, sweetened condensed milk (SCM). A cocaine (both LgA and ShA) but not saccharine history was associated with heightened SCM seeking and intake, characterized by heightened resistance to extinction, increased workload, and punishment, as described for drug addiction. Importantly, this occurred without significant changes in bodyweight and baseline feeding. The failure to show weight gain and overeating per se suggests that the addiction-like appetitive behavior or 'compulsive appetite' that developed in rats with a cocaine history is due to dysregulation of non-homeostatic (non-metabolic) rather than homeostatic (metabolic) control of food intake. Consistent with this hypothesis, rats with a cocaine history exhibited heightened resistance to punishment when responses reinforced by a non-caloric palatable reinforcer, saccharine, were paired with electric footshock. Moreover, rats with a cocaine history showed functional upregulation of group II metabotropic receptors (mGluR2/3) in the medial prefrontal cortex (mPFC) and amygdala, brain sites implicated in non-homeostatic control of food intake. These receptors negatively modulate neural excitability, possibly contributing to the impaired functional connectivity between mPFC and amygdala observed in drug addicts. Overall, the current findings suggest that the nosology of addiction is most applicable to phenotypes of eating disorders that are characterized by compulsive appetite such as binge-eating disorder and bulimia nervosa. Neurobiological irregularities in the non-homeostatic pathway - abnormalities akin to those observed in drug addicts - possibly provide a neuroregulatory basis for compulsive appetite. **Support:** NIDA/NIH DA007348 (F.W.), DA008467 (F.W.), DA033533 (N.S), DA033344 (R.M.F.), DA032898 (T.C.J.).

Disclosures: **F. Weiss:** None. **Y. Hao:** None. **A. Matzeu:** None. **G. de Guglielmo:** None. **P. Panday:** None. **T. Kerr:** None. **R. Martin-Fardon:** None. **T.C. Jhou:** None. **R.C. Ritter:** None. **N. Suto:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.20/Y28

Topic: C.17. Drugs of Abuse and Addiction

Title: Cocaine-conditioned locomotor response is mediated by reactive oxygen species signaling

Authors: *J. D. NGUYEN, M. J. FORSTER;
Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstracts: We have previously shown that compounds that produce alterations in cellular redox state can modulate the cocaine-conditioned locomotor response; however, the role of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-generated reactive oxygen species (ROS) has not been well characterized. Compounds that modulate intracellular and extracellular ROS signaling mechanisms were tested for their effects on the acquisition and expression of context-dependent increases in locomotion produced by a single exposure to cocaine. Cocaine (40 mg/kg) was administered to different groups of Swiss-Webster mice via intraperitoneal injection (i.p) in either a locomotor activity testing apparatus or the home cage, 2 hours following an activity test under saline. Mice placed in the testing chambers were given 30 minutes to explore freely and locomotion was monitored. A conditioned effect of cocaine was inferred by an increase in horizontal activity counts relative to home cage cocaine controls during a test in the same apparatus on the following day. N-acetylcysteine (10-250 mg/kg), dimethylthiourea (5-50 mg/kg), L-ascorbic acid (25-500 mg/kg), alpha-tocopherol (25-100 mg/kg), apocynin (1-50 mg/kg), or vehicle was administered prior to placement into the activity chamber on the test day. N-acetylcysteine and dimethylthiourea inhibited the expression and the acquisition of cocaine-conditioned locomotion, L-ascorbic acid and apocynin increased acquisition and expression of the conditioned effect, respectively, and alpha-tocopherol failed to affect the conditioned response. These results suggest that (i) attenuation of cocaine-conditioned behavior may be achieved by modulation of cellular redox state, (ii) a facilitation of the conditioned response may be due to pro-oxidizing redox state or inhibition of NADPH oxidase, and (iii) attenuation of the actions of ROS in cell membranes is not sufficient to prevent conditioning. Thus, ROS signals may mediate context associations during the development of addiction. These findings further support the potential value of redox modulating compounds as targets for addiction treatment.

Disclosures: J.D. Nguyen: None. **M.J. Forster:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.21/Y29

Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC Discovery Grant

Title: Cocaine pre-treatment induces a shift in the balance of motivational control over behavior in approach-avoidance conflict paradigms

Authors: *D. NGUYEN, S. ERB, R. ITO;
Psychology, Univ. of Toronto Scarborough, Toronto, ON, Canada

Abstracts: Addiction is a disease characterized by persistence to seek drug reinforcement despite negative consequences. Such maladaptive behaviors are attributable to drug-induced neuronal adaptations in the cortico-limbic-striatal circuitry implicated in learning, motivation, inhibitory control, and decision making. Dysregulation of neurotransmission in this circuitry is suggested to contribute to aberrant approach and avoidance processing for motivationally relevant stimuli, thus producing a bias towards appetitive behaviors and facilitating the development and sustenance of an addiction phenotype. Although enhanced reward seeking may result from a shift in balance of behavioral control by competing neuronal processes, the effects of repeated drug exposure on the resolution of motivational conflict under conditions wherein appetitive and avoidance motivations are simultaneously evoked have yet to be thoroughly investigated. In the present study, male Long Evans rats received daily IP injections of 30 mg/kg cocaine or saline for 7 d. Following a 10 d drug-free period, rats were tested in one of three behavioral paradigms. In the runway paradigm, rats learned that entering the goal compartment of a runway apparatus would lead to sucrose reward along with shock administrations. Latency to enter the goal compartment was taken as a measure of motivational conflict. In our radial arm maze paradigm, rats were conditioned to associate separate texture bar cues with either the delivery of sucrose or shock in the arms in which the cues were presented. Exploration time was then assessed in a final conflict test during which rats freely explored two maze arms containing either a neutral cue or a superposition of the appetitive and aversive cues for 5 min. For our active avoidance paradigm, rats were conditioned to lever press to avoid impending shock upon presentation of a conditioned tone stimulus. They were then tested in a conflict situation in which they could lever press to either avoid impending shock or to receive sucrose reward. Our results revealed that cocaine pre-treated rats display lower latencies to enter the mixed valence goal compartment in the runway. Cocaine pre-treated rats also showed a delay in learning for the aversive cue association in the radial arm maze. Finally, our preliminary data suggest that

cocaine pre-treated rats show impairment in acquisition of avoidance responding when motivational conflict is evoked in the active avoidance paradigm. Together, we conclude that neuronal imbalances induced by repeated cocaine exposure allow appetitive motivations to gain greater influence over behavioral output *in situ*ations of motivational conflict.

Disclosures: D. Nguyen: None. **S. Erb:** None. **R. Ito:** None.

Poster

616. Cocaine: Behavioral Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 616.22/Y30

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant P30GM32128

UW CHS travel grant

Title: Exposure to a high-fat diet attenuates the locomotor-stimulating effects of cocaine

Authors: *P. M. DINGESS[1], B. J. ANDERSON[2], A. E. CREAGER[2], R. A. DARLING[1], E. K. DOLENCE[2], T. E. BROWN[1];
[1]Neurosci., [2]Pharm., Univ. of Wyoming, Laramie, WY

Abstracts: According to the National Institute of Health over consumption of diets rich in high-fat (HF) is one of the contributing factors to the obesity epidemic. Various laboratories have shown that exposure to a HF diet evokes changes in dopaminergic signaling within the reward circuitry. However, the effect HF has on general reward processing is still unclear. In our first set of experiments, we examined the effects of HF diets on cocaine-induced locomotion to assess whether HF may influence the sensitivity to the stimulating effects of cocaine. Rats were placed on either a normal chow (NC), HF *ad libitum*, or HF calorically restricted diet for 1 or 3 weeks. Animals were then tested in locomotor boxes to assess activity via photobeam breaks. Cumulative activity induced by novelty, saline injection (intraperitoneal (i.p.)) or cocaine (5 or 15mg/kg, i.p.) was assessed for each dietary condition. At 1 week there was a significant gain in body weight but no significant differences in any of the dietary groups with regard to locomotor activity. However at 3 weeks, there was a significant increase in body weight and decrease in beam breaks in the HF group injected with 15 mg/kg cocaine (21,128 ± 1,721, n=14) compared to NC (33,705 ± 2,965, n=8). The same reduction in cocaine-induced (15mg/kg) locomotor

activity was observed in the HF calorically restricted group (20,286 ± 2,623, n=14) when compared to normal chow controls (30,342 ± 3241, n=9). Our results indicate that the HF diet is having an effect on cocaine-induced locomotor activity and this effect is independent of body weight. To address whether there is a general hypo-responsiveness of the reward circuitry in animals exposed to a HF diet an additional set of rats were placed on *ad libitum* HF or NC diets for 3 weeks, fasted for a 24-hr period and subsequently tested in an overnight sucrose self-administration task. Our results show that rats fed a HF diet have a significant attenuation in lever responses (429 ± 50, n=6) compared to the NC controls (858 ± 45, n=8). In our final experiment, animals were fed either a HF calorically restricted or NC *ad libitum* diet for 3 weeks. Following dietary exposure, extracellular basal dopamine levels were assessed using high-performance liquid chromatography (HPLC) in the prefrontal cortex (PFC), nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus, and amygdala. Our results demonstrate a significant increase in basal dopamine in the PFC in the HF group (979.487 ± 410.584, n=8) relative to NC controls (100.000 ± 40.176, n=8). Overall, our results lead us to conclude that exposure to the HF diet elicits changes in reward processing that manifests as a reduction in reward sensitivity.

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Poster

617. Cocaine: Neural Mechanisms III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 617.01/Y31

Topic: C.17. Drugs of Abuse and Addiction

Support: JPB Foundation for Medical Research

the Department of Defense (W81XWH-09-1-0381)

National Institute on Drug Abuse (P01 DA008227)

Title: Characterization of the role of limbic p11 on addiction and depression

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Abstracts: The high rate of comorbidity between depression and cocaine addiction suggests shared molecular mechanisms and anatomical pathways. Here we investigate the contribution of p11 (S100A10) in two limbic structures, the Nucleus Accumbens (NAc), and the infralimbic Cortex (ILc) in both disorders. We have previously shown that downregulation of p11, a critical adaptor protein, specifically in the NAc elicits depressive-like behaviors in mice but its role in drug addiction is unknown. Conversely, human imaging has evidenced the involvement of the NAc and ILC on depression, yet the contribution of ILc p11 on depression is unknown. We combine mouse genetics and viral strategies to titrate p11 levels within the ILc, the NAc or its specific cell types. We investigate how p11 in the NAc affects the rewarding actions of cocaine on behavior and molecular correlates; and how ILc p11 contributes to ILc connectivity and tricyclic antidepressant response. We demonstrate that p11 knockout mice have enhanced cocaine conditioned place preference, which is reproduced by the focal downregulation of p11 in the NAc of wild-type mice. In wild-type mice, cocaine inhibited p11 expression in the NAc, while p11 overexpression exclusively in the NAc reduced cocaine CPP. Finally, we identify dopamine receptor-1 expressing medium spiny neurons as key mediators of p11's effects on cocaine reward. We show that ILc p11 does not mediate depressive-like phenotypes, but ILc p11 defficiency facilitates rapid onset antidepressant effects of imipramine, and mimic the effect of chronic imipramine treatment on the novelty induced hypophagia test. Our data provide evidence that disruption of p11 homeostasis in the NAc and ILc may underlie pathophysiological mechanisms of comorbid ailments as depressive disorders and of cocaine addiction.

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Poster

617. Cocaine: Neural Mechanisms III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 617.02/Y32

Topic: C.17. Drugs of Abuse and Addiction

Support: R21DA035592

R01DA035055

Title: Epigenetic readers of lysine acetylation regulate cocaine-induced behavioral plasticity

Authors: *G. C. SARTOR, S. K. POWELL, S. P. BROTHERS, C. WAHLESTEDT;
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Abstracts: Epigenetic processes that regulate histone acetylation play a critical role in behavioral and molecular responses to cocaine. However, to date, only a small fraction of the mechanisms involved in the addiction-associated acetylome have been investigated. 'Readers' of acetylated histone, referred as bromodomain containing proteins, have recently been shown to be key regulators of chromatin dynamics and disease state, but their role in addiction is unknown. Here, we found that chronic cocaine exposure and cocaine self-administration significantly increased expression of the BET bromodomain protein, Brd4 (but not Brd2 or Brd3) in the nucleus accumbens (NAc) of rats and mice. Behaviorally, we found that systemic and intra-accumbal administration of the BET bromodomain (BRD2, BRD3, BRD4, and BRDT) inhibitor, JQ1, reduced acquisition of cocaine conditioned place preference (CPP) in mice, whereas a similar bromodomain inhibitor that does not cross the blood brain barrier (I-BET 151, 50 mg/kg, i.p.) had no affect on cocaine CPP. JQ1 (50 mg/kg, i.p.) alone did not produce a condition place preference or aversion, nor did JQ1 (50 mg/kg, i.p.) alter LiCl-induced (150 mg/kg, i.p.) conditioned place aversion, indicating that JQ1 does not affect all types of contextual learning. Additionally, JQ1 attenuated the expression of brain-derived neurotrophic factor (BDNF) in the NAc. Ongoing studies are investigating potential mechanisms by which BETs regulate BDNF expression in response to cocaine. In summary, these observations support a role for BET bromodomain proteins as novel epigenetic regulators of cocaine-induced behavioral plasticity.

Disclosures: G.C. Sartor: None. **S.K. Powell:** None. **S.P. Brothers:** None. **C. Wahlestedt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of Epigenetix.

Poster

617. Cocaine: Neural Mechanisms III

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NIH R01DA027664

Title: The role and regulation of histone deacetylase 4 (HDAC4) in cocaine-related behaviors

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Abstracts: Repeated exposure to drugs of abuse alters the function of the nervous system and produces states that encourage continued drug use. Changes to chromatin landscape and transcriptional accessibility, mediated in part by nuclear enzymes such as histone deacetylases (HDACs), are thought to play important roles in the development and persistence of addiction-related behaviors. HDAC4, a member of the Class IIa HDAC family, shuttles between the nucleus and cytoplasm in response to neuronal activity, and overexpression studies have implicated it in drug-related behaviors in the nucleus accumbens (NAc). As such, we sought to determine whether and how HDAC4 is regulated by cocaine, and whether this regulation is important for its suspected role(s) in addiction-related behavioral plasticity. Our findings indicate that acute and chronic cocaine differentially regulates HDAC4's phosphorylation state and nuclear/cytoplasmic localization, suggesting that the change in cellular localization might contribute to changes in cocaine-induced behaviors. We find that nuclear, but not cytoplasmic, HDAC4 suppresses cocaine reward behavior, and this was associated with strong repression of MEF2-dependent transcription activity. As a complementary approach to mutant expression within the NAc, we generated conditional gene deletion of HDAC4 from the adult nucleus accumbens using viral-mediated expression of Cre recombinase. Our preliminary findings indicate that HDAC4 regulates sensitivity to cocaine and the development of psychomotor sensitization, and ongoing work is evaluating its role in the extinction and reinstatement of cocaine conditioned place preference.

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Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant: 1R01 DA03505501.

Title: A role for natural antisense transcripts in cocaine reward and addiction

Authors: *S. K. POWELL[1,2], G. C. SARTOR[2], D. VELMESHEV[2], S. P. BROTHERS[2], C. WAHLESTEDT[2];
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Abstracts: Growing evidence indicates an important role of epigenetic regulation in the mechanisms of cocaine addiction. Chronic cocaine use causes long-lasting changes in gene expression in the brain's reward areas that contribute to persistent drug-seeking and drug-taking behaviors. Recent reports indicate that epigenetic processes are key factors in such neuroadaptations and behaviors. Thus, understanding epigenetic mechanisms of addiction is crucial to develop targeted therapeutic interventions. However, the majority of epigenetic regulators and associated mechanisms have yet to be investigated. Representing an unexplored yet promising addiction-related target, natural antisense transcripts (NATs), transcripts encoded on the strand opposite to the sense strand on either protein-coding or non-protein coding genes, have recently been shown by our laboratory and others to be key regulators of chromatin state. However, to date, little is known about the expression of NATs in the context of addiction. Here, we investigated a role for NATs in response to cocaine by injecting c57bl/6 male mice with cocaine (20 mg/kg, i.p.) or saline once a day for 10 days. The nucleus accumbens (NAc) and other brain regions were collected at multiple time points after the last injection. Of addiction-related genes investigated, many were found to have natural antisense transcripts, and the expression of several of these antisense transcripts was also found to be significantly altered following chronic cocaine administration. These data indicate that cocaine disrupts the expression of addiction-related NATs, and that NATs may play an important role in cocaine addiction. Ongoing studies will determine the role of these NATs in the molecular mechanisms of cocaine reward and to explore their functions in animal models of addiction.

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Poster

617. Cocaine: Neural Mechanisms III

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Program#/Poster: 617.05/Z3

Topic: C.17. Drugs of Abuse and Addiction

Support: ANPCyT PICT 227-2008

Title: Involvement of Wnt/βcatenin pathway in cocaine induced sensitization

Authors: *S. CUESTA, S. B. ROSSO, A. M. PACCHIONI;
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Abstracts: Wnt factors are cistein rich secreted proteins which interact with one of 2 membrane receptors: Frizzled and Ryk. As a result of the interaction Dishevelled (DVL) is activated, and consequently, one of three pathways: canonical or Wnt/βcatenin, Planar Cell Polarity, and Wnt/calcium pathways. These three ways participate in different cell fates decisions like synaptogenesis, cell and tissue polarity and cell movement. Despite all the information about these factors in mammalian brain development, little is known regarding its role in adulthood. In the last years it has been revealed that Wnt pathways are involved in neuropsychiatric diseases. In schizophrenia, antipsychotic and amphetamine treatments lead to opposite changes in Wnt's effectors. Taking into account that all these evidence involves the dopaminergic pathways, our main goal was to evaluate the role of Wnt pathway in the long-lasting neuroadaptations induced by cocaine. According to recent evidence, we started evaluating the Wnt/βcatenin pathway, where the activation of DVL inhibits GSK3β and lead to the stabilization of βcat. We have already found that development of cocaine induced sensitization after 7 days of cocaine treatment (2x15 mg/kg i.p and 5x30 mg/kg i.p.) is associated with modifications in βcatenin (βcat) levels in prefrontal cortex (PFC), amygdala (Amyg) and dorsal striatum (DS). Moreover we have also found that a systemic treatment with a non-specific inhibitor of the Gsk3β blocks the development of cocaine sensitization by restoring βcat´s modifications. Our new data reveals that changes in βcat levels are only present when an animal showed behavioral sensitization after a cocaine treatment. On the other hand, we found that behavioral sensitization is not only related to a reduction in βcat in PFC, DS and Amyg, but also to an increase in Gsk3β activity and a reduction in Axin2 mRNA levels (target gene of the pathway) in PFC, suggesting an inhibition of Wnt/βcatenin pathway in this area. Then, we evaluate if these PFC changes were necessary for cocaine sensitization. In order to do that, rats received an intra-PFC infusion of Sulindac an hour before cocaine between day 2 and 6 of a 7 i.p. injections, administered once a day, of 15mg/kg of cocaine. The results showed that blocking PFC Wnt/βcatenin pathway with Sulindac, prior to cocaine injections, enhances the development of behavioral sensitization. So far our data suggests that cocaine sensitization is associated with modifications of Wnt/βcatenin pathway, and particularly, with an inhibition in PFC.

Disclosures: S. Cuesta: None. **S.B. Rosso:** None. **A.M. Pacchioni:** None.

Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA R01 DA023988

Title: CLOCK interacts with co-repressor protein complexes to negatively regulate tyrosine hydroxylase: Mechanisms underlying the circadian control of dopamine and cocaine reward

Authors: *R. W. LOGAN[1], W. P. WILLIAMS, III[1], S. WAPLINGER[1], S. SPENCER[2], M. M. SIDOR[1], C. A. MCCLUNG[1];
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Abstracts: Mood and addiction disorders are associated with disruptions to the circadian system, although the mechanisms underlying these associations are poorly understood. The circadian gene, Clock, is highly expressed in the ventral tegmental area (VTA), a brain region that sends major dopaminergic projections with the mesocorticolimbic system that is implicated in mood and drug circuitry. Previously, we showed that mice carrying a mutation in exon 19 of this gene, ClockΔ19, exhibited increased levels of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, and enhanced sensitivity to rewarding stimuli, including cocaine. Here, we sought to determine the precise regulatory mechanisms by which CLOCK impacts the dopaminergic system that may be critical for behavioral responses to cocaine reward. Under normal conditions, CLOCK acts as a transcription factor by binding to enhancer elements (E-boxes) within gene promoters to promote transcription. However, we found that CLOCK may act as a transcriptional repressor of TH in the VTA, potentially through interactions with phosphoactive CRE-element binding protein (pCREB), the principle driver of TH transcription, and the recruitment of repressor proteins, Cryptochrome 1 (CRY1), and the histone deacetylase, sirtuin 1 (SIRT1). Using chromatin immunoprecipitation (ChIP) assays in wild-type mice, we found that CLOCK and pCREB bound the TH promoter in antiphase_maximum CLOCK and pCREB binding at ZT4 and ZT16, respectively. In the VTA of ClockΔ19 mice, however, pCREB binding was constitutively high, which was mirrored by elevated expression of TH mRNA across the entire day. Additionally, we found that mutation of the E-box sites in the TH promoter significantly increased TH-Luciferase reporter activity *in vitro*, suggesting CLOCK binds to these loci to repress TH transcription. In the cocaine conditioned place preference test, we also found that viral-mediated gene transfer of mutant CREB (mCREB) into the VTA normalized the enhanced reward sensitivity to cocaine of ClockΔ19 mice. To determine whether

CLOCK may recruit co-repressor proteins, we measured the protein levels of CRY1 and SIRT1, along with their protein-protein interactions. Both CRY1 and SIRT1 levels were reduced in the VTA of ClockΔ19 mice. Ongoing studies are investigating whether these proteins are recruited by CLOCK and/or pCREB to regulate TH transcription. Together, these experiments provide a molecular mechanism by which diurnal rhythms of TH expression are regulated by CLOCK and may represent a circadian-regulated pathway of drug reward.

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Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant P50 DA00266

Title: Inositol polyphosphate multikinase regulates cocaine-induced behavioral effects

Authors: *M. M. HARRAZ, R. XU, I. AHMED, S. H. SNYDER;
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Abstracts: Transient induction of immediate early genes in response to various stimuli mediate synaptic plasticity and long-term changes in neural function. Drugs of abuse including cocaine induce immediate early genes expression. However, the molecular signals that link cocaine to immediate early genes induction are not well characterized. Recently, we demonstrated that the inositol polyphosphate multikinase (IPMK) is required for immediate early genes induction. Here we investigated the role of IPMK signaling pathway in cocaine-mediated actions utilizing IPMK conditional knockout mice. Our findings demonstrate that IPMK regulates cocaine-induced behavioral effects and suggest that IPMK signaling is a potential therapeutic target in cocaine abuse disorders.

Disclosures: M.M. Harraz: None. **R. Xu:** None. **I. Ahmed:** None. **S.H. Snyder:** None.

Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: ZONMW TOP subsidie 91211002

Title: Investigation of the PFC-amygdala pathway and its role in addiction in serotonin transporter knockout rats

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Abstracts: During their lifetime only a subset of cocaine users make the transition to true addiction. One factor that may contribute to individual differences in liability to this transition is the serotonin transporter polymorphism (5-HTTLPR). Indeed, it has been demonstrated that the low activity short (s) allelic variant of this polymorphism increases risk for cocaine addiction. It has also been observed that carriers of the s-allelic version show an uncoupling between their prefrontal cortex (PFC) and the amygdala. Here we hypothesize that vulnerability to cocaine addiction in low 5-HTT expressing individuals is at least in part caused by this uncoupling. To test this hypothesis we use the 5-HTT knockout (5-HTT-/-) rat, an extreme model for 5-HTTLPR s-allele carriers. These animals model the human s-allele carrying population in many of their behaviors. In light of addiction these animals show increased responding to drugs as mirrored by the human DSM IV criteria. More specifically, we previously showed that these 5-HTT-/- rats show increased cocaine self-administration under short (1 hour, regular drug taking) and long access (6 hours, compulsive drug taking) conditions, an increased motivation to self-administer the drug under a progressive ratio schedule of reinforcement, and a failure to extinguish cocaine-seeking behavior. To test whether this increased addictive phenotype is caused by PFC-amygdala uncoupling, 5-HTT-/- and wild-type rats were trained in the long access paradigm of cocaine self-administration for 4 weeks. As controls a naïve group and a group trained to self-administer sucrose were included. All animals were perfused using 4% paraformaldehyde and brains were analyzed using a 9.4 Tesla rat MRI scanner focusing on the PFC and amygdala volumes and pathways connecting these areas. Here, we present the preliminary findings of this work.

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Poster

617. Cocaine: Neural Mechanisms III

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Fondation de l'Avenir

NIDA

Title: Loss of control over cocaine intake: The subthalamic nucleus as the critical actor

Authors: Y. PELLOUX[1], C. COHEN[1], A. TIRAN-CAPPELLO[1], S. LARDEUX[2], O. GEORGE[3], G. F. KOOB[4], S. H. AHMED[5], *C. BAUNEZ[1];
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Abstracts: The subthalamic nucleus (STN) is critically involved in reward-related behavior. Lesion or high frequency stimulation (HFS) of the STN decreases the motivation to obtain cocaine, without decreasing that to obtain more natural rewards (Baunez et al., 2005; Rouaud et al., 2010). This selective reduction in the motivation for cocaine use suggests that the STN might be necessary for reinforcement sustained by drug of abuse and suggests that it could represent a promising brain target for therapy against cocaine addiction. The present study sought to further validate this hypothesis by testing the effects of bilateral inactivation of STN, by either lesions or high frequency stimulation (HFS), on establishment of escalation of cocaine self-administration. Escalation of cocaine use is a hallmark of drug addiction that is observed in rats when availability of the drug increases (Ahmed and Koob 1998). Here we show first that both STN lesions and HFS prevent escalation of cocaine intake. By recording local field potentials within the STN of rats escalating their cocaine intake, we also show that increased oscillations in the STN could serve as a marker of loss of control over cocaine intake. This outcome suggests a role

for the STN in the development of cocaine addiction and confirms that acting at the level of STN may be an interesting strategy for the treatment against cocaine addiction.

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Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Optogenetic stimulation of red nucleus glutamate neurons inhibits cocaine self-administration in mice

Authors: Y. HE, H.-Y. ZHANG, G.-H. BI, H.-J. YAU, H. SHEN, E. GARDNER, A. BONCI, *Z. XI;
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Abstracts: It was recently reported that optogenetic activation or inactivation of glutamate neurons in the prefrontal cortex, ventral hippocampus (VH) or amygdala alters cocaine-seeking and reward-seeking behavior. However, little is known as to whether activation or inactivation of glutamate neurons in these and other brain regions similarly alters cocaine self-administration. The red nucleus (RN) in the midbrain is a structure anatomically adjacent to both the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), and the majority of RN neurons are glutamatergic. Interestingly, while RN neurons have been shown to modulate locomotion, little is known on which RN neurons modulate locomotion and whether RN glutamate neurons modulate drug-taking and drug-seeking behavior. In the present study, we used optogenetic strategies to explore these questions. We delivered Cre-dependent adeno-associated viral (AAV) vectors [AAV-EF1a-DIO-hChR2 (H134R)-EYFP] into distinct brain regions of vGluT2-IRES-cre mice to selectively express light-sensitive channelrhodopsin-2 (ChR2) in glutamatergic neurons. We found that, 1) optogenetic activation of glutamate neurons in the RN (targeted by both the AAV and optical fibers) significantly inhibited intravenous cocaine self-administration. 2) photostimulation of ChR2-containing glutamatergic terminals in the nucleus accumbens (NAc) in mice that had received intra-VTA AAV microinjections produced a similar inhibitory

effect on cocaine self-administration; 4) optogenetic activation of RN glutamate neurons failed to alter basal or cocaine-enhanced locomotion; and 5) photostimulation of glutamate neurons in the RN or glutamatergic terminals from the VTA to NAc failed to maintain intracranial self-stimulation behavior. Taken together, these behavioral findings suggest that RN glutamate neurons appear to play an important role in cocaine self-administration, and therefore, may constitute a new therapeutic target for treatment of drug abuse and addiction.

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Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: CAPES

PROPPI-UFF

FAPERJ

Title: GABA uptake changes in mice prefrontal cortex after a cocaine Exposure: effects on anxiety-like behavior

Authors: ***M. P. CARVALHO**[1], R. MARTINS[2], N. PENICINALLI[1], A. C. MANHÃES[3], R. C. C. KUBRUSLY[1];
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Abstracts: Aim: Cocaine is a psychostimulant, which acts in central nervous system, increasing the levels of catecholaminergic neurotransmission. Moreover, this drug can modulate different neurotransmitters systems, like GABAergic synapses. We are investigating the molecular mechanisms related to GABA uptake induced by acute cocaine (COC) exposure and its effects on anxiety-like behavior in adolescent mice. Methods: Swiss Webster mice in P40 were treated with cocaine (10; 20; 25; 30 mg/kg/ip) or saline. The pre frontal cortex (PFC) was used for [3H]-GABA uptake. To evaluate the anxiety-like behavior we use elevated plus-maze apparatus (EPM). Animals were randomly selected into four groups: (A) saline, (B) treated with COC 20

mg/kg/ip, (C) treated with 25 mg/kg/ip, (D) cocaine 30 mg/kg/ip. The animals were free to explore the apparatus for 5 minutes. To estimate the locomotor activity Open Field apparatus (OF) was used. All experiments were conducted in accordance with ethical committee, under the protocol CEUA/065/2012. Results: The % of time in open arms in all of the groups tested were not different (A: 8.959 ± 1,941 B: 15.35±3.33; C: 11.12±2.25; D: 14.67±2.84; n=16). The % of entries in the open arm had no difference between the groups (B: 26.57±5.57 C: 27.06±4.84 D: 34.17±4.62; n=16) compared to group A; (25.64±3.06; n=16). The % of time in Center has showed no modifications among the groups (B: 21.27±4.03; C: 25.11±2.952; D: 19.07±2.44; n=16) and saline (17.46±1.60; n=16). Although the analysis in the OF to the distance traveled revealed no differences among the tested groups (A: 79.31±11.87; b: 102.0±10.4; C: 103.2±8.81; D: 80.85±7.25; n=16) the rearing analyses showed that groups B (49.40±2.75; n=16) and C (37.19±4.13; n=16) had an increase in this behavior compared to saline group (18.86±3.22; n=16). Moreover, the % of time in the Center demonstrated that animals in group C (62.56±3.31; n=16) spent higher time than control group (35.65±8.2 n=16). COC 25mg/kg inhibited GABA uptake (97.93±6.95 n=14) compared to control group (148.4±7.80 n=14). After a short withdraw 24h or 48h GABA uptake levels were restored (24h: 188.0±18.83; 48h: 182.4±12.13; n=8) Conclusion: The acute cocaine exposure inhibited GABA uptake just after its administration however after a short-withdrawal GABA uptake levels were similar to the saline group showing that the system seems to be sensitized. This changes in the uptake could lead to an anxiolytic and impulsive behavior as was shown in the OF apparatus.

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Poster

617. Cocaine: Neural Mechanisms III

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Title: Estradiol rapidly enhances dopamine in nucleus accumbens shell of female but not male rats

Authors: K. E. YOEST[1], J. A. CUMMINGS[1], K. N. CHAMBERLAIN[4], Y. A. ALONSO[2], B. J. ARAGONA[1], *J. B. BECKER[3];
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Abstracts: There are well established sex differences in the susceptibility to addiction, where women show faster escalation of drug use and are more prone to relapse than their male counterparts. Similar findings have been reported in rodent models, where female rats acquire cocaine-taking behavior more rapidly than males. Gonadal hormones modulate this sex difference, as the positive subjective effects of psychomotor stimulants are enhanced by estradiol treatment in women, and estradiol also enhances the acquisition of and motivation for cocaine self-administration in female rats. Our laboratory has demonstrated that acute estradiol administration also enhances cocaine-induced dopamine in the striatum, but not the nucleus accumbens (NAc) measured via microdialysis, (Cummings et al., Drug and Alcohol Dependence, 135:22-28, 2014). In this previous experiment, the microdialysis probe was in the boundary of the core and shell of the NAc. We postulated that our failure to see an effect of estradiol in the NAc was due to probe placement, since estradiol has been reported to have opposite effects in the NAc core and shell. With this experiment, we tested the effect of acute estradiol treatment on stimulated cocaine-induced dopamine release and reuptake in the NAc shell. Ovariectomized females and intact males were anesthetized with urethane and treated with estradiol benzoate (EB) or vehicle 30 min prior to administration of cocaine (10 mg/kg i.p.). Fast scan cyclic voltammetry (FSCV) was used to measure stimulated dopamine release in the NAc shell both prior to and after cocaine administration. We found that acute EB treatment significantly increased stimulated dopamine release as measured by FSCV in females but not males after cocaine. We also found that acute EB significantly reduced reuptake of dopamine after stimulation in cocaine-treated males but not females. These findings demonstrate a significant sex difference in the mechanism by which estradiol enhances dopamine release in NAc shell. Importantly, dopamine signaling in the NAc has been implicated in acquisition and escalation of drug taking behaviors, aspects of addiction that are more pronounced in female addicts. Elucidating the mechanism by which estradiol may render women more susceptible to compulsive drug use will help to develop gender-specific treatments in clinical settings.

Disclosures: K.E. Yoest: None. **J.A. Cummings:** None. **Y.A. Alonso:** None. **B.J. Aragona:** None. **J.B. Becker:** None. **K.N. Chamberlain:** None.

Poster

617. Cocaine: Neural Mechanisms III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 617.13/Z11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant GM-08224

NIH Grant R25 GM-061838

Title: Alpha-1 adrenergic receptor modulation of ventral tegmental area dopamine neurons: role in cocaine sensitization

Authors: *M. C. VELASQUEZ-MARTINEZ[1], M. E. VELEZ-HERNANDEZ[2], B. SANTOS-VERA[2], A. VAQUER-ALICEA[2], R. VAZQUEZ-TORRES[2], C. A. JIMENEZ-RIVERA[2];
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Abstracts: The ventral tegmental area (VTA) plays an important role in reward and motivational processes that facilitate drug addiction. Previous investigations indicate that presynaptic alpha1-adrenoreceptors (α1-AR) modulate glutamate and GABA release on VTA dopaminergic (DA) neurons. We hypothesized that chronic but not acute cocaine treatment alters presynaptic α1-AR modulation of glutamate and GABA neurotransmission on VTA DA neurons. We recorded VTA DA neuronal excitatory (EPSCs) and inhibitory postsynaptic currents (IPSCs) using whole-cell voltage clamp in brain slices from male rats previously treated with one or five daily cocaine injections. We also recorded EPSCs from brain slices of animals after a 7 day withdrawal period. After acute cocaine treatment, α1-ARs increased EPSCs amplitude. In contrast, following chronic cocaine injections α1-ARs failed to increase EPSCs amplitude. Therefore, subsequent to a chronic cocaine administration paradigm, α1-ARs were desensitized and their pharmacological stimulation did not augment EPSCs amplitude. Moreover, PKC stimulation with phorbol 12-myristate 13-acetate failed to increase glutamate release in slices obtained from cocaine sensitized animals. However, after acute or chronic cocaine treatments α1-ARs stimulation decreased IPSCs amplitude. , α1-ARs actions on glutamatergic transmission on VTA DA neurons were desensitized following a 7 day withdrawal period. These data suggest that α1-ARs enhance DA neuronal excitability after repeated cocaine administration most probably by reducing GABA inhibition onto VTA DA neurons. A better understanding of α1-AR modulatory changes in cocaine sensitization will increased our knowledge of the role of the noradrenergic system in cocaine addiction and might provide possible avenues for therapeutic pharmacological interventions (Supported by GM-08224 to CAJR and R25 GM-061838 to MVH).

Disclosures: **M.C. Velasquez-Martinez:** None. **M.E. Velez-Hernandez:** None. **B. Santos-Vera:** None. **A. Vaquer-Alicea:** None. **R. Vazquez-Torres:** None. **C.A. Jimenez-Rivera:** None.

Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: NARSAD GTS 35482

NIDA DA025279

Title: Sex differences in hypocretin modulation of dopamine signaling in the nucleus accumbens

Authors: ***J. K. SHAW**, R. A. ESPAÑA;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstracts: Women progress to cocaine dependence more rapidly and are more prone to stress-induced relapse than men. Preclinical evidence is consistent with this, indicating that females acquire self-administration more readily, display higher motivation for cocaine and are more likely to reinstate cocaine use than their male counterparts. The mechanisms underlying these sex-differences have yet to be fully elucidated but likely involve the mesolimbic dopamine (DA) system_a pathway that is modulated by hypocretin (HCRT) projections to DA neurons in the ventral tegmental area (VTA). Although increased HCRT expression in the female hypothalamus has been described, sex differences in HCRT regulation of dopamine transmission have not been sufficiently investigated. Here we used *in vivo* voltammetry to detect HCRT-mediated sex differences in DA release and uptake. We implanted HCRT knockout (KO) or wild-type (WT) male and female mice with a bipolar stimulating electrode in the VTA and a carbon fiber electrode in the caudate and nucleus accumbens (NAc) core, where DA release was electrically evoked every 5 minutes. Following collection of at least three stable baseline samples we administered 10mg/kg cocaine i.p. and monitored subsequent DA transmission. Results from a subset of animals confirm data in rats that females demonstrate higher concentrations of DA in caudate relative to males regardless of genotype. In the NAc, our data suggest an interaction between sex and genotype in regulating DA transmission; whereas HCRT KO males demonstrate reduced evoked DA relative to WT males, KO females instead demonstrated higher

concentrations of evoked DA that WT females. Our results also indicate an interaction between sex and genotype on DA responses to cocaine. Taken together, these observations suggest a differential role for HCRT regulation of DA reward pathways based on sex.

Disclosures: J.K. Shaw: None. **R.A. España:** None.

Poster

617. Cocaine: Neural Mechanisms III

Location: Halls A-C

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Program#/Poster: 617.15/Z13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA (DA025279)

NIDA (DA031900)

Title: Isoflurane is an alternative anesthetic for *in vivo* voltammetry

Authors: *Z. D. BRODNIK[1], K. HARRIS[2], R. ESPANA[3];
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Abstracts: Electrophysiology and voltammetry experiments in anesthetized animals commonly use urethane as an anesthetic of preference. Urethane produces an extended surgical-level anesthesia with only modest changes in circulation, respiration, and reflex responses, and therefore is thought to minimally interfere with the physiological relevance of data. Despite these benefits, there are also disadvantages to the use of urethane for *in vivo* experiments. In particular, urethane is considered to be cytotoxic, carcinogenic and immunosuppressive and thus is limited to use in terminal experiments. Isoflurane is an inhalation anesthetic drug that holds advantages over injectable agents. For example, isoflurane anesthesia requires minimal animal handling, has a large margin of safety, and can be rapidly adjusted to maintain an appropriate level of anesthesia throughout an experiment. To examine whether isoflurane could serve as an anesthetic for use during *in vivo* voltammetry experiments, we tested the effects of isoflurane on the uptake kinetics of stimulated dopamine release in the nucleus accumbens and compared results to those obtained with urethane. Uptake parameters Km and V_{max} were modeled, and we determined that these parameters did not differ between low doses of isoflurane and urethane. Furthermore, we found that the same dose of isoflurane did not alter changes in dopamine uptake

in response to intravenous cocaine. Together these data identify isoflurane as viable for use in anesthetized voltammetry experiments.

Disclosures: **Z.D. Brodnik:** A. Employment/Salary (full or part-time):; Drexel University College of Medicine. **K. Harris:** None. **R. Espana:** A. Employment/Salary (full or part-time):; Drexel University College of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIDA (DA025279), NIDA (DA031900).

Poster

617. Cocaine: Neural Mechanisms III

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Title: Ventral tegmental area regulation of the prefrontal cortex is superactivated by chronic cocaine self-adminstration

Authors: *W. BUCHTA[1], A. RIEGEL[2];
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Abstracts: Dopamine (DA) release in the prefrontal cortex (PFC) is thought to gate sensory information important for interpreting the saliency of environmental stimuli. In addiction, the prefrontal cortex (PFC) becomes hyper-responsive to drug-associated sensory cues, disrupting executive function. Although DA terminals emanating from the Ventral Tegmental Area (VTA) participate in cue processing, the underlying mechanism and its role in addiction remains unclear. To investigate this relationship, Cre-dependent AAVs were injected into the VTA of transgenic (TH:Cre) rats to selectively transfect VTA DA cells with channel rhodopsin (ChR2) or Gs/Gq-coupled synthetic receptors (DREADDs). After ~30d, PFC (prelimbic area) brain

slices were prepared. Whole cell patch clamp electrophysiological recordings were performed in L5 pyramidal cells and VTA terminals were stimulated with either transient pulses of blue light (to activate ChR2) or bath application of CNO (to activate DREADDs). Under baseline conditions, cells displayed robust spike-frequency adaptation (accommodation) and a large slow after-hyperpolarization (sAHP). Activation of ChR2 or DREADDs reduced the sAHP, reduced accommodation, and increased firing. Similar changes were observed with bath application of DA, the D1 agonist SKF81297 or direct activation of cyclase activity with forskolin. Co-application of cocaine increased the percentage of responsive cells, but not the magnitude of firing. D1-antagonism or depletion of intracellular Ca2+ stores blocked the actions of DA. Thus, VTA terminals regulate PFC accommodation via a DA/D1/AC/PKA/Ca2+ cascade, and DA's influence is expanded during an acute application of cocaine. To evaluate this mechanism in a model of addiction, recordings were made in slices from animals with a history of chronic cocaine self-administration. Under these conditions, cells demonstrated (a priori) a compromised sAHP and accommodation, as well as hyperexcitable firing rates_an adaptation that was absent in slices from matched yoked-cocaine animals that received identical amounts of cocaine but were not subjected to drug-related cues. Acute blockade of DA-D1 receptors, inhibition of PKA, depletion of intracellular Ca2+, or stabilization of KCNQ (KV7) ion channels restored accommodation following chronic cocaine self-administration. Taken together these data suggest that VTA terminals utilize a DA-D1 receptor mechanism to regulate PFC excitability and this mechanism is superactivated following chronic cocaine self-administration. This neuroadaptation may underlie the enhanced saliency of drug-related cues that trigger relapse in cocaine addicts.

Disclosures: W. Buchta: None. **A. Riegel:** None.

Poster

617. Cocaine: Neural Mechanisms III

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Title: Role of 5-HT1A and 5HT2A in cocaine-mediated modulation of GABA release from the thalamic reticular nucleus

Authors: B. GOITIA[1], N. WEISSTAUB[2], J. GINGRICH[3], E. GARCIA-RILL[4], V. BISAGNO[5], *F. J. URBANO[1];
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Abstracts: Methylphenidate (MPH), a drug widely used to treat children diagnosed with ADHD, and cocaine (Coc) inhibit the re-uptake of dopamine and norepinephrine. Cocaine, unlike MPH, also inhibits the re-uptake of serotonin (5-HT). Previously, we observed that the frequency of spontaneous GABA release from thalamic reticular nucleus (Ret) neurons is increased in slices from mice treated with a Coc binge (3 i.p. injections, 15mg/kg each, 1 hour apart) but not in those from animals treated with an MPH binge, suggesting that the effect of Coc is mediated by changes in serotonergic transmission. Thus, we investigated the effect of 5-HT receptors agonists and antagonists in control and Coc treated thalamocortical slices from mice using patch clamp. Additionally, we administered Coc to mice lacking 5-HT2A receptors. We first recorded miniature inhibitory post-synaptic potentials (mIPSPs) from ventrobasal neurons using thalamocortical slices from control mice in the presence of 5-HT and 5-HT1A or 5HT2A/2C agonists ((±)-8-OH-DPAT, (±)-DOI hydrochloride; both 10μM) and antagonists (NAN-190 hydrobromide, ketanserin; both 25μM). Our results show that the effect of bath-applied 5-HT (100 μM, >15min) on mIPSP frequency resembles that of Coc (5-HT, Coc vs. control, Kruskal Wallis test, $p<0.05$), and that even though both types of agonists led to an increase in frequency, the effect of the 5-HT1A agonist was stronger (8-OH-DPAT=347% increase, DOI=129% increase; 8-OH-DPAT vs. DOI vs. control, Kruskal Wallis test, $p<0.05$). Surprisingly, the 5HT2A antagonist also had the same effect (Kruskal Wallis test, $p<0.05$), which could be explained by a biphasic action of 5HT2A receptors at the GABAergic afferents terminals from Ret neurons. We then analyzed the effect of 5-HT 100 μM applied locally ("puff") onto ventrobasal neurons during recordings of mIPSCs from mice lacking 5-HT2A receptors (both control or injected with a Coc binge). In both cases, the application of 5-HT resulted in a reduction of the mIPSPs frequency that after the puff recovered to initial values only in the control group (post-puff frequency was 96% of the initial value in control group vs. 58% in Coc binge group, Wilcoxon Mann-Whitney test, $p=0.0075$). Our findings suggest that Coc-induced effects on thalamic serotonergic transmission might be mediated by presynaptic 5-HT receptors (on the terminals of Ret neurons), and that these effects could be responsible for thalamocortical abnormalities observed in cocaine addicts and animal models of Coc intake.

Disclosures: **B. Goitia:** None. **N. Weisstaub:** None. **J. Gingrich:** None. **E. Garcia-Rill:** None. **F.J. Urbano:** None. **V. Bisagno:** None.

Poster

617. Cocaine: Neural Mechanisms III

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA031916

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Title: Fluoxetine potentiates methylphenidate-induced gene regulation in the striatum: Role of 5-HT1B serotonin receptor

Authors: V. VAN WAES, S. EHRLICH, J. BEVERLEY, *H. STEINER;
Chicago Med. School/RFUMS, North Chicago, IL

Abstracts: The psychostimulant methylphenidate (Ritalin) is used in the treatment of attention-deficit hyperactivity disorder (ADHD) and as a cognitive enhancer in the healthy. Methylphenidate, like cocaine, acts by blocking the reuptake of dopamine. However, unlike cocaine, methylphenidate does not affect serotonin. Serotonin contributes to addiction-related gene regulation by cocaine. Thus, the lack of a serotonin effect may explain methylphenidate's more moderate gene regulation effects and addiction liability. Our previous studies indeed show that enhancing serotonin action by adding a selective serotonin reuptake inhibitor (SSRI), fluoxetine (Prozac), to methylphenidate potentiates methylphenidate-induced gene regulation in the striatum. The 5-HT1B serotonin receptor subtype facilitates cocaine-induced gene regulation. Here, we investigated a role for 5-HT1B in the SSRI potentiation of methylphenidate-induced gene regulation, by *in situ* hybridization histochemistry. Our results show that repeated methylphenidate treatment (5 mg/kg, 5 days) induces a minor increase in 5-HT1B expression in the striatum. Repeated fluoxetine treatment (5 mg/kg) by itself had no effect, but fluoxetine significantly potentiated the methylphenidate-induced increase in 5-HT1B expression. We also assessed the effects of 5-HT1B receptor-selective agents on methylphenidate-induced gene regulation. The 5-HT1B antagonist GR 55562 (2 mg/kg) attenuated the fluoxetine potentiation of methylphenidate-induced zif 268 expression. Conversely, the 5-HT1B agonist CP94253 (3-10 mg/kg) alone had minimal effects on zif 268 expression but dose-dependently potentiated

methylphenidate-induced zif 268 induction, thus mimicking the fluoxetine effects. Methylphenidate plus SSRI concomitant therapies are indicated in ADHD/depression comorbidity and other disorders, and co-exposure also occurs with cognitive enhancer use by patients on SSRIs. Our findings demonstrate that SSRIs potentiate addiction-related gene regulation by methylphenidate; SSRIs may thus enhance the addiction liability or other behavioral effects of methylphenidate. Our results show that the 5-HT1B receptor contributes to this SSRI potentiation of gene regulation. The 5-HT1B receptor may thus serve as a pharmacological target to prevent SSRI-induced behavioral effects.

Disclosures: V. Van Waes: None. **S. Ehrlich:** None. **J. Beverley:** None. **H. Steiner:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Program#/Poster: 618.01/Z17

Topic: C.17. Drugs of Abuse and Addiction

Support: DA025785 SW

Title: The role of 5-HT-mediated Glycogen synthase kinase 3β signaling pathway in the nucleus accumbens core in cocaine addiction

Authors: *I.-J. YOU, S. WEE;
Dept. of Mol. Therapeut., The Scripps Res. Inst., Jupiter, FL

Abstracts: In our previous study, we hypothesized that enhanced 5-HT_{1A} autoreceptor activity with chronic cocaine use underlay cocaine addiction and found that the inhibition and deletion of 5-HT_{1A} autoreceptors attenuated motivation for compulsive cocaine seeking behavior and cocaine reward, respectively. However, the key signaling components in the forebrain that mediate cocaine addiction via the increased 5-HT_{1A} autoreceptor function have not been examined. The present study, therefore, examined whether decreased serotonergic output in the nucleus accumbens (NAc) by the increased 5-HT_{1A} autoreceptor function is related to compulsive cocaine seeking and reward. The literature suggests that the increased activity of glycogen synthase kinase (GKS)-3β in the NAc is associated with synaptic plasticity in psychiatric disorders and addictive behaviors. Serotonin (5-HT) negatively regulates the activity of GSK3β. Accordingly, we also investigated the role of GSK 3β in the NAc core in cocaine addiction. Using the DREADD (designer receptors exclusively activated by designer drug)

technique, we determined the effect of the stimulation of the raphe to the nucleus accumbens 5-HT circuit on cocaine-induced conditioned place preference in mice. In a rat model of cocaine self-administration with extended access, which mimic compulsive cocaine intake in humans, we measured the level of phosphorylated GSK3β, an inactive form, and the total GSK3β in the NAc core. Lastly, we determined whether a selective GSK-3β inhibitor in the NAc core decreased cocaine self-administration in rats with extended access. The stimulation of the raphe to the nucleus accumben 5-HT projection abolished cocaine-induced conditioned place preference in mice, similar to our previous finding with the deletion of 5-HT_{1A} autoreceptors. Additionally, we found that the level of phosphorylated GSK3β was significantly decreased in the NAc core of LgA rats compared with ShA rats. Moreover, the inhibition of GSK3β in the NAc core attenuated compulsive-like cocaine self-administration in LgA rats, but not in ShA rats. Altogether, these results show that increased motivation for cocaine is associated with increased 5-HT_{1A} autoreceptor function, resulting in decreased serotonergic output to the NAc and suggest that the increased activity of GSK3β as the downstream signaling of decreased 5-HT neurotransmission in the NAc contributes to the transition to cocaine addiction perhaps by promoting a pro-depressive state.

Disclosures: I. You: None. **S. Wee:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Title: Prefrontal correlates of addiction and the effect of subconvulsive electrical stimulation on cue-induced relapse in rats

Authors: *I. GOLDENBERG[1], R. GAL[2], S. ZIBMAN[2], N. BARNEA-YGAEL[2], A. ZANGEN[2];
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Abstracts: A major hallmark of drug (and especially cocaine-) addiction is relapse to drug use following a period of self-imposed abstinence. In humans and animal models, relapse can be reliably triggered by presenting cues previously associated with drug self-administration, a phenomenon termed cue induced relapse (CIR). It has been suggested that changes in neuronal activity in the medial prefrontal cortex (mPFC) following repeated drug use may play a key role

in CIR. Here we used a chronically-implanted bilateral linear microelectrode array (14 recording electrodes + 2 stimulating electrodes) to record local field potentials (LFPs) and induce subconvulsive electrical stimulations (SCES) in the mPFC of behaving rats. Following array implantation, rats were trained to self-administer cocaine by pressing a reinforcing lever, and each reinforcement was coupled with a light + tone cue. Next, 2/3 of the cage floor was electrified such that the rats had to tolerate a foot shock to reach and press the reinforcing lever. The shock intensity was increased over daily sessions until an individual Abstinence Threshold (AT) was reached and the rats abstained from lever pressing for 3 consecutive days. Next, mPFC-SCES (20 Hz, 0.01 ms pulse duration, 400 μAmp) was applied daily for two weeks. The rats then entered a 30 min CIR-test, during which the cues were non-contingently presented under 85% AT conditions. The CIR-test was repeated for 4 consecutive days and we recorded the number of lever presses each day. Cue-induced mPFC-LFP activity was recorded throughout the experiment. Whereas 36% of the rats that did not receive SCES (n=11) 'relapsed' to lever-pressing (i.e., pressed the lever at least twice during the CIR-test), only 16% of the rats that received SCES (n=6) 'relapsed'. Additionally, during the CIR-tests as compared with prior to the cocaine self-administration training, an increased power was observed in the theta and beta frequency ranges in response to the cues. This physiological response decreased in consecutive CIR-test days, suggesting extinction associated with the non-contingent cue presentation. It therefore seems that repeated cocaine self-administration is associated with increased saliency of the drug-associated cues manifested, at least, as increased cue-induced theta/beta mPFC activity. Results obtained in this study may provide insights to the mechanisms underlying CIR and offer novel therapeutics alternatives for relapse to drug use in human addicts.

Disclosures: I. Goldenberg: None. **R. Gal:** None. **S. Zibman:** None. **N. Barnea-Ygael:** None. **A. Zangen:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: Mercer University Seed Grant

Title: Blockade of TrkB receptors attenuates D-AMPH withdrawal-induced increases in dynorphin expression in the nucleus accumbens and striatum

Authors: *K. A. HORNER[1], R. C. MURRAY[2], J. J. L. FULLER[2];
[1]Div. Basic Med. Sci., Mercer Univ. Sch. Med., MACON, GA; [2]Mercer Univ. Sch. of Med., Macon, GA

Abstracts: Several lines of evidence suggest that withdrawal from chronic, escalating doses of D-amphetamine (D-AMPH) induces a syndrome with symptoms that are similar to those seen in major depressive disorder (MDD). Animals and humans undergoing psychostimulant withdrawal exhibit a loss of interest or pleasure in all or most activities (anhedonia) and depressed mood (dysphoria), which are also core symptoms of MDD. Increased dynorphin (DYN) expression in the nucleus accumbens (NAc) is thought to contribute to the anhedonia and dysphoria that is seen following psychostimulant withdrawal and in MDD. Brain derived neurotrophic factor (BDNF) is also increased in the NAc following psychostimulant withdrawal and in MDD, and is thought to have a pro-depressive effect in this region. Interestingly, increased BDNF in the NAc could contribute to the up-regulation in DYN that is observed with psychostimulant withdrawal and MDD. Specifically, activation of tropomyosin related kinase B (TrkB) receptors by BDNF increases *cAMP response element-binding (CREB)* protein activity via activation of the mitogen-activated protein/extracellular signal regulated kinase (MAP/ERK) pathway. Furthermore, CREB has been shown to positively modulate DYN levels in the NAc. Therefore, enhanced BDNF-CREB activity in the NAc may result in anhedonia and pro-depressive effects via up-regulation of DYN activity and abrogation of BDNF-CREB signaling may have a beneficial, antidepressant effect. However, it is not known whether blockade of TrkB receptors following psychostimulant withdrawal will alter DYN expression in the NAc. Thus, the goal of the current study was to determine the effects of TrkB receptor blockade on DYN expression in the NAc, as well as the adjacent striatum (STR) following withdrawal from D-AMPH. Animals were treated 3 times a day for 4 days with escalating doses of D-AMPH (1-10 mg/kg). Twenty-four hours after the final dose of D-AMPH, animals were treated with the TrkB receptor antagonist, ANA-12, sacrificed and the tissue processed for *in situ* hybridization histochemistry. Chronic, escalating doses of D-AMPH, coupled with 24h of withdrawal resulted in a significant increase receptor DYN mRNA expression in the core and shell of the NAc and the STR. Blockade of TrkB receptors attenuated D-AMPH-withdrawal induced increases in DYN expression in the core, but not shell of NAc, and in the STR. These data indicate blockade of BDNF receptors reverses the increase in DYN expression seen following chronic D-AMPH exposure and could alleviate the anhedonia and dysphoria observed during psychostimulant withdrawal, as well as in MDD.

Disclosures: K.A. Horner: None. **R.C. Murray:** None. **J.J.L. Fuller:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Burroughs Wellcome Fund 9550300872

Title: Transcriptome analysis and gene targeting of a QTL influencing methamphetamine sensitivity

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Abstracts: We previously utilized interval-specific congenic lines derived from C57BL/6J (B6) and DBA/2J (D2) alleles to fine map a quantitative trait locus (QTL) influencing methamphetamine (MA)-induced locomotor activity. We identified a 0.23 MB critical interval on chromosome 11 containing only two protein-coding genes, Rufy1 and Hnrnph1. Notably, Rufy1 contains three missense SNPs and Hnrnph1 contains 1 SNP near the 5' UTR. We are currently generating null mutant lines for both genes using transcription activator-like nucleases (TALENs) to determine the quantitative trait gene(s) that influence MA sensitivity. In an attempt to identify transcriptome signatures associated with strain differences in MA sensitivity, we carried out RNA sequencing (RNA-seq) with striatal samples from a B6.D2 congenic line (chr.11: 50-60 Mb) that captures the QTL and WT B6 littermates. Results depict an overrepresentation of cis-regulated, differentially expressed genes within the congenic interval (4 out of 92 differentially expressed genes; FDR < 0.05) and widespread genomic regulation on all autosomes. Using Ingenuity Pathway Analysis (IPA), the top canonical pathways were "glutamate receptor signaling" and "GalphaQ signaling," while our top gene networks were "Behavior, Nervous System Development and Function, Tissue Morphology" and "Behavior, Neurological Disease, Cell-to-Cell Signaling and Interaction." In B6.D2 congenics, we uncovered down-regulation in the expression of critical genes involved in midbrain dopaminergic neuron development (e.g. Nr4a2, Bdnf) and striatal glutamate neurotransmission (e.g. Slc17a6, Slc17a7, Homer2, Gls, Gng2, Gria4). Dopamine signaling in the striatum plays a modulatory role on innervating glutamatergic neuron synapses on principal medium spiny neurons (MSNs), and this interplay has been shown to be sensitive to acute MA. Thus, we hypothesize that Rufy1 is the quantitative trait gene (QTG) responsible for variation in behavioral sensitivity to MA, which is mediated by a mechanism involving decreased

dopaminergic striatal innervation and dampened glutamatergic neurotransmission, cumulating to altered MSN morphology and MA-induced function.

Disclosures: **N. Yazdani:** None. **Y. Shen:** None. **W. Johnson:** None. **C.D. Bryant:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 618.05/Z21

Topic: C.17. Drugs of Abuse and Addiction

Support: DA036331

EB014539

DA022340

Title: Amphetamine-induced phasic dopamine release requires dopamine cell firing and endocannabinoid signaling

Authors: ***D. P. COVEY**[1], K. BUNNER[2], J. F. CHEER[1], P. A. GARRIS[3];
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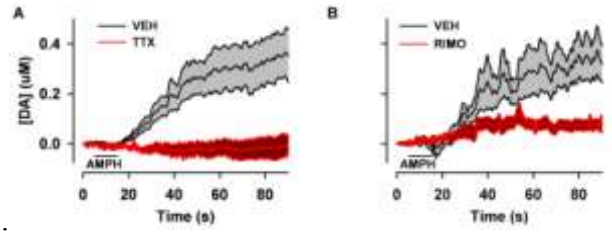
Abstracts: Similar to all other classes of abused drugs, the rewarding and reinforcing effects of amphetamine (AMPH) arise from its ability to increase dopamine levels in the nucleus accumbens (NAc). Recent *in vivo* measures showing AMPH to increase phasic dopamine release events - i.e., dopamine transients - challenge the accepted mechanism by which AMPH exerts this effect. Yet, the respective contributions of action potential-dependent transient signaling and non-exocytotic efflux to AMPH effects on NAc dopamine levels are unknown. These two processes were assessed by administering AMPH intravenously to awake rats while monitoring dopamine levels in the NAc with fast-scan cyclic voltammetry. AMPH elicited an immediate and robust increase in NAc dopamine concentration (Figure, A). This effect was abolished by intracerebral infusions of tetrodotoxin (TTX), but not vehicle (VEH), onto ventral tegmental dopamine cell bodies. Thus, cell activity is required for AMPH to increase NAc dopamine levels, indicating that non-exocytotic efflux is not the major action of AMPH in this region. Next, we assessed how AMPH elevates NAc dopamine concentration by administering the cannabinoid CB1 receptor inverse agonist/antagonist rimonabant (RIMO) prior to AMPH (Figure, B). In

contrast to TTX, which eliminates action potential generation in dopamine neurons, RIMO selectively disrupts the increase in dopamine transient frequency in the NAc - supporting similar work with cocaine, ethanol and nicotine. Terminal effects of AMPH remained intact following RIMO, as evidenced by persistence of AMPH-induced increases in the amplitude and duration of dopamine concentration transients. Thus, blocking CB1 receptors with RIMO selectively disrupt AMPH's ability to elicit dopamine transients. Collectively, these results provide strong evidence that a predominant action by which AMPH increases NAc dopamine levels is by activating dopamine cell firing through mobilization of endocannabinoid tone.



Disclosures: **D.P. Covey:** None. **K. Bunner:** None. **J.F. Cheer:** None. **P.A. Garris:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA018165

NIH Grant OD011092

Title: M1 muscarinic and α4β2-containing nicotinic receptor ligands modulate oral methamphetamine self-administration in DBA/2 mice

Authors: *M. M. FORD;
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Abstracts: Cholinergic transmission modulates several aspects of stimulant abuse, including initial exposure, transition to dependence, and relapse risk. Recent work from our laboratory determined that the discriminative stimulus effects of methamphetamine (MA) in mice is influenced by M1 muscarinic and α4β2-containing nicotinic receptor activities, but the potential

therapeutic value of drugs with activity at these receptor subtypes in reducing MA self-administration has yet to be evaluated. The goal of the current work was to evaluate the influence of the M1-preferring agonist xanomeline, the M1-preferring antagonist dicyclomine, the α4β2-preferring nicotinic antagonist DHβE, and the α4β2 nicotinic partial agonist varenicline in a binge-like model of MA consumption. Thirty-two male DBA/2 mice were exposed to progressively higher concentrations of MA (10, 20 and 40 mg/L) versus water over consecutive 5-day blocks during 2-hr limited access sessions, starting 2-hr into the dark phase. Drinking patterns were monitored in custom lickometer chambers. Following a 3-week maintenance period with access to 40 mg/L MA, sub-groups of mice were treated with multiple doses of a test drug (i.p.) in a Latin square design. Xanomeline reduced MA intake by 40% at each dose tested (1, 1.7 and 3 mg/kg), and this decrease was attributable to a comparable decline in the number of MA bouts per session. While dicyclomine treatment (10 and 17, but not 3 mg/kg) similarly decreased MA intake, this treatment effect was associated with a diminished latency to first MA bout and smaller mean bout size, but no difference in the number of bouts. In contrast, no dose of DHβE (0.3, 1 or 3 mg/kg) altered MA self-administration or underlying drinking patterns. Varenicline (2 mg/kg) attenuated MA intake by 40%, which was primarily attributable to a decreased mean MA bout size. Results are congruent with earlier evidence showing that xanomeline and dicyclomine elicited rightward and leftward shifts in the stimulus dose-response curve of MA, respectively. In conjunction with the present findings, xanomeline and dicyclomine treatment may diminish and enhance the reinforcing properties of MA, respectively, while similarly decreasing self-administration via distinct mechanisms. Supported by DA018165 and OD011092.

Disclosures: M.M. Ford: None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: PR-110146

Title: Toll-like receptor 4 activation by methamphetamine contributes to increased dopamine concentrations

Authors: *T. A. COCHRAN[1], A. L. NORTHCUTT[1], T. J. FABISIAK[1], X. WANG[1], M. HAAS[1], J. AMAT[1], M. R. HUTCHINSON[2], S. F. MAIER[1], K. C. RICE[3], L. R. WATKINS[1]; [1]Univ. of Colorado, Boulder, Boulder, CO; [2]Discipline of Physiol., Univ. of Adelaide, Adelaide, Australia; [3]Chem. Biol. Res. Br., Natl. Inst. on Drug Abuse, Rockville, MD

Abstracts: Historically, the rewarding effects of drugs have been attributed to neuronal responses, particularly those of the mesolimbic dopamine pathway. Recent evidence suggests that CNS immune activation within the brain is also required for drug reward. Morphine and cocaine can activate Toll-Like Receptor 4 (TLR4), resulting in proinflammatory CNS immune signaling. TLR4 is an innate immune pattern recognition receptor, expressed principally on microglia and astrocytes in the CNS. In these instances, TLR4 appears to recognize morphine and cocaine as xenobiotics, or foreign invaders, triggering CNS immune signaling, akin to the immune response elicited by bacteria. The ensuing proinflammatory cascade can have neuroexcitatory consequences. In the case of morphine or cocaine, blockade of TLR4 (1) prevents induction of CNS immune activation, (2) suppresses conditioned place preference, (3) blocks drug-induced dopamine increases within the nucleus accumbens and (4) attenuates drug self administration. These findings provide the foundation for our recently proposed xenobiotic hypothesis. This hypothesis suggests that in serving its immune-surveillance role, TLR4 detects and identifies morphine and cocaine as foreign, invading compounds and initiates proinflammatory immune signaling in response to the perceived threat. Further, this drug-induced proinflammatory CNS immune signaling may be necessary for drug reward and reinforcement. Methamphetamine is thought to exert its rewarding effects via inhibition and reversal of dopamine transporters in the mesolimbic dopamine pathway. Repeated methamphetamine use is also associated with neurotoxicity. There is some indication that CNS immune activation may contribute to methamphetamine's rewarding and neurotoxic effects. However, the mechanism by which methamphetamine triggers a CNS immune response is unknown. Here, we demonstrate that methamphetamine can bind to TLR4 to initiate CNS immune activation. Systemic methamphetamine induces upregulation of proinflammatory markers in the brain, notably within the ventral tegmental area. Further, we demonstrate that systemic TLR4 antagonism attenuates methamphetamine-induced increases of dopamine within the nucleus accumbens. To date, our data suggest that methamphetamine-activation of TLR4 contributes to its dopaminergic effects, as well as implicating a possible mechanism underlying neurotoxicity. These findings also provide further support for the xenobiotic hypothesis, and indicate that TLR4 may be a promising target for pharmacological intervention to treat drug abuse.

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Poster

618. Cocaine and Amphetamine Reinforcement

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Support: CONACYT GRANT 180919

Title: Acute high dose of methylphenidate increases the amount of synaptic NMDAR but decrease its association with lipid raft in nucleus accumbens

Authors: A. D. CABELLO ARREOLA, L. GARZA OCAÑAS, *I. DELINT-RAMÍREZ; Pharmacol., Univ. Autonoma De Nuevo Leon, Monterrey, Mexico

Abstracts: Methylphenidate is a stimulant drug with dopamine and noradrenaline reuptake inhibition properties. It is mainly prescribed for attention deficit hyperactivity disorder. However, high doses of methylphenidate are potentially addictive. Growing numbers of young people use high dose of this drug for pleasurable enhancement. It is known that acute administration of drugs of abuse, such as cocaine, increase synaptic NR2B containing NMDAR in nucleus accumbens. To address whether high doses of methylphenidate also induces insertion of NMDAR at synapses, we measured the amount of NMDAR in the synaptosomal fraction isolated from nucleus accumbens, 30 minutes after an intraperitoneal injection of 10mg/kg of methylphenidate or cocaine in rats. Like cocaine, methylphenidate treatment increased the amount of NR2B contain NMDAR in the synaptosomal fraction, which suggest an increase in the NMDAR signaling. Their association with lipid rafts also regulates NMDAR signaling. Previously, we have showed that spatial training increases the association of NMDARs with lipid raft, where they can interacts with proteins such as SRC family proteins, which regulates the NMDAR function. We measured the effect of Methylphenidate treatment on the association of NMDAR with lipid raft isolated by density gradient. Unlike what happen during spatial training, we found that the association of NMDAR with lipid rafts decreases after the acute injection of Methylphenidate.

Disclosures: A.D. Cabello Arreola: None. **I. Delint-Ramírez:** None. **L. Garza Ocañas:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Program#/Poster: 618.09/Z25

Topic: C.17. Drugs of Abuse and Addiction

Title: Differential effects on reward produced by synthetic cathinones

Authors: S. B. DOLAN, *M. J. FORSTER, M. B. GATCH;
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Abstracts: Since 2007, there has been a dramatic rise in the frequency of abuse of and consequential emergency room visits related to synthetic cathinones, an emerging class of designer drugs. Despite the classification of a number of synthetic cathinones as Schedule I controlled substances, numerous analogs have been synthesized and continue to remain a popular legal alternative to methamphetamine and MDMA. Using a conditioned place preference assay, dose-response curves for the rewarding effects of four synthetic cathinones in male Swiss-Webster mice were generated: MDAI (0.1, 0.3, 1, 3, 10 mg/kg), flephedrone (4-FMC, 3, 10, 30 mg/kg), butylone (1, 3, 10 mg/kg), and naphyrone (0.3, 1, 3, 10 mg/kg). Conditioning with each of the drugs produced a significant increase in time spent on the drug-paired floor, which suggests that this class of drugs has a high potential for abuse. MDAI was the most potent compound tested, followed by naphyrone, butylone, then flephedrone. MDAI, butylone, and naphyrone produced a plateau in the dose-response pattern of reward, with significant effects across a broad range of doses. Flephedrone produced a narrow inverted-U-shaped dose-response curve with 10 mg/kg resulting in a significant increase in time spent on the drug paired floor, but not 3 or 30 mg/kg. These results suggest that, although each of these drugs produce rewarding effects, MDAI, naphyrone, and butylone may be more likely to be abused over flephedrone, given their broad range of effective doses compared to flephedrone's narrow window of efficacy. When considered together with previous findings that these compounds produce increases in locomotor activity and discriminative effects comparable to cocaine and methamphetamine, the synthetic cathinones pose a significant risk for abuse and warrant further study.

Disclosures: S.B. Dolan: None. **M.J. Forster:** None. **M.B. Gatch:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant P51RR-000165

NIH Grant R37 DA010344-18

Title: Attenuation of the sleep-disrupting effects of methamphetamine by the selective 5-HT2C agonist WAY 163909 and the selective 5-HT2A antagonist M100907 in rhesus monkeys

Authors: *M. PEREZ DIAZ[1], M. ANDERSEN[2,1], L. L. HOWELL[1,3];
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Abstracts: Methamphetamine (METH), one of the most widely abused illicit drugs, causes adverse mental, behavioral and physical effects. One of the most prominent symptoms of amphetamine use is sleep deprivation, which negatively impacts cognitive function and mood, and may contribute to on-going drug use. METH self-administration (SA) reliably disrupts measures of sleep in rhesus monkeys. Interestingly, evidence indicates that serotonin (5-HT) can modulate sleep and the neurochemical and behavioral effects of abused stimulants. Selective 5-HT2A inhibition attenuates the effects of acute amphetamine on arousal and dopamine overflow in rhesus monkeys, and selective 5-HT2C inhibition increases wakefulness and decreases REM sleep in rodents. In the present study, we investigated whether a selective 5-HT2C agonist, WAY163909, and a selective 5-HT2A antagonist, M100907, could attenuate the sleep-disrupting effects of METH SA. Four rhesus macaques with a previous history of METH SA self-administered 0.03mg/kg/injection of METH on a fixed ratio (FR) 20 schedule of reinforcement on Mon.-Fri. between 9-11am. Daily METH intake per subject was 8.97±0.11mg (mean ± SEM) and did not vary throughout study. Subjects then received I.M. injections of M100, WAY, or both between 6-6:15pm. Each dose (or combination of doses) was given for one week (Mon.-Fri.). Sleep was monitored using actigraphy-based monitors attached to the subjects' collars that measure slight changes in motion and translate these into measures of sleep using a temporal-smoothing algorithm. For all subjects, both WAY and M100 treatments decreased latency to fall asleep and increased sleep efficiency compared to vehicle. In addition, attenuation of sleep disruption did not decrease METH SA. These data further inform previous studies by demonstrating that 5-HT2C activation and 5-HT2A inhibition can improve sleep that has been disrupted by METH SA as well as sleep in general, suggesting effectiveness across different neurochemical states. The results of this study suggest that improved sleep may not be sufficient to disrupt ongoing drug-taking behavior. Further, results also suggest that these compounds may prove useful in the treatment of the sleep-related side-effects of psychostimulant abuse.

Disclosures: **M. Perez diaz:** None. **M. Andersen:** None. **L.L. Howell:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant 1R01DA033610 (A.E.W.)

Title: Amphetamine-induced behavioral and neuronal plasticities are regulated by Parvalbumin-positive GABAergic interneurons of the nucleus accumbens

Authors: ***X. WANG**[1,2], J. DENG[3], A. WEST[2];
[1]Neurosci., [2]Neurobio., Duke Univ., Durham, NC; [3]Dept. of Psychiatry, Univ. of Florida, Col. of Med., Gaines Ville, FL

Abstracts: Psychostimulant drugs of abuse induce plasticity of the functional circuitry within the brain's reward circuits, leading to the expression of addictive-like behaviors. While the majority of the literature has focused on the plasticity of medium spiny neurons (MSNs) in the nucleus accumbens (NAc), as they are critical mediators of motivated behaviors, MSN firing is also gated by local interneurons. Parvalbumin (Pv)-containing GABAergic interneurons make strong inhibitory synapses onto the somata and proximal dendrites of MSNs. These interneurons fire strongly following psychostimulant administration and changes in their activity alter striatal circuit properties. However the functional impact of PV+ GABAergic interneuron in the induction or/and expression of addictive behavior remains unknown. We have shown that both acute and chronic exposure to psychostimulants induce phosphorylation of the methyl-DNA binding protein MeCP2 at Ser421 (pMeCP2), selectively in Pv+ GABAergic interneurons of the NAc. Genetic data show that MeCP2 functions in the NAc to limit the rewarding properties of psychostimulants. Thus these data have led us to investigate whether pMeCP2-dependent plasticity of the Pv+ GABAergic interneuron population in the NAc might underlie these effects. We find that mice bearing a germline MeCP2 knockin (KI) mutation in which Ser421 is replaced by a non-phosphoryatable Ala residue, show enhanced locomotor sensitization to repeated amphetamine injection. Therefore, pMeCP2 functions in the NAc to limit psychostimulant-induced behavioral plasticities. Consistent with the possibility that pMeCP2 regulates adaptations of NAc interneurons, we also find that amphetamine-induced expression of the immediate-early gene Fos is reduced in the Pv+ GABAergic interneurons of the KI mice

compared with their WT littermates. The correlation we observe between Fos regulation in NAc Pv+ GABAergic interneurons and locomotor sensitization in these mice suggests that adaptations in the Pv+ GABAergic interneuron may contribute to psychostimulant-induced behavioral plasticities in these mice. To test this hypothesis we are using an intersectional genetic strategy to express tetanus toxin light chain selectively in Pv+ interneurons of the NAc to determine the behavioral consequences of inhibiting the contribution of these neurons to NAc circuit regulation. These studies are increasing our understanding of the cellular mechanisms that underlie addictive behaviors.

Disclosures: **X. Wang:** None. **J. Deng:** None. **A. West:** None.

Poster

618. Cocaine and Amphetamine Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA027960 (KTB)

Title: Tolerance to the locomotor-activating effects of 3,4-methylenedioxymethamphetamine (MDMA) predicts escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking in rats

Authors: *K. T. BALL, M. SLANE;
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Abstracts: Pre-clinical studies of individual differences in addiction vulnerability have been increasing over recent years, but the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) has received relatively little attention in this regard. Previously, we reported large individual differences both in rats' initial behavioral response to experimenter-administered MDMA and their degree of behavioral sensitization to repeated administration. To determine whether these differences could predict subsequent patterns of MDMA-taking or -seeking behaviors we used the self-administration-extinction-reinstatement model to examine addiction-like behavior (i.e., escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking) in rats a priori characterized for either locomotor sensitization or tolerance to MDMA. Rats that developed tolerance to the locomotor-activating effects of MDMA had a significantly larger locomotor response to the first

MDMA injection relative to rats that developed sensitization. Importantly, rats that developed tolerance subsequently displayed an escalation of MDMA self-administration over days, as well as clear cue-induced reinstatement of MDMA seeking following extinction. Conversely, rats that developed locomotor sensitization to MDMA subsequently maintained relatively stable levels of MDMA self-administration over days and showed no cue-induced reinstatement of MDMA seeking. These results show that differences in the level of psychomotor activation following acute and repeated MDMA administration can reliably predict two important addiction-like behaviors in rats, which may have implications in the prediction of compulsive MDMA use in humans.

Disclosures: K.T. Ball: None. **M. Slane:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA029815

Title: The effects of repeated amphetamine exposure during adolescence on psychomotor activity and neuronal function in the medial prefrontal cortex in young adulthood

Authors: *L. K. SHERRILL, T. KRISHNAMANI, D. O'HEARN, M. WU, Y. KUDAIMI, J. M. GULLEY;
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Abstracts: Amphetamine (AMPH) use typically begins in adolescence and a strong association exists between adolescent-onset drug use, cognitive dysfunction, and a high lifetime prevalence rate of drug dependence. Exposure to drugs during adolescence may have long-lasting neurophysiological effects given the significant, yet normal, changes in dopamine signaling and prefrontal cortex (PFC) development during this developmental time period. Previous studies from our lab and others indicate that repeated exposure to AMPH during adolescence produces long-lasting changes in executive function that may be mediated by alterations in dopaminergic circuits in the medial PFC. Here, we investigated the protracted effects of repeated AMPH exposure early in life on psychomotor behavior and dopamine function in the medial PFC using single-unit recordings from awake behaving male rats. During adolescence, all rats were given

10 injections of saline or AMPH (3.0 mg/kg, i.p.), once every other day, from postnatal day (P) 27-45. In Experiment 1, motor behavior was assessed in an open-field arena following challenges with dopamine D1 or D2 receptor-selective agonists (1.0 mg/kg SKF 82958 and 0.5 mg/kg quinpirole, respectively). In Experiment 2, separate rats were re-exposed to 3.0 mg/kg AMPH in the open-field and after 45 min were given a challenge injection of the D1 receptor antagonist (SKF 83566, 0.03 mg/kg, i.p.). A subset of these rats underwent stereotaxic surgery approximately one week prior wherein a microwire electrode array was implanted into the prelimbic or infralimbic medial PFC. Our preliminary results suggest that repeated AMPH exposure during adolescence produces sensitization to the motor stimulant effects of AMPH and D1- and D2-selective agonists. In the mPFC of AMPH pre-treated rats, neurons excited during AMPH challenge showed a greater increase in firing rate compared those from controls. In addition, this increased activity tended to persist during challenges with SKF 83566 in the AMPH pre-exposed group. Taken together, these preliminary findings provide evidence that exposure to AMPH during adolescence leads to long-lasting plasticity in the mPFC and may be mediated in part by increased responsiveness at D1 receptors. Follow-up studies are underway to assess the effects of repeated exposure to AMPH on D2 receptor function in the mPFC.

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Poster

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Support: DA025606

Dissertation Completion award from The College of Graduate Education at Arizona State University

Title: Abuse liability and toxicity of synthetic cathinones (“bath salts”) as revealed by intravenous drug self-administration, *ex vivo* MRI, and immunohistochemistry

Authors: *L. R. WATTERSON[1], S. B. TAYLOR[2], F. BUDIN[3], S. F. ALI[4], P. KUFAHL[1], N. NEMIROVSKY[1], M. OLIVE[1];

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Abstracts: In the last few years, designer drugs known as synthetic cathinones, often colloquially referred to as “bath salts”, have emerged as popular “legal high” replacements of illicit stimulants such as methamphetamine, cocaine, and MDMA (ecstasy). Acute use of synthetic cathinones has led to numerous documented incidences of intoxication and toxicity, adverse psychological and behavioral effects, and death. However, while adverse acute effects are well documented, little scientific data exists detailing their abuse liability or potential neurotoxic effects. In the first set of experiments, we present data collected in our laboratory using rodent intravenous self-administration (IVSA) showing that both MDPV and methylone, two first-generation synthetic cathinones, were both robustly self-administered during 2-hour limited access conditions. In 6-hour extended access conditions, however, only MDPV led to escalated intake, a pattern most similar to that of methamphetamine. This finding suggests that MDPV has a higher degree of addiction potential compared to methylone. In addition, we show that self-administration of both MDPV and methylone intake is highly correlated with alterations in monoamine and monoamine metabolite levels in both cortical and hypothalamic brain tissue. Next, in a separate set of experiments, we present additional evidence of toxicity with *ex vivo* MRI data revealing macro-scale changes in brain structures resulting from chronic self-administration of MDPV and methamphetamine as compared to sucrose controls. Furthermore, brains that underwent MRI imaging were further processed in histological procedures to assess changes in the expression of dopamine and serotonin transporters, microgliosis, and neurodegeneration. Collectively, these results indicate that synthetic cathinones possess a significant abuse liability and exert numerous CNS effects on both macro-structural and micro-structural levels.

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Poster

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Support: Methamphetamine Abuse Research Center P50DA018165

the Portland Alcohol Research Center P60AA010760

the Portland Alcohol Research Center R24AA020245

Title: The role of trace amine associated receptor 1 in behaviors

Authors: X. SHI[1], N. WALTER[1], K. J. BUCK[1], J. K. BELKNAP[1], T. J. PHILLIPS[1], *A. J. JANOWSKY[2];
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Abstracts: Trace amine associated receptor 1 (TAAR1) is a G-protein coupled receptor and is activated by a number of endogenous ligands including tyramine and β-phenethylamine. The receptor is also a direct target of psychostimulants, including methamphetamine (MA), amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA). Despite a growing number of studies, the role of this receptor in specific symptoms of drug abuse is not well understood. Interestingly, there is one nonsynonymous single nucleotide polymorphism (SNP) in the TAAR1 that distinguishes various inbred mouse strains. One allele is apparently unique to the DBA/2J (D2) strain, while an alternative allele is shared by C57BL/6J (B6) and at least 17 additional strains. This SNP (C229A) results in allelic isoforms with either proline or threonine at amino acid position 77. This amino acid residue is situated at the putative cytoplasmic end of the second transmembrane domain of TAAR1. This has tremendous implications for TAAR1 function and its role in behavioral and physiological phenotypes. To determine if there is a difference in function between the B6 and D2 TAAR1, site-directed mutagenesis was used to create the D2 construct. Both B6 and D2 constructs were stably transfected into HEK-293 cells, cells were prepared and treated with β-phenethylamine or tyramine, and cAMP accumulation was measured using ELISA. Both β-phenethylamine and tyramine elicited a dose-dependent response in cells expressing the B6-like TAAR1 (EC_{50}~100nM), and the effect was blocked by EPPTB, a TAAR1 antagonist. However, agonist effects were absent in the cells expressing the D2 TAAR1, suggesting that the receptor is non-functional. RT-PCR was used to verify that both receptors are transcribed. Additionally, confocal microscopic analysis of GFP-tagged constructs indicated that both B6 and D2 TAAR1 are cytosolic, consistence with previous reports. Correlational analyses of B6 x D2 recombinant inbred (BXD RI) strain data using published and unpublished behavioral trait databases indicated that TAAR1 function (or its absence) is strongly correlated with behaviors related to psychostimulant, opiate, and ethanol responses. These findings have significant implications for understanding the role of TAAR1 in drug seeking or withdrawal behaviors, as well as in normal physiology. This work was supported by the Department of Veterans Affairs, the Methamphetamine Abuse Research Center (P50DA018165), the Portland Alcohol Research Center (P60AA010760), and R24AA020245.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.01/Z32

Topic: C.17. Drugs of Abuse and Addiction

Support: VR grant 2009–2289

VR grant 2009–4477

VR grant 2010–3100

Title: Nicotine causes long-lasting behavioral sensitization and changes in accumbal neurotransmission

Authors: *J. E. MORUD, L. ADERMARK, M. ERICSON, B. SÖDERPALM;
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Abstracts: Chronic tobacco use is often associated with life-long addiction as well as high risk of relapse after cessation. This is suggestive of persistent neural adaptations, but very little is known about the long-lasting effects of nicotine on neural circuits. In order to investigate the long-term effects of nicotine exposure Wistar rats were treated during three weeks with nicotine (0.36 mg/kg), and the duration of behavioral and neurophysiological adaptations were evaluated seven months later. We found that increased drug-induced locomotion persisted seven months after the initial behavioral sensitization. *In vitro* analysis of synaptic activity in the nucleus accumbens (nAc) revealed a decrease in nicotine-sensitized rats as compared to vehicle-treated control rats. In addition, administration of the dopamine D2 receptor agonist quinpirole significantly increased evoked population spike amplitude in nicotine-sensitized rats as compared to vehicle-treated control rats. These results suggest that nicotine sensitization produces long-lasting neural effects, which are detectable both in behavior and in field potential recordings.

Disclosures: J.E. Morud: None. **L. Adermark:** None. **M. Ericson:** None. **B. Söderpalm:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.02/Z33

Topic: C.17. Drugs of Abuse and Addiction

Title: Role of histone modifying enzyme PRDM2 in alcohol-related behaviors

Authors: *C. PITCAIRN[1,2], E. BARBIER[2], A. BORICH[2], J. TAPOCIK[2], A. JOHNSTONE[2], J. SCHANK[2], Z. ZHOU[3], Q. YUAN[3], D. GOLDMAN[3], C. WAHLESTEDT[2], M. HEILIG[2]; [1]1-5288, NIH-NIAAA, Bethesda, MD; [2]Lab. of Clin. and Translational Studies, Natl. Inst. of Alcohol Abuse and Alcoholism, Natl. Inst. of Hlth., Bethesda, MD; [3]CTI, Dept. of Psychiatry, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstracts: Exposing rats to repeated cycles of alcohol intoxication and withdrawal will produce persistent changes in voluntary alcohol consumption that model characteristics of human alcohol use disorder (AUD). Although the neural adaptations mediating these behavioral changes remain unclear, recent data suggest that modifications in histone and DNA methylation may play a role. Therefore, we wanted to investigate how the expression of enzymes related to such epigenetic modifications is changed following alcohol-exposure and whether these expression differences are associated with behaviors characteristic of post-dependent rats. To test this, alcohol dependence was induced in male Wistar rats by exposing them to alcohol vapor 14 hours a day for 7 weeks. Three weeks after conclusion of alcohol exposure, total RNA extracted from the dorsal medial prefrontal cortex (dmPFC) was sequenced using the whole transcriptome sequencing protocol (n=4/group). Whole transcriptome sequencing identified 783 genes that were differentially expressed between control and post-dependent rats, including several genes coding epigenetic enzymes that showed significantly decreased expression in rats chronically exposed to alcohol vapor. We chose to investigate the effect of reducing expression of one of these enzymes - the histone lysine 9 methyltransferase PRDM2. Thus, a shRNA lentivirus specific to prdm2 was injected into the dmPFC of naïve rats and behavioral tests for alcohol consumption, aversion resistant alcohol seeking, and stress induced reinstatement were performed. We found that inhibition of PRDM2 within the dmPFC was associated with increased alcohol self-administration, a resistance to quinine adulteration, and reinstatement of self-administration at a lower shock intensity. These results suggest that PRDM2 may regulate specific behaviors observed in post-dependent rats and suggest a role for histone methylation in alcohol-related behaviors more generally.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.03/Z34

Topic: C.17. Drugs of Abuse and Addiction

Title: Neuronal dna-methylation in the medial prefrontal cortex regulates alcohol-induced behavior and plasticity

Authors: *A. L. BORICH, E. BARBIER, J. TAPOCIK, N. JUERGENS, C. PITCAIRN, J. HANSON, J. SCHANK, H. SUN, A. THORSELL, K. SCHUEBEL, Z. ZHOU, Q. YUAN, M. EMMERT-BUCK, D. GOLDMAN, M. HEILIG;
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Abstracts: Recent studies have suggested an association between alcoholism and DNA methylation, a mechanism that can mediate long-lasting changes in gene transcription. Here, we examined the contribution of the DNA methyltransferase 1 (DNMT1) to the long-term behavioral and molecular changes induced by a history of alcohol dependence. In search of mechanisms underlying persistent rather than acute dependence-induced neuroadaptations, we studied the role of DNA methylation and DNMT1 activity for medial prefrontal cortex gene expression and for alcohol self-administration (SA) in post dependent (PD) rats. Male wistar rats (200-400g) became alcohol dependent with exposure to intermittent alcohol vapor for 14h each day for 7 weeks until a stable blood alcohol concentration (BAC) of 150-300 mg/dl was established. Controls were housed in identical chambers with normal airflow. Three weeks after exposure, baseline SA of 10% alcohol was measured. PD rats showed escalated alcohol intake, which was associated with increased DNMT1 expression as well as decreased expression of genes encoding synaptic proteins involved in neurotransmitter release in the mPFC. Infusion of the DNMT1 inhibitor RG108 prevented both escalation of alcohol consumption and dependence-induced down-regulation of 4 out of 7 transcripts modified in PD rats. Specifically, DNMT1 treatment directly reversed the down-regulation of syt2, which was caused by alcohol-induced hypermethylation on exon1. Lentiviral inhibition of syt2 expression in the mPFC increased aversion-resistant alcohol drinking, supporting a mechanistic role of syt2 in alcohol seeking. Treatment with the methyl-donor, L-Methionine, induced escalation in alcohol consumption in naïve rats similar to that of PD rats. Our findings show a functional role of DNMT1 in persistent alcohol-induced behaviors, and identify a candidate gene network that may mediate its effects. DNMT1 expression is persistently increased in mPFC with chronic intermittent exposure to

alcohol. Inhibition of DNMT1 normalized alcohol consumption and restored expression levels of neurotransmission-related genes, indicating that DNA hypermethylation is causally related to their suppressed expression in PD rats. Together, these data provide novel evidence for DNMT1 as a potential therapeutic target for alcoholism.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

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Program#/Poster: 619.04/Z35

Topic: C.17. Drugs of Abuse and Addiction

Support: AA019455

Title: GluN2B-NMDA receptor targeted proteomic approach unveils ethanol-induced changes in molecular signaling cascades involved in plasticity

Authors: ***T. A. WILLS**[1], K. M. LOUDERBACK[2], A. J. BAUCUM[3], R. J. COLBRAN[1], D. G. WINDER[1];
[1]Mol. Physiol. and Biophysics, [2]Neurosci., Vanderbilt Univ., Nashville, TN; [3]Dept of Biol., Indiana Univ. Purdue Univ. in Indianapolis, Indianapolis, IN

Abstracts: GluN2B-containing NMDA receptors (NMDARs) govern synaptic efficacy at glutamate synapses, and are major acute and chronic targets of alcohol. In the BNST, the acute and chronic effects of ethanol are dependent on the GluN2B subunit of the NMDAR. Further, withdrawal from chronic intermittent ethanol (CIE) in this region causes an enhancement of long-term potentiation (LTP) that may involve activation of extrasynaptic GluN2B-NMDARs. This enhancement was unexpected in light of the wealth of data suggesting that extrasynaptic GluN2B-NMDARs promote long-term depression (LTD) in regions like the hippocampus. To investigate how this plasticity might be differentially regulated in different regions by ethanol, we utilized a GluN2B-NMDAR-immunoprecipitation-based proteomic approach to identify GluN2B associated proteins. In the hippocampus, we find a constellation of ethanol-induced protein changes indicative of induction of group I mGluR regulated LTD (Arc, Homer, GluA2).

In support of this proteomic data, we find that the group I mGluR agonist DHPG fails to produce LTD in area CA1 following CIE. These data suggest *in vivo* induction of group I mGluR-LTD by CIE and a possible connection between NMDAR and mGluR signaling. Current proteomic work in the BNST will help determine whether divergent mechanisms I these two regions are responsible for the district regional influence on plasticity.

Disclosures: T.A. Wills: None. **K.M. Louderback:** None. **R.J. Colbran:** None. **D.G. Winder:** None. **A.J. Baucum:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: P60AA011605

F31AA020132

ABMRF/The Foundation for Alcohol Research

David Bray Peele Memorial Research Award from the Department of Psychology, University of North Carolina at Chapel Hill

Title: Family history of alcohol use disorder and large-scale intrinsic network connectivity in adulthood

Authors: M. H. PARRISH[1], C. T. SMITH[2], M. MENCELOGLU[1], S. H. OPPLER[1], *C. A. BOETTIGER[4,3];
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Abstracts: Alcohol use disorders (AUDs) are highly heritable, with 40-60% of AUD risk estimated to be genetic. Despite known heritability, to date, few biomarkers of family history of AUDs have been identified. Moreover, although a few neuroimaging studies have compared adolescent brain connectivity between those with a family history of AUD (FHP) and those without (FHN), no studies to date have compared intrinsic brain connectivity in FHP and FHN adults. Our objective in the present investigation was to identify differences in large-scale adult

brain network connectivity associated with AUD family history status. To do so, we used functional connectivity analysis of resting state fMRI data to quantify the coactivation of predefined brain regions in identified intrinsic functional networks (salience network: SN; central executive network: CEN; default mode network: DMN) in 58 healthy adults, comparing those classified as FHP (n=22) or FHN (n=36). Participants were screened for psychiatric diagnoses, including lifetime substance use disorders. The FHP and FHN groups did not differ significantly in terms of age, sex, socioeconomic status, education, IQ, or substance use. We first specifically compared covariation in the activity of a key SN node, the right frontoinsular cortex (rFIC), and frontoparietal nodes of the CEN between FHP and FHN groups. We observed significantly greater SN-CEN synchrony in the FHN group ($Z=0.23 \pm 0.03$) relative to FHP group ($Z=0.06 \pm 0.05$); $t(56)=3.24$, $p=.004$). We next compared coactivation of another SN seed, the ventral tegmental area (VTA) and the medial orbitofrontal cortex (mOFC) between FHP and FHN groups. We found significantly greater VTA-mOFC connectivity in the FHP group ($Z=0.24 \pm 0.03$) relative to the FHN group ($Z=0.11 \pm 0.03$); $t(56)= 2.85$, $p=.004$). Additional exploratory seed-to-voxel analyses evaluating the effect of FH status on connectivity with rFIC or VTA seeds identified multiple loci within the CEN and DMN that were differentially synchronous with the rFIC or VTA seeds between the FHP and FHN groups. Loci within the DMN showed greater synchrony with the VTA among the FHP group, whereas sites within the CEN were less coactive with the rFIC among the FHP group. These findings predict impaired switching between exogenous and endogenous attention in FHP relative to FHN individuals, an idea to be empirically tested. These data also suggest adult patterns of resting state connectivity between salience processing regions and the DMN and CEN as novel biomarkers for familial AUD risk or resilience. Future work to determine whether these patterns of network interconnectivity predict future alcohol problems may shed light on this issue.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.06/AA1

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Nakatomi Fondation, Grants for scientific research relating to health promotion focusing on physical exercise

Title: Exercise-induced positive activated affect is dependent on work intensity and modulated by opioid receptor system distributed in the mesolimbic pathway. A positron emission tomography study

Authors: *M. HIURA[1,2], T. NARIAI[3,2], K. ISHII[2], M. SAKATA[2], K. ODA[4,2], J. TOYOHARA[2], K. ISHIWATA[2];
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Abstracts: [Introduction] The opioid receptor system is involved in the regulation of pain and emotion and the underlying mechanism has been studied using positron emission tomography (PET) with the selective μ-opioid agonist radiotracer [^{11}C]carfentanil (CFN). Physical exercise is associated with changes in mood states and altered pain perception, and the underlying mechanism has not been well elucidated. Considering that exercise is a physiological stressor, as with pain and emotion, it is speculated that exercise-induced mood change might share common neuroanatomical pathways of the opioid receptor system. The present study investigated the effect of exercise intensity on the μ-opioid receptor (MOR) availability in the brain by CFN PET and the association between the exercise-induced altered mood and the opioid receptor system. **[Methods]** Seven healthy young males performed two bouts of the constant work rate cycling exercise for 20 min on separate days with different intensities and underwent two CFN PET imaging sessions on each day: a resting state PET scan and a post exercise PET scan. The exercise of moderate and heavy intensities (ExM and ExH, respectively) was applied, which corresponded to 60% and 80% of their peak pulmonary oxygen uptake, respectively. Mood status was monitored before and after the exercise bouts. The binding potential (BP_{ND}) of CFN was determined by a modified Logan graphical analysis. For an anatomical reference, individual brain MRI scans were acquired and the image data were analyzed using Statistical Parametric Mapping software. **[Results]** Decrease of the BP_{ND} was identified in broad areas including the amygdala, insular cortex, anterior cingulated cortex, caudate nucleus, precuneus region and frontal and temporal cortexes ($P<0.001$, uncorrected) after the ExM but only in the anterior cortex, precuneus region and orbital gyrus after the ExH. With regards to the altered mood in the ExM, the scales of depression positively correlated with the BP_{ND} in the regions such as the amygdala and anterior cingulated cortex which are involved in the mesolimbic pathway ($P<0.001$, uncorrected). This correlation was not identified after the ExH. **[Discussion]** Decreased BP_{ND} was considered to reflect the endogenous opioid release as the main effect of the ExM and ExH as well. However, an anxiolytic effect was specific to the ExM which might be attributable to the MOR system in the mesolimbic pathway. The results of the present study suggest that leg movement, respiratory effort and elevation in body temperature caused by exercise might have effect on interoceptive network of feelings from the body which partially shares the mesolimbic pathway.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.07/AA2

Topic: C.17. Drugs of Abuse and Addiction

Support: VR 2009-2289

brain foundation

Title: Acute and long-term effects on neurotransmission and plasticity in striatal subregions after intermittent nicotine exposure

Authors: *L. ADERMARK[1], J. MORUD[1], K. DANIELSSON[1], M. PEREZ ALCAZAR[2], M. ERICSON[1], A. LOTFI[1], B. SÖDERPALM[1];
[1]Addiction Biol. Unit, Gothenburg, Sweden; [2]Neurosci. and Physiol., Gothenburg, Sweden

Abstracts: Repeated administration of drugs of abuse appears to recruit serial and dopamine-dependent striatal ascending spirals from the nucleus accumbens (nAc) to more dorsal regions of the striatum. This progressive shift might be of importance for the development of compulsive drug seeking and the high vulnerability to relapse. The aim of this study was to explore acute and long-term effects of repeated nicotine administration on synaptic efficacy and plasticity in striatal subregions of adult male Wistar rats. Daily nicotine injections for 3 weeks (0.36 mg/day s.c., 15 days) produced robust locomotor sensitization to the drug. Field potential recordings reviled a decrease in input/output function in the dorsomedial striatum (DMS) for up to one month after the nicotine sensitization-period, while the dorsolateral striatum (DLS) and nucleus accumbens (nAc) remained unaffected. After three, and up to six months of nicotine abstinence, however, synaptic efficacy was recovered in the DMS but significantly decreased in the nAc of nicotine-sensitized rats as compared to vehicle-treated controls. In parallel to the decrease in input/output function the number of dendritic spines was increased, suggesting that repeated administration to nicotine might increase the number of silent synapses in striatal sub-regions. Synaptic plasticity induced by high frequency stimulation sustained in nicotine-treated rats acutely after the sensitization-period, but was significantly impaired in the DLS following one and three months of nicotine abstinence. In conclusion, the data presented here indicates that repeated nicotine-

administration produces long-lasting, sub-region specific and time-dependent effects on striatal neurotransmission, which could be related to behavioral sensitization to nicotine and/or to the development of addiction.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: the CURE grant

NIH R21DC011074

Title: A hypo-status revealed by multi-modal neuroimaging in drug addicted Brain

Authors: ***Z. WANG**[1], J. SUH[2], C. P. O'BRIEN[2], T. FRANKLIN[2], A. R. CHILDRESS[2]; [1]Psychiatry, Univ. of Pennsylvania, PHILADELPHIA, PA; [2]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstracts: Addiction is a chronic brain disorder. Finding brain signatures sensitive to disease is a high research priority. Previous studies suggested a reduced baseline activity in addicted brain, but it is unclear whether it is a static change or a dynamic baseline dysfunction and whether it overlaps with structural alterations related to drug use. The purpose of this study was to address these unexplored questions. We hypothesized that drug users have 1) tissue loss in orbitofrontal cortex (OFC), a critical area in the development of addictive behavior, and 2) hypo-perfusion in prefrontal cortex (PFC) and limbic area; 3) hypo-dynamic activity in PFC and limbic area; 3) the 3 modal brain alterations show overlaps. To test the hypotheses, we acquired structural MRI, arterial spin labeled (ASL) perfusion MRI, and resting state fMRI (rsfMRI) data from 23 cocaine treatment seeking patients and 25 race/education/age matched controls. Individual brains were registered into a standard space for group level analysis. Brain volume was measured using the voxel-based morphormetry technique. Static baseline activity was measured with cerebral blood flow using ASL MRI, which is known to be related to regional brain function. Dynamic baseline was characterized by rsfMRI reflected brain complexity, which has been shown to be altered in

disease conditions. Statistical results were collected using a threshold of p100 (corrected using Monte Carlo simulations with $\alpha<0.05$). Compared to controls, patients showed reduced grey matter volume in OFC, consistent with findings published by us and others; patients had hypo-perfusion in medial and bilateral OFC, ventral striatum (VS), anterior cingulate cortex (ACC), insula, temporal cortex, and dorso-lateral PFC (dlPFC), indicating a static hypo-activity in these mesa-limbic-cortical area, which is a major circuit involved in drug addiction; hypo-resting state functional complexity was found in mOFC, VS, insula, and dlPFC, but also in the sensory-motor system consisting of precentral cortex, parietal cortex, and thalamus. The hypo-complexity patterns indicate a loss of functional capacity of integrating either external or internal input such as inhibition or attentional control signal for drug use. Hypo-complexity in ACC/OFC and dlPFC was related to higher risk-taking (an addiction-relevant behavior). Hypo-perfusion and hypo-complexity overlapped with atrophy patterns only in the anterior part of mOFC. Suggesting a dis-association of structural and functional contribution to addiction related brain alterations. These multi-modal hypo- signatures may have potential clinical significance. Supported by the CURE grant.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

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Program#/Poster: 619.09/AA4

Topic: E.05. Stress and the Brain

Support: T32 DA28874

R01 DA033646

Title: The transgenerational effects of nicotine and chronic stress in mice

Authors: *N. L. YOHN[1], C. KRAPP[2], M. S. BARTOLOMEI[2], J. A. BLENDY[1];
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Abstracts: Objective: Evidence suggests that nicotine and stress can influence behavior within and across generations. Thus, the goal of this work is to determine if nicotine and stress

administered in separate generations interact to influence behavioral manifestations of drug and stress response in subsequent generations. Methods: Male C57Bl/6 mice (F0) were administered chronic saline or nicotine via osmotic minipump for four weeks starting at 4 weeks of age. Following 1 month of nicotine administration, minipumps were removed and males were mated to produce F1 offspring. Half of the F1 males and females underwent chronic unpredictable stress (CUS) for 2 weeks starting at 4 weeks of age. Following CUS, both male and female mice were mated with naïve partners to produce the F2 generation and these offspring were used to produce the F3 generation. All generations (F1, F2, and F3) were administered a series of tests for anxiety-like phenotypes and stress responsivity between 10-14 weeks of age. Brain regions of interest were harvested from the F2 generation. A candidate gene approach was employed to determine the molecular changes in subsequent generations of offspring following parental nicotine exposure by probing for the expression of CRF, CRFR1, CRFR2, and GR in brain regions that directly influence behavior on the acoustic startle response (ASR) in F2 mice. Results: In F1 males, adolescent stress increased anxiety-like behavior in the MB and EZM. Increase in anxiety-like behavior extended to the F2 male offspring derived from F1 female mothers that received stress. In addition, in the male F1 mice a decrease in ASR was found due to stress and nicotine lineage. Finally, preliminary evidence suggests, the effect of stress in the F1 generation extends to males in the F3 generation. In F1 females, adolescent stress increased anxiety-like behavior in the EZM, however this phenotype was not transmitted to additional generations. Instead, in both F1 and F2 female mice, nicotine lineage increased startle amplitude in ASR. In addition, F2 male mice derived from stress and/or nicotine showed altered expression of CRFR1 and GR in the hypothalamus and amygdala in a region specific manner. Summary: The clinical and public health implications of life-long or transgenerational effects after preventable “exposures” such as nicotine indicate an urgent need to understand the mechanisms that mediate these effects. This work was supported by T32 DA28874 and R01 DA033646.

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Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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Program#/Poster: 619.10/AA5

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

Support: R01 DA025674 (to EB)

Title: Effects of adolescent exposure to morphine in female rats on cognitive function in male offspring

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Abstracts: In humans, opiate use during adolescence is more common in girls than in boys. Previous research in rodents has determined that the male offspring of females exposed to morphine during adolescence show sensitized behavioral response to morphine. With acute morphine administration, prepubescent male offspring have prolonged sedation and adult male offspring have increased morphine analgesia. We explored the possibility that exposure to morphine in female adolescent rats may affect measures of cognitive function in male offspring. Female Sprague-Dawley rats were administered an increasing dose regimen of morphine (5-25 mg/kg; subcutaneously) or saline from post-natal day 30-40, and then allowed to recover for at least 3 weeks before mating with a naïve male. Differences between male offspring of saline-exposed females (SAL-F1) and male offspring of morphine-exposed females (MOR-F1) were assessed in the 5-Choice Serial Reaction Time Task (5CSRTT), a well-validated method of studying learning, impulsivity, and motivation. Specifically, 5CSRTT measures include %Correct, %Omitted, %Accuracy, head entries, response types, and latencies. Rats were first evaluated in terms of baseline (pre-drug) performance by 3 days at criteria (>60% Correct, <20% Omissions). Following baseline assessment, rats received intraperitoneal injections of cocaine (1.0, 3.0, 10.0 mg/kg) in ascending order. After cocaine administration rats were given at least one session to meet criteria before the next dose, if criteria was not met baseline was re-established. There were no inherent differences between MOR-F1 and SAL-F1 rats as assessed by 5CSRTT. On the day of cocaine treatment, both groups performed similarly in %Correct, %Omitted, and %Accuracy. There was a main effect of cocaine dose and of maternal treatment on head entries, often considered an index of stereotypic behavior. Post-hoc tests revealed that 10mg/kg cocaine increased head entries significantly in the MOR-F1 group when compared to SAL-F1. Moreover, MOR-F1 rats exhibited greater head entries than SAL-F1, independent of dose. Interestingly, on the day following cocaine treatment, the MOR-F1 group performed better in %Correct, %Omitted, and %Accuracy than while on cocaine. In contrast, the SAL-F1 group performed the same or worse the day following cocaine treatment than while on cocaine. One possibility is that performance on the day following cocaine reflects acute withdrawal, and the MOR-F1 rats are less sensitive to withdrawal-associated dysphoria. Further exploration into the transgenerational effects of female adolescent morphine use on responsiveness to adverse and rewarding stimuli is warranted.

Disclosures: C. Webber: None. **F. Vassoler:** None. **R. Donahue:** None. **E. Byrnes:** None. **W.A. Carlezon:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.11/AA6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA016959

Radford University

Title: Alterations in cell proliferation following combined binge alcohol and chronic nicotine exposure in adult rats

Authors: R. T. LINGG[1], C. B. HARTLESS[1], K. Y. CHEN[2], K. NIXON[2], *D. M. HAYES[1]; [1]Radford Univ., Radford, VA; [2]Univ. of Kentucky, Lexington, KY

Abstracts: Excessive alcohol consumption has consistently been associated with drastic impairments in neurological functioning ranging from structural to complex behavioral abnormalities, but the long term cognitive effects of nicotine use are less clearly established. Within the adult brain, researchers have shown impairments from alcohol and nicotine abuse in neural progenitor cell (NPC) proliferation, as well as the subsequent differentiation, maturation, and integration of NPCs into existing neuronal functioning; a process known as neurogenesis. Specifically, research has shown that both binge alcohol consumption and chronic nicotine abuse result in a substantial depression of cell proliferation within the subgranular zone (SGZ) of the dentate gyrus of the hippocampus; an area associated with ongoing plastic developments involved in spatial memory and learning. Furthermore, reduced neurogenesis has been hypothesized to be a potential mechanism underlying impairments in structural and functional integrity seen in many chronic drug users. As alcohol and nicotine are the most commonly co-abused substances, further elaboration regarding the neural mechanisms of their combined use is critical. Adult male Sprague-Dawley rats were injected with nicotine (0.3 mg/kg in 0.9% saline) or vehicle every 8 hours for 10 days. For the final four days of exposure, rats also received intragastric intubations of an ethanol-containing diet (25% w/v in Vanilla Ensure Plus®) or control diet thrice daily (mean dose: 9.17 ± 0.31 g/kg/day, BEC: 276.07 ± 12.43 mg/dL). Immediately after the final drug administration, brains were extracted and coronal tissue sections (40 μm) were used for Ki67 immunohistochemistry in order to identify cells in all active phases of the cell cycle. As expected, cells expressing Ki67 were located in non-homogenous cell clusters along the SGZ of the dentate gyrus. Consistent with previous reports, a significant reduction in Ki67+ cells was observed following binge ethanol treatment as compared to

controls. Furthermore, exposure to nicotine expectedly resulted in significant reductions in cell proliferation in the SGZ. Importantly, the dual ethanol and nicotine treatment yielded substantially reduced numbers of proliferating cells as compared to all other groups. Thus, dual exposure to alcohol and nicotine resulted in enhanced deficits in cell proliferation, which may compromise hippocampal integrity more drastically than either drug alone. Future research will investigate whether the additive damage to hippocampal circuitry following dual drug exposure results in behavioral deficits on a Morris Water Maze task.

Disclosures: **R.T. Lingg:** None. **C.B. Hartless:** None. **K.Y. Chen:** None. **K. Nixon:** None. **D.M. Hayes:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.12/AA7

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of chronic nicotine administration on hippocampal and striatal acetylcholinesterase activities

Authors: *P. U. NWOHA[1], O. M. IJOMONE, Male[2];
[1]Anat. & Cell Biol., Obafemi Awolowo Univ., Ile-Ife, Nigeria; [2]Human Anat., Cross River Univ. of Technol., Okuku, Nigeria

Abstracts: The present study investigated the effects of chronic administration of nicotine on the activities of acetylcholinesterase (AChE) in the hippocampus and striatum. Adult male albino Wistar rats, obtained from the Animal Holding of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria, were divided into two groups - experimental and control groups. Rats in the experimental group were given daily subcutaneous injections of nicotine at doses of 0.25, 2 or 4 mg/kg body weight for 28 days, while those in the control received similar injections of physiological saline for the same period. At the end, animals were anaesthetised and brains excised and processed for histochemical demonstration of AChE. Results obtained revealed significant decrease in AChE activities in the hippocampus and striatum following 2, and 4 mg/kg nicotine ($P < 0.01$) but not 0.25 mg/kg administration. This shows that nicotine may inhibit AChE activities in the brain, thereby affecting acetylcholine availability, with consequent motor effect.

Disclosures: **P.U. Nwoha:** None. **O.M. Ijomone:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.13/AA8

Topic: C.17. Drugs of Abuse and Addiction

Support: Virginia Youth Tobacco Projects

Virginia Tobacco Settlement Foundation

Virginia Foundation for Healthy Youth

Title: The gene expression response of Snca and Cdk5 to nicotine dosing is adolescent-specific and correlates with nicotine preference

Authors: R. T. HALLENBERG[1], N. S. DHARKER[1], *K. J. FRYXELL[2];
[1]Sch. of Systems Biol., George Mason Univ., Manassas, VA; [2]Sch. of Systems Biol., George Mason Univ., MANASSAS, VA

Abstracts: We injected inbred adolescent and adult mice (A/J and C57BL/6J strains) with a single subcutaneous dose of 0.5 mg/kg nicotine (or saline vehicle). We sacrificed them 3 hr or 24 hr later, and dissected specific brain areas for gene expression analysis. An additional set of mice were subjected to 4 injections at 2 day intervals, then sacrificed 24 hr after the last injection. Here we show that *Snca* and *Cdk5* were up-regulated in multiple brain areas of adolescent (but not adult) mice, by nicotine (but not saline) injections. In some cases, this up-regulation was significant after a single nicotine injection. After multiple nicotine injections, up-regulation was further increased, and was most pronounced in the mouse strains, ages, and sexes most vulnerable to nicotine dependence. In particular, *Snca* and *Cdk5* up-regulation was greater in adolescent mice than adults, greater in females than males, and greater in C57BL/6J than in A/J mice, all of which matched reported differences in nicotine preference. These observations, together with previous evidence that *Snca* and *Cdk5* were up-regulated by cocaine, suggest that both genes may have been up-regulated by nicotine-stimulated dopamine signaling. If so, then the probable result would be homeostatic reductions in dopamine signaling, which are among the best-documented functions of both *Snca* and *Cdk5*.

Disclosures: **R.T. Hallenberg:** None. **N.S. Dharker:** None. **K.J. Fryxell:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.14/AA9

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of kolaviron on the histology of the hypothalamus, pituitary, and testes of adult male Wistar rats

Authors: A. U. OBI[1], P. U. NWOHA[2], *C. A. ONYEKA[3,4];
[1]Dept. of Anat. and Neurobio., Imo State Univ., Owerri, Nigeria; [2]Dept. of Anat. and Cell Biol., Obafemi Awolowo Univ., Ile-Ife, Nigeria; [3]Dept. of Anat., Univ. of Lagos, Lagos, Nigeria; [4]Anat., Madonna Univ., Elele, Nigeria

Abstracts: Garcinia kola Linn is consumed widely in sub-Saharan Africa for various purposes, and kolaviron is its main product. This study determined the effects of kolaviron on the histology of organs of the hypothalamic-pituitary-gonadal axis, mainly the hypothalamus, pituitary and testis. The aim was to ascertain if its consumption has deleterious effects on these organs. Thirty-six adult male Wistar albino rats, obtained from the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria, with mean weight of 220g, were divided into six groups (A, B, C, D, E, F) of six animals each. Groups A, B, C, and D were the experimental groups and were given 100, 200, 400 and 800 mg/kg body weight of kolaviron respectively. Animals in group E served as control 1 and were given physiological saline, while those in group F served as control 2 and were given corn oil in which the kolaviron was dissolved. All administration was done daily by gastric intubation for 8 weeks. Adequate care was taken of all the animals. At the end, animals were anaesthetised, and the organs processed, stained with H & E and studied. Cells were counted with Image J. Compared to the controls, results showed gross cellular depletion and desquamation in the testis of groups C and D. Also in groups C and D, but not A, and B, there was significant reduction in the number of cells of the hypothalamus, and pituitary, compared to the controls ($P<0.05$). These findings suggest that kolaviron, at high doses could affect these organs and be detrimental to the body.

Disclosures: **A.U. Obi:** None. **P.U. Nwoha:** None. **C.A. Onyeka:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.15/AA10

Topic: C.17. Drugs of Abuse and Addiction

Support: DA017949

Title: Chronic nicotine administration in adolescent C57BL/6J mice results in deficits in trace fear conditioning in adulthood

Authors: *D. A. CONNOR[1], T. J. GOULD[2];
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Abstracts: The consequences of nicotine exposure during adolescence on cognition are not well understood. However, according to the CDC, 23 percent of high school students currently use tobacco products. Therefore, there is a need to develop a better understanding of the immediate and long-lasting effects of nicotine exposure during adolescence on cognition. Previous work indicates that exposure to chronic nicotine during adolescence leads to long-lasting deficits in contextual fear conditioning, 30 days after cessation of nicotine treatment (in adulthood). In contrast, adult mice do not show long-term deficits in contextual fear conditioning 30 days after exposure to chronic nicotine. Previous work from our lab has shown that immediate abstinence results in deficits in trace fear conditioning in adult mice. Thus, we sought to investigate how chronic nicotine administration might alter learning in a different hippocampus-dependent task, trace fear conditioning. In addition to recruitment of the hippocampus, trace fear conditioning also requires the medial prefrontal cortex and is thought to share characteristics with declarative memory. Therefore, trace fear conditioning is a useful behavioral assay to assess changes higher order cognitive function. We tested both long-term (30 days) and immediate (24 hr) abstinence after chronic administration during either adolescence or adulthood. An age-dependent effect was seen after long-term abstinence from chronic nicotine. Adolescent mice chronically administered nicotine showed deficits in trace fear conditioning 30 days after cessation. However, when mice were chronically treated during adulthood, and tested 30 days after cessation, no deficit was observed. In contrast, adolescent mice showed no immediate abstinence deficit, while mice treated in adulthood did show immediate deficits. Our findings support previous data showing administration during adolescence leads to deficits in hippocampus-dependent conditioning and attention. However, our results diverge from work showing that chronic nicotine treatment during adolescence leads to immediate abstinence-associated deficits in hippocampus-dependent contextual fear conditioning. This difference may result from the recruitment of extrahippocampal prefrontal regions during trace fear conditioning. In sum, our results represent a novel assessment of the long and short-term effects of prior chronic nicotine administration in adolescent and adult mice on cognition. Furthermore, this work demonstrates

that abstinence-associated deficits in hippocampus/cortex-dependent conditioning may impact adolescents differently than adults.

Disclosures: D.A. Connor: None. **T.J. Gould:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.16/AA11

Topic: C.17. Drugs of Abuse and Addiction

Support: Virginia Foundation for Healthy Youth

Title: The role of context preference on single trial nicotine conditioned place preference, and the role of dosing context on MAPK activation in the ventral striatum

Authors: *G. M. FERNANDEZ[1], D. G. EHLINGER[1], H. C. BERGSTROM[2], C. G. MCDONALD[1], R. F. SMITH[1];
[1]George Mason Univ., Fairfax, VA; [2]Lab. of Behavioral and Genomic Neurosci., Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstracts: Adolescents are prone to risky behaviors that lead to experimentation with drugs, such as nicotine. This behavioral profile coincides with a sensitization of the dopamine reward pathway. Drug-seeking behaviors are also maintained through reinforced drug- cue associations, tested via conditioned place preference (CPP). CPP is a behavioral measure of drug reward where a drug- context relationship is established through associative learning. Developmental differences in the establishment of a nicotine- context association could be due to differences in the cellular mechanisms underlying plasticity. The mitogen activated protein kinase (MAPK) pathway has been implicated in drug-induced plasticity. Adult (~P70) and adolescent (P28) Sprague Dawley rats were trained for single trial nicotine CPP (0.5 mg/kg), and their initial CPP chamber bias was correlated with the strength of the drug-cue relationship. Adolescents with relatively higher dark chamber preference (HDP) on day 1 formed single trial nicotine CPP, an effect not seen in HDP adults. Adults with relatively lower dark chamber preference (LDP) on day 1 of testing also formed single trial nicotine CPP, an effect not seen in LDP adolescents. Single trial nicotine CPP was abolished in HDP adolescents administered a MAPK inhibitor (SL327; 50 mg/kg) prior to conditioning. Adult and adolescent Sprague Dawley rats were also exposed to either a single CPP conditioning session or a saline versus nicotine injection in the

homecage. Brains were processed for phosphorylated MAPK (pMAPK) immunohistochemistry in the nucleus accumbens (NA). In the NA shell, there was a trend for increased pMAPK in adolescence following a nicotine injection in the CPP chamber compared to the homecage. There was a significant increase in pMAPK labeled cell counts in adults after a saline injection in the CPP versus homecage context. In the NA core, there was a significant increase in pMAPK with CPP versus homecage dosing in both age groups, regardless of drug. Our results suggest that preexisting context preference modulates the strength of a drug cue relationship across age groups, and during adolescence, this modulation involves MAPK. Down regulation of the NA MAPK pathway may modulate age related differences in response to a stimulus by flagging the significance of a novel event within a context.

Disclosures: G.M. Fernandez: None. **D.G. Ehlinger:** None. **H.C. Bergstrom:** None. **C.G. McDonald:** None. **R.F. Smith:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

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Program#/Poster: 619.17/AA12

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant NS065385

NIH Grant MD007597

NIH Grant AA021262

Title: Evidence for maternal deprivation stress and gestational nicotine exposure as risk factors for affective disorders and drug abuse

Authors: *M. C. GONDRE-LEWIS[1], K. WARNOCK[2], H. WANG[1], H. L. JUNE[2]; [1]Anat., Howard Univ. Col. of Med., WASHINGTON, DC; [2]Psychiatry and Behavioral Sci., Howard Univ. Col. of Med., Washington, DC

Abstracts: Individuals exposed to abuse, neglect, or drugs of abuse during gestational development and infancy are maladaptive to many social situations and are chemically sensitized to subsequent drug exposure. In addition, a high percentage of individuals receiving care for mood disorders like major depressive disorder, bipolar disorder and schizophrenia report experiencing these early life stressors during vulnerable periods of brain development. We have

previously shown that early postnatal maternal deprivation (MD) alone or in combination with prenatal nicotine (NIC) exposure alters neuronal development in the hippocampus, primarily the ventral hippocampus associated with emotional memory. Here, we explored the role of MD, NIC or the combination of the two in inducing behaviors correlated with neuropsychiatric disease and drug seeking in adolescents and adults. Pregnant Sprague Dawley female rats were prenatally exposed to saline or 4mg/kg/day of nicotine during the gestational period and their offspring were subjected to MD or normal maternal care at P2 until weaning. During adolescence, P28-P42, their performance on the open field test, elevated plus maze, forced swim test (FST), sucrose drinking, nicotine bottle drinking and alcohol binge drinking was assessed. NIC or MD exposure resulted in significantly increased locomotor activity. Subcutaneous nicotine during adolescence (sq-NIC) enhanced locomotor activity in controls, but NIC + sqNIC or MD + sqNIC were always significantly more active than sqNIC or sqPBS controls. NIC or MD exposure also decreased sucrose preference, and caused higher immobility times in the FST in adolescents. MD had a more robust effect on these parameters than NIC, and when combined the effect was mostly additive, but sometimes synergistic. Notably, both MD and NIC induced a high and sustained preference for nicotine by bottle drinking and alcohol via operant intracranial self-administration lever-pressing. Interestingly, MD binge drinking was elevated to levels close to that of alcohol-preferring rats. Neuroanatomical assessment of neurons in the amygdala or ventral tegmental area (VTA), brain loci associated with emotion and reward, showed an increase in the number of amygdala neurons in MD and NIC, and a decrease in VTA neurons of MD but not NIC groups, corresponding to dopamine neurons of alcohol preferring rats. We conclude that NIC and MD are confounders for behaviors associated with mood disorders and are risk factors for increased alcohol and nicotine substance use. We further conclude that the mechanisms for MD-induced drug seeking behavior may act through the dopamine reward pathway by a different mechanism than NIC.

Disclosures: **M.C. Gondre-Lewis:** None. **K. Warnock:** None. **H. Wang:** None. **H.L. June:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

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Program#/Poster: 619.18/AA13

Topic: C.17. Drugs of Abuse and Addiction

Support: NASA Grant

Title: A single nicotine injection leads to neural tube defects in chicken model

Authors: V. HUMPHREY, N. BOHN, *N. V. OMELCHENKO;
West Liberty Univ., West Liberty, WV

Abstracts: A large proportion of the United States population has been exposed to maternal smoking *in utero*. Mounting data suggests that nicotine can have a negative impact on neural system development. There is a known list of negative consequences of nicotine exposure, including neural tube defects. The goal of this study was to evaluate effects of nicotine exposure on chicken neural system development. The early chick embryo is an established model of the first month of embryonic development in mammals. Nicotine (nicotine hydrogen bitartrate) or vehicle (sodium bitartrate monohydrate) solutions were injected in eggs prior to incubation to match blood plasma levels observed in heavy smokers. Three cohorts of 24 eggs distributed between treatment groups were generated. After injections, eggs were sealed and placed in the incubator (35.5°C). Embryos were harvested on day 5 after injections. Our data indicates that the nicotine treatment does not affect viability, weight, or length of the embryos. Nonetheless, nicotine notably affects the axial rotation of the embryos. Axial rotation is defined as a change in the dorsoventral orientation of the head during development ("head turning"). In our study, atypical axial rotation was observed in nicotine treated groups 4-5 times more often than in controls ($p<0.05$). In order to elucidate the mechanism of such changes, the animals were embedded in paraplast, sectioned, and stained with hematoxylin and eosin for histological analysis. Preliminary data indicates that altered axial rotation linked to incomplete closing of the embryonic neural tube in the cervical region, but not in other areas of the tube. The development of neural tube and surrounding mesoderm is currently being evaluated using modern techniques of stereological analysis.

Disclosures: V. Humphrey: None. **N. Bohn:** None. **N.V. Omelchenko:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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Program#/Poster: 619.19/AA14

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant RO1AA018037

Title: Epigenetic and mutant analysis of alcohol tolerance in *Drosophila*

Authors: *A. GHEZZI, N. S. ATKINSON;
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Abstracts: Exposure to alcohol is known to trigger homeostatic adaptations in the brain that lead to the development of drug tolerance and dependence. These adaptations are believed to be of central importance in producing the addictive state. Accumulating evidence from genomic studies performed over the last several years suggests that these alcohol phenotypes are not the product of changes in the activity of single genes, but rather, these responses seem to be choreographed by multi-gene networks. It has thus become increasingly clear that proper understanding of these adaptations will require a comprehensive analysis of the molecular pathways that coordinate the regulation of these complex networks. Epigenetic histone modifications have recently emerged as important modulators of gene expression and are thought to represent a form of transcriptional memory that is directly imprinted on the chromosome. By altering the structural arrangement of chromatin regions, these modifications regulate the accessibility of transcription factors to the underlying DNA. Using a novel genomic approach that exploits the analysis of epigenetic modifications and the power of *Drosophila* genetics, we have recently identified a network of genes with a direct role on the development of alcohol tolerance. The genes in this network not only show a common alcohol-induced histone acetylation response but also share a highly correlated expression profile in response to distinct environmental stimuli. These results suggest that the genes in this network are coordinately regulated. Here, we explore the epigenetic landscape of a 'tolerance' gene network to decipher potential regulatory epigenetic signatures involved in adaptation to alcohol.

Disclosures: A. Ghezzi: None. **N.S. Atkinson:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NSFC of China U1132602

SF of Ningbo,China 2013A610252

Title: Significant association of rs17189632 in the glutamate receptor subunit gene (GRIN3A) with heroin dependence

Authors: *X. XIE[1,2,3], H. LIU[1,2,3], W. ZHOU[1,2,3];
[1]Ningbo Inst. of Microcirculation and Henbane, Zhejiang, China; [2]Lab. of Behavioral Neuroscience, Sch. of Medicine, Ningbo Univ., Ningbo, China; [3]Ningbo Addiction Res. and Treatment Ctr., Ningbo, China

Abstracts: Objective The N-methyl-D-aspartate receptor is consisting of three subfamilies (NR1, NR2A, 2B, 2C, 2D and NR3A, 3B). The ability of NR3A to modulate the NMDAR function makes it an attractive candidate gene of heroin addiction. The purpose of this study is to explore the association between two single nucleotide polymorphisms (SNPs) (rs3739722, rs17189632) in the glutamate receptor subunit gene (GRIN3A) and heroin addiction. Methods The genotypes of the two SNPs (rs3739722, rs17189632) in 332 heroin dependent patients and 200 normal control subjects in the male Han Chinese population were detected by TaqMan SNP genotyping method, and the association between heroin dependence and the two SNPs was analyzed. Results The distributions of genotype and allele at rs3739722 were not significantly different between in the cases and in the control group (P> 0.05). The frequencies of genotype and allele at rs17189632 were significantly different between the cases and the controls (x2 = 7.237, P = 0.0268; x2 = 4.278, P = 0.0386). In addition, the A allele frequency of rs17189632 was significantly lower in cases compared with the control group (OR = 0.752, 95% CI 0.574-0.986, P = 0.0386). And the frequency of AA genotype of rs17189632 was significantly lower in cases compared with the control group (OR = 0.457, 95% CI 0.255-0.818, P = 0.0073). Conclusion Our study indicates that the rs17189632 of the glutamate receptor subunit gene (GRIN3A) play a major role in heroin dependence.

Disclosures: **X. Xie:** None. **H. Liu:** None. **W. Zhou:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

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NIH Grant DA005010

NIH Grant NS048004

NIH Grant DA027309

Title: Hybrid mouse diversity panel reveals genetic correlations between impulsivity and intravenous drug self-administration

Authors: *J. D. JENTSCH[1], M. C. CERVANTES[1], R. LAUGHLIN[2], A. S. JAMES[1];
[1]Univ. Calif, Los Angeles, Los Angeles, CA; [2]Williamette Univ., Salem, OR

Abstracts: Impulsivity, which often reflects a loss of inhibitory control over reward-seeking behaviors, has been implicated as a heritable risk factor for drug and alcohol use disorders. That said, impulsivity has often been conceived of as a multi-dimensional construct, spanning several partially related behavioral phenomena with separate biological substrates. For example, heightened propensity to engage in intravenous drug self-administration has been linked to an elevated tendency to respond early in a choice serial reaction time task (Dalley et al. Science, 15:1267-70, 2007) and to difficulty with suppressing or updating behavior in a reversal learning task (Cervantes et al. Psychopharmacol., 229:515-25, 2013). Yet other studies have linked the tendency to discount delayed rewards to self-administration. We sought to determine whether premature responding in choice reaction time tasks and/or response inhibition in a reversal test were genetically correlated with intravenous cocaine self-administration, using the hybrid mouse diversity panel - a large genetic reference panel consisting of inbred mouse strains. Published data on impulsive responding in a 5-choice serial reaction time from Loos et al. (Biol. Psychiatry, 2014; doi: 10.1016/j.biopsych.2014.02.011) were combined with novel data on reversal learning and intravenous cocaine self-administration acquired from many of the same strains. Genetic correlations were performed at the strain level to identify phentoypes with common genetic influences. Anticipatory responding in the 5-choice task (from Loos et al. 2014) and from our reversal learning task were negatively correlated with one another but each was positively correlated with a measure of response inhibition in reversal learning, suggesting that these measures index a common construct. Moreover, anticipatory responding and response inhibition in the reversal learning task were positively related to drug self- administration behaviors, while anticipatory responding in the 5-choice task was not. Gene co-expression network analyses revealed novel data about transcriptomic influences that mediate these relationships, and genome-wide scans for each phenotype reveals evidence for both conserved and distinct genetic determinants. These data provide new evidence suggesting that multiple measures of impulsivity are, in part, related to one another, while simultaneously having partially distinct genetic/genomic influences and relating to drug self-administration propensity in non-overlapping fashions.

Disclosures: **J.D. Jentsch:** None. **M.C. Cervantes:** None. **R. Laughlin:** None. **A.S. James:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Grant P50AA03510

NIH Grant RO1 AA015606-01

Title: Studying the role of epigenetic marks in a gene cluster associated with alcohol use disorders using induced pluripotent stem cell-derived neural cultures

Authors: *M. GROSS, R. LIEBERMAN, J. COVAULT;
Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstracts: Alcohol use disorders affect approximately 8.5% of the US population during a one-year period. Studies have found strong genetic effects playing a role in the transition of heavy drinking to alcohol dependence; however, understanding the mechanisms behind this switch has been difficult. Induced pluripotent stem cells (iPSCs) have become a tremendous tool for studying neurological disease. Before their appearance, neurobiological disease was studied solely on the use of neuroimaging studies, post-mortem human tissue, and animal models, all of which have their limitations. Using iPSCs, our lab has differentiated iPSCs from control and alcoholic subjects into forebrain glutamatergic neurons. After examining mRNA expression of key genes involved with alcohol dependence susceptibility, it was discovered that a cluster of genes encoding four subunits to the $GABA_A$ receptor on chromosome 4p12 were expressed at low levels in close to half of the lines we examined. Further, this low expression was correlated with the presence of a synonymous *GABRA2* exon 5 T to C SNP (rs279858), which has been associated with alcohol dependence in several studies. However, mRNA for subunits encoded by $GABA_A$ receptor genes located on other chromosomes were expressed. Existing data shows that in embryonic stem cells and iPSCs, the promoter region of *GABRA2* is poised, meaning it has both repressive and active epigenetic chromatin/histone modifications, but that post-mortem neuronal tissue shows a loss of *GABRA2* promoter region repressive histone marks. We hypothesize that the low expression of the chromosome 4 $GABA_A$ receptor gene cluster in a subset of iPSC-derived neural cultures may be associated with the retention of repressive histone modifications, or possibly long distance chromatin looping by CTCF binding sites. Using chromatin immunoprecipitation followed by quantitative PCR, epigenetic marks commonly associated with activate gene expression (H3K4me3), marks associated with repressed gene

expression (H3K27me3 and H3K9me3), and CTCF binding sites were explored in subject's iPSCs and iPSC-derived neural cultures. Preliminary results show that 12-week old iPSC-derived neural cultures that show low expression of the chromosome 4p12 $GABA_A$ gene cluster and that also have the rs279858 C-allele have a greater amount of repressive histone marks than lines that express the $GABA_A$ gene cluster and have the TT genotype at rs279858. Our results suggest that the rs279858 polymorphism may moderate, via chromatin epigenetic mechanisms, the developmental regulation of the chromosome 4p12 $GABA_A$ gene cluster.

Disclosures: M. Gross: None. **R. Lieberman:** None. **J. Covault:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.23/AA18

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant 5P01DA021633 (HA)

ONR Grant N00014-09-1-0598 (HA)

ONR Grant N00014-12-1-0366 (HA)

'Plan Nacional sobre Drogas' (MSC, Madrid, Spain) Grant 2012I011 (MJGF)

NIDA Grant T32 DA007268

Title: Adolescent cocaine exposure alters acetylation and tri-methylation on histone 3 lysine 9 in the hippocampus and nucleus accumbens exclusively in selectively bred rats that are typically resilient to addiction

Authors: *A. PARSEGIAN[1], J. GARCIA-FUSTER[2], S. CHAUDHURY[1], P. BLANDINO[1], S. J. WATSON[1], S. FLAGEL[1,3], H. AKIL[1];
[1]The Mol. & Behavioral Neurosci. Inst., Ann Arbor, MI; [2]Univ. Res. Inst. on Hlth. Sci., Univ. of the Balearic Islands, Palma de Mallorca, Spain; [3]Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstracts: Only a small minority of those who experiment with drugs of abuse goes on to become drug addicts. Although there is no single factor that confers addiction liability, it is clear

that genetic predisposition and prior experience with the drug have predictive validity. Notably, early initiation of drug use reliably predicts the likelihood of addiction in adulthood. Thus, adolescence may be a particularly critical period when drug use causes persistent neuroadaptations that might render one more susceptible to addiction. Recently, an important relationship has emerged between certain chromatin modifications, the genes they modify, and the enduring neurobiological impact of cocaine in promoting addiction. Here, we utilized a unique genetic rat model that captures individual differences in vulnerability to addiction to determine whether adolescent drug exposure alters their genetic predisposition via modification of epigenetic chromatin marks. Rats selectively bred based on locomotor response to novelty differ on a number of addiction-related traits. Specifically, relative to bred low-responder (bLR) rats, bred high-responders (bHR) are more sensitive to the psychomotor activating effects of cocaine and reinstate drug-seeking more readily following a prolonged period of abstinence. We exposed these bred rats to a sensitizing regimen of cocaine (15 mg/kg) or saline for 7 days during adolescence (PND 33-39) and assessed phenotypic differences in the acute and sensitized response to cocaine during this period. Further, we used a novel approach to measure two histone modifications, acetylation (ac) and tri-methylation (me3) on histone lysine 9 (H3K9), in the hippocampus (HC) and nucleus accumbens (NAc) during either adolescence, or after three weeks of abstinence. As expected, bHRs showed greater sensitivity than bLRs to both acute and repeated cocaine exposure during adolescence. In the saline-treated bLRs, expression of acH3K9 was higher and H3K9me3 was lower in the HC and NAc, as compared to bHRs. Interestingly, adolescent cocaine eliminated these differences, rendering bLRs more similar to bHRs. Further, these effects appear to be sub-region specific, as no differences were seen in the ventral HC, and epigenetic changes in the NAc shell were distinct from those in the core between the phenotypes. These findings suggest that adolescent cocaine exposure may render bLRs more susceptible to addiction, without affecting bHRs. Additional epigenetic and behavioral analyses will be performed to determine associations linking adolescent cocaine-induced epigenetic modifications to neurobiological and behavioral alterations that persist into adulthood

Disclosures: **A. Parsegian:** None. **J. Garcia-Fuster:** None. **S. Chaudhury:** None. **P. Blandino:** None. **S.J. Watson:** None. **S. Flagel:** None. **H. Akil:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.24/AA19

Topic: C.17. Drugs of Abuse and Addiction

Support: UTHSC Pilot grant.

Title: Strain-specific differences in microrna expression in the hippocampus following exposure to stress and/or alcohol

Authors: *K. M. HAMRE, S. LATTIMER, J. INGELS, L. LU;
Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstracts: Genetic differences in response to stress and/or alcohol exposure are well documented although the specific genetic pathways that mediate these differences remain elusive. Moreover, it is becoming clear that these pathways include more than simply alterations in gene expression, but also include other changes such as differences in microRNA (miR) expression. The present experiment was conducted to determine whether there were strain-specific differences in miR expression following exposure to either stress and/or ethanol. Adult male C57BL/6J (B6) and DBA/2J (D2) mice were examined. Stress was given via a 4-week chronic, unpredictable mild stress paradigm that included tilted cages, wet bedding, mild restraint, exposure to odor from another male and 24- hour constant light. Alcohol was given as an acute exposure to 1.8 g/kg given via IP injection. Four groups were examined: 1) stress only, 2) ethanol only, 3) stress + ethanol, and 4) control given neither. Prior to tissue collection, mice were examined for anxiety phenotypes in an elevated plus maze, with bigger effects seen in the D2 than in the B6 mice. Hippocampal tissue was collected and expression of selected miRs was examined using real-time qPCR. MicroRNAs were selected because they had previously been shown to be altered following ethanol exposure or because they are proposed members of stress-related pathways such as the CRH pathways. As expected, the different groups exhibit differentially-expressed miRs. Interestingly, exposure to stress alters the miRs that change expression following alcohol exposure and this effect is observed in a strain-dependent fashion with more changes observed in the D2 mice than in the B6 mice. Analysis showed that miRs involved in several stress- or ethanol-related pathways showed changes following ethanol and/or stress treatment. For example, changes were found in 1) miR-206, a miR related to the BDNF (brain derived neurotrophic factor) signaling pathway, and 2) miR-335 that has strain-specific alterations in expression. These results demonstrate that microRNAs are excellent candidates for mediating genetic differences in responses to stress and ethanol. Future studies will expand these analyses to evaluate other miRs as well as their downstream mRNA targets.

Disclosures: **K.M. Hamre:** None. **S. Lattimer:** None. **J. Ingels:** None. **L. Lu:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.25/AA20

Topic: C.17. Drugs of Abuse and Addiction

Support: P50AA03510

RO1 AA015606-01

Title: Examining regulators of the glucocorticoid receptor in human neural cells derived from alcoholics

Authors: *R. LIEBERMAN[1], E. S. LEVINE[2], H. R. KRANZLER[4], J. COVAULT[3]; [2]Neurosci., [3]Psychiatry, [1]Univ. of Connecticut Hlth. Ctr., Farmington, CT; [4]Univ. of Pennsylvania, Philadelphia, PA

Abstracts: The glucocorticoid receptor (GR) is a ligand-activated transcription factor that is the primary target for the glucocorticoid cortisol. Cortisol is released by the adrenal gland following hypothalamic-pituitary-adrenal (HPA) axis activation in response to stress. Termination of the stress response is facilitated by GR activation in the brain, which is a complex process involving numerous co-chaperones that can regulate activity of the GR by modulating folding, translocation into the nucleus, and affinity for cortisol. A common finding in psychiatric disorders including depression, post-traumatic stress disorder, schizophrenia, bipolar disorder, and alcoholism is an alteration in the subject's response to stress. Studies of human post-mortem brain suggest that differences in the expression of the glucocorticoid receptor and its regulatory co-factors between control and patient samples may underlie some of the alterations to the stress response observed in these psychiatric disorders. Alcohol use disorders are common in society, affecting 8.5% of the U.S. population. Recent RNA-seq findings from post-mortem hippocampal samples suggest that genes involved in the stress-response pathway, including the glucocorticoid receptor and its co-factors, are altered in alcoholics. Furthermore, alcoholics have higher basal levels of cortisol and a blunted cortisol response to acute stress, and dysregulation of the stress response can be seen in the children of alcoholics, suggesting a genetic component. To further elucidate differences in glucocorticoid receptor regulation in alcoholism, we examined human neural cells differentiated from induced pluripotent stem cells generated from 12 alcoholic and 10 non-alcoholic donor subjects. Using an established neural differentiation protocol, we generated mixed neural cultures containing physiologically active forebrain glutamatergic neurons and glial cells. After 6 weeks of neural maturation, cells were exposed for 6 hours to 10μM, 100μM, or 1000μM dexamethasone, a synthetic agonist with a high affinity for the glucocorticoid receptor. Following exposure, RNA was extracted and the expression of genes encoding the GR and its co-factors FKBP51, FKBP52, and Bcl-2-associated athanogene

(encoded by *NR3C1, FKBP5, FKBP4* and *BAG1*, respectively) was analyzed using quantitative PCR. Differences were observed in *FKBP5*, but not *NR3C1*, *FKBP4,* or *BAG1* gene expression following acute dexamethasone exposure between cells derived from alcoholic and non-alcoholic donor subjects. These results suggest expression of certain GR co-factors may be regulated differently in neural cells from alcoholics.

Disclosures: R. Lieberman: None. **E.S. Levine:** None. **H.R. Kranzler:** None. **J. Covault:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.26/AA21

Topic: C.17. Drugs of Abuse and Addiction

Title: A genetic reduction in the serotonin transporter differentially influences self-administration of 3,4-methylenedioxymethamphetamine (MDMA) and heroin

Authors: B. W. BROX, *B. A. ELLENBROEK;
Victoria Univ. of Wellington, Wellington, New Zealand

Abstracts: Addiction to drugs of abuse is a ubiquitous phenomenon that places a tremendous financial and psychological burden on society, families and the individual. Interestingly, only a small percentage of individuals (~20%) who use drugs of abuse go on to develop the compulsive behaviours that define addiction. Clinical studies have shown that there is a subset of the population with a genetically determined reduction in the serotonin transporter that may increase vulnerability to developing addiction. To investigate the influence of reduced serotonin transporter function in the laboratory we studied the reinforcing properties of MDMA ('ecstasy') and heroin in a genetically modified animal model: the serotonin transporter (SERT) knockout rat. Homozygous ($SERT^{-/-}$) animals lack SERT function completely while heterozygous ($SERT^{+/-}$) have about 50% SERT function compared to the wild type ($SERT^{+/+}$). Importantly, MDMA, in addition to its effects on the SERT, directly stimulates dopamine release. Heroin, on the other hand, via stimulation of μ-opioid receptors, indirectly stimulates dopamine release. To understand the interaction between reduced serotonin transporter function and the development of addictive behaviours we utilized a self-administration behavioural paradigm. One hundred per cent of $SERT^{-/-}$ and $SERT^{+/-}$ rats acquired MDMA self-administration compared to only 50% of $SERT^{+/+}$. Of the animals that met the criterion for self-administration the $SERT^{-/-}$ took more

infusions during maintenance as well as reached greater breakpoints when tested on a progressive ratio schedule compared to $SERT^{+/+}$. On the other hand, there were no significant differences between genotypes during acquisition and maintenance of heroin self-administration. These results clearly indicate that genetically reduced SERT function is a risk factor for the development of self-administration of some drugs and further underline the fact that significant differences exist between disparate drugs of abuse.

Disclosures: B.W. Brox: None. **B.A. Ellenbroek:** None.

Poster

619. Exposure to Addictive Drugs: Genetic and Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 619.27/AA22

Topic: C.17. Drugs of Abuse and Addiction

Support: UWM RGI Program

NIEHS ES016513

NIEHS 2P30ES004184

Title: Dorsal root ganglion development in nicotine-exposed zebrafish

Authors: *J. SCHULD[1], K. R. SVOBODA[2];
[1]Joseph J. Zilber Sch. of Publ. Hlth., Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Joseph J. Zilber Sch. of Publ. Hlth., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstracts: During vertebrate development including humans, multipotent cells known as neural crest cells (ncc) give rise to a variety of different cell types such as neurons, pigment/melanocytes, bone, cartilage, and glia. The development of ncc is likely altered in children born to mothers who smoke during pregnancy which could result in congenital birth defects. These birth defects may also be linked to nicotine, a main ingredient of cigarettes. Our research group utilizes zebrafish as a model to study nicotine toxicity, a phenomenon seen in humans. Our unpublished results reveal that embryonic nicotine exposure in zebrafish alters the development and migration of melanocytes and dorsal root ganglion (DRG) neurons. In zebrafish and other vertebrates, the dorsal root ganglia lie adjacent to the spinal cord and are segmentally positioned along the length of the body. We are utilizing Tg(Isl2B:gfp) transgenic zebrafish to study the consequences of nicotine exposure on DRG development. In non-exposed,

96 hpf larvae, cells within individual DRG are organized in a pattern that resembles either a cluster of grapes or in a more elongated pattern, resembling a chili pepper. About 50% of the ganglia analyzed exhibited the cluster of grapes phenotype, ~ 40% exhibited the chili pepper phenotype, and about 10% exhibited a phenotype not easily categorized. In nicotine exposed larvae, roughly 10% of the ganglia analyzed exhibited the cluster of grapes phenotype, whereas ~ 70-80% of the ganglia analyzed exhibited the chili pepper phenotype. When nicotine-exposed larvae were raised out to 1.5-3 weeks of age and imaged, the patterning of neural processes extending from DRG neurons and projecting within the skin was found to be altered. This analysis was done using a Zeiss, Axio Zoom.V16 Microscope. This imaging platform is designed for imaging in thick tissue with high-resolution. Our results show that an early exposure to nicotine during times when the dorsal root ganglia are developing, may permanently alter their development.

Disclosures: J. Schuld: None. **K.R. Svoboda:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.01/AA23

Topic: A.04. Stem Cells

Support: NIH DC011534

Title: Defining a novel intermediate progenitor cell class in the embryonic olfactory epithelium

Authors: *E. M. PARONETT, D. W. MEECHAN, T. M. MAYNARD, A.-S. LAMANTIA; Pharmacol. and Physiol., George Washington Univ., Washington, DC

Abstracts: The identity of olfactory epithelial (OE) intermediate progenitors remains uncertain, despite the potential role of this class of cells in ongoing OE regeneration. We addressed this issue in the early developing OE using inducible Cre-driven mouse lines for two critical proteins -Pax7 and Ascl1_that are likely associated with OE stem and transit amplifying precursors, respectively. Using brief exposure to tamoxifen, we induced Cre driver expression in a subset of cells expressing either Pax7 or Ascl1 at embryonic day 10.5 (E10.5) and studied lineage-derived cell expression patterns at E11.5 for early OE development, and E16.5 for late OE development. The numbers of Pax7 recombined progeny seen at E11.5 after E10.5 tamoxifen exposure is consistent with the slowly dividing stem cell identity of the Pax7 cells. Nevertheless, E11.5

Pax7-derived cells give rise to Ascl1-expressing cells. We are currently evaluating the fates of these Pax7 progeny at later survivals after E10.5 tamoxifen exposure. At E11.5, some, but not all, Ascl1-derived cells are proliferatively active. The proliferatively active cells are primarily located in the dorsomedial OE and are likely to be derived from the slowly-dividing Pax7 progenitors in the ventrolateral OE. They are proximal to differentiating neurons on either side of the zone in which they are actively dividing, suggesting that Ascl1 acts late in the transit-amplifying niche of the stem cell lineage. Discontinuous patterns of Ascl1 in the OE at E11.5 and E16.5 after E10.5 tamoxifen indicate that Ascl1-mediated expansion may reflect local morphogenesis that guides emergence of OE turbinates. These results indicate that there is a likely intermediate progenitor that emerges between the Pax7 slowly dividing stem cells and the Ascl1 near-terminally neurogenic intermediate progenitors. We are currently determining whether these novel intermediate progenitors are a subset of high Six1-expression proliferative cells in the E11.5 OE. Dissociated cells of the medial OE analyzed by pair cell assay show that cells expressing Six1 as well as Ascl1 are capable of both symmetric and asymmetric division and thus have the potential to be both self-renewing and terminally neurogenic. Therefore, it seems likely that there is a novel OE intermediate progenitor class that is essential for the progression from slowly-dividing stem cell to Ascl1-expressing late neural progenitor, and ultimately newly generated olfactory receptor, vomeronasal receptor and GnRH neurons. These precursors, or a closely related class, may be retained in the mature OE to facilitate ongoing regeneration.

Disclosures: E.M. Paronett: None. **D.W. Meechan:** None. **T.M. Maynard:** None. **A. LaMantia:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.02/AA24

Topic: D.01. Chemical Senses

Support: FONDECYT-1111046

Proyecto Instituto Milenio PO9-022-F

CONICYT Doctoral Fellowship

Title: Genomic Plasticity in the olfactory epithelium is correlated with odorant exposure during early development in the zebrafish (Danio rerio)

Authors: C. CALFÚN[1], C. DOMINGUEZ[2], T. PÉREZ-ACLE[2], *K. E. WHITLOCK[1];
[1]Univ. de Valparaiso, Valparaiso, Chile; [2]Fundacion Ciencia y & Vida, Santiago, Chile

Abstracts: Zebrafish form olfactory memories of odors experienced during early development (imprinting) including the artificial odorant phenylethyl alcohol (PEA). Correlated with this olfactory memory we have shown that the transcription factor (TF) *otx2* is upregulated in the olfactory epithelium (OE) of PEA-imprinted fish, demonstrating a role for peripheral nervous system (PNS) in olfactory memory. In order to further analyze odorant modulation of the gene expression in the OE, we focused on the olfactory receptor (OR) as potential odorant regulated genes through regulation by *otx2.* We identified ORs containing Otx2 binding sites in their control regions and analyzed their expression by qPCR. Exposure to PEA resulted in the down regulation of five of the eight ORs analyzed. In order to identify regulatory sequences for ORs, we performed a bioinformatic analysis looking TF for binding sites. We observed multiple binding sites for transcription factors, including Otx2, in regions flanking ORs sequences. Interestingly, according the literature, some of these TFs can to form repressive complexes with Otx2 to control gene expression, and are located upstream the odorant-modulated ORs. Currently we are performing a RNA seq analysis at three weeks post fertilization and adult stage, in order to study odorant effects on the whole transcriptome of the zebrafish OE, including the OR repertoire. At three weeks post fertilization, our preliminary data show that the exposure to PEA is correlated with changes in different receptors expressed in the olfactory sensory neurons, including some genes of the OR family. This study supports the model where the odor environment can modulate gene expression during early development and suggests a role for the ORs in the memory formation.

Disclosures: C. Calfún: None. **C. Dominguez:** None. **K.E. Whitlock:** None. **T. Pérez-Acle:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.03/BB1

Topic: D.01. Chemical Senses

Support: NIH R01 DC005782

Title: Crucial role of olfactory receptor accessory proteins RTP1 and RTP2 in receptor gene choice, development and odor detection

Authors: *R. SHARMA[1], Y. ISHIMARU[2], I. DAVISON[3], M. EHLERS[4], H. MATSUNAMI[1]; [1]Duke Univ., Durham, NC; [2]Univ. of Tokyo, Tokyo, Japan; [3]Boston Univ., Boston, MA; [4]Pfizer Neurosci., Cambridge, MA

Abstracts: Receptor transporting proteins (RTP1 and RTP2), specifically expressed in the olfactory sensory neurons (OSNs), have been shown to greatly increase the cell surface expression of olfactory receptors (ORs) when expressed in heterologous cells. We have generated RTP1 and RTP2 double knockout mice (RTP1,2-/-) to test the function of RTP1 and RTP2 *in vivo*. RTP1,2-/- mice are viable and show no gross morphological defects. Consistent with the role of RTP1 and RTP2 in OR trafficking, cilia localization of a specific OR is lost in RTP1,2-/- mice. OMP and ACIII expression indicate fewer mature OSNs in the RTP1,2-/- olfactory epithelium which can be explained by a four fold increase in apoptosis of the olfactory epithelium in these mice. Strikingly, expression of ATF5, an indicator of the unfolded protein response and ongoing OR gene choice is vastly expanded in the olfactory epithelium of the knock out mice suggesting that these neurons may be unable to stably express a single OR. This is further reinforced by the expanded expression of LSD1, a histone modifier responsible for the desilencing and initiation of the OR transcriptional machinery. Surprisingly, while expression of many ORs is diminished in RTP1,2 -/- mice, some ORs are overexpressed in the mutant, suggesting a biased OR choice in the absent of RTP1 and 2. Electroolfactogram recordings as well as odor-induced c-Fos induction in the olfactory bulb suggest that RTP1,2-/- mice show dramatically diminished responses to odors. Furthermore axons of OSN expressing OR-M71 do not converge in the olfactory bulb,, indicating that the axons are unable to target specific glomeruli. Together, these results show that RTP1 and RTP2 are crucial for development and function of the olfactory system.

Disclosures: **R. Sharma:** None. **Y. Ishimaru:** None. **I. Davison:** None. **M. Ehlers:** A. Employment/Salary (full or part-time):; Pfizer Neurocience. **H. Matsunami:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.04/BB2

Topic: D.01. Chemical Senses

Support: Jill Barrett Summer Research Program, Mills College

Mills College Faculty Research Grant

Title: Identifying odor receptors in *C. elegans*

Authors: *J. J. YOUNG[1], S. APOSTOL[1], S. NATHAN[1], E. NEWMAN[1], A. COX-HARRIS[1], F. TAN[1,2], C. BRUEGGEMANN[2], N. L'ETOILE[2];
[1]Mills Col., OAKLAND, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstracts: Although scientists have been studying olfaction in *C. elegans* for decades, olfactory receptor proteins remain largely uncharacterized. We are currently using two approaches to address this knowledge gap. A forward genetic screen was carried out to identify genes involved in odor signaling. Mutants were selected if they displayed repeated lack of attraction to benzaldehyde (which is sensed by the olfactory neuron AWC) after exposures to benzaldehyde and E. coli. 27 worm strains were isolated as potentially interesting mutants. We are currently analyzing these mutants for olfactory defects, and have identified two such lines. We are also pursuing localization of candidate proteins. Data generated by Yen-Ping Hsueh in the Sternberg lab identified a set of putative odor receptor genes that are expressed in AWC. We selected eight of these genes for localization analysis and obtained GFP-reporter constructs from the Transgenome Project. We are producing tagged lines and determining which of these proteins localize to the olfactory cilia.

Disclosures: J.J. Young: None. **S. Apostol:** None. **S. Nathan:** None. **E. Newman:** None. **A. Cox-Harris:** None. **F. Tan:** None. **C. Brueggemann:** None. **N. L'Etoile:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.05/BB3

Topic: D.01. Chemical Senses

Support: NIH Grant DC02736

Title: Odorant receptor activation patterns *in vivo*

Authors: *T. S. MCCLINTOCK[1], K. ADIPIETRO[2], P. BREHENY[3], A. WALZ[4], P. MOMBAERTS[5], H. MATSUNAMI[2];

[1]Dept Physiol., Univ. Kentucky, LEXINGTON, KY; [2]Duke Univ., Durham, NC; [3]Univ. of Iowa, Iowa City, IA; [4]Rockefeller Univ., New York, NY; [5]Max Planck Inst. of Biophysics, Frankfurt, Germany

Abstracts: Analogous to distinguishing colors by differential activation of opsins, the patterns of *in vivo* activation of receptor proteins by odorants must be fundamental to odor discrimination. However, these patterns are as yet undefined. We identified receptors activated by odorants *in vivo* by measuring the enrichment of all receptor mRNAs after activity-dependent capture of olfactory sensory neurons, an approach made possible by the fact that these neurons each express only one receptor. Receptors for the odorants muscone (a base note in perfumes) and eugenol (a flavoring) were identified first. These odorants activated similar, but nonoverlapping, patterns consisting of core sets of a few related odorant receptors, with weaker evidence of activation of additional receptors. For example, the receptors most strongly activated by muscone both *in vivo* and in heterologous expression assays belonged to the mOR214 and mOR215 families, with Olfr1440 (mOR215-1) and Olfr235 (mOR214-3) showing the strongest responses. These receptors were not activated by polycyclic musk odorants. The homologous human receptor, OR5AN1, was similarly selective for macrocyclic musk odorants over polycyclic musks. As expected, easily discriminated odorants showed distinct patterns of receptor activation. Some patterns were atypical, however, such as the activation of both odorant receptors and trace amine-associated receptors (TAARs) by 2-phenethylamine.

Disclosures: T.S. McClintock: None. **K. Adipietro:** None. **P. Breheny:** None. **A. Walz:** None. **P. Mombaerts:** None. **H. Matsunami:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.06/BB4

Topic: D.01. Chemical Senses

Support: German Research Foundation

Title: Odor responses of *Drosophila* receptor neurons - Response profiles, mixtures and individual response dynamics

Authors: *D. MÜNCH, J. S. IGNATIOUS RAJA, T. LAUDES, A. NISSLER, C. G. GALIZIA; Biol., Univ. of Konstanz, Konstanz, Germany

Abstracts: Olfactory sensory systems usually consist of multiple classes of olfactory receptor neurons (ORNs) that are tuned to different but overlapping sets of odorants. Thus, when odors are presented, specific activation patterns consisting of all the activated, inhibited or non-activated ORNs arise. Tuning profiles of individual ORN classes differ in the number of odorants they respond to, ranging from generalist ORNs that are sensitive to hundreds or thousands of chemical compounds, to specialist ORNs that respond only to a handful or to single substances only. On a single cell level, ORN responses vary in strength as well as in their dynamics, e.g. response polarity or response duration. ORN responses become even more complex when one considers more natural odor-stimuli i.e. mixtures. Two substances might interfere when stimulating an ORN, generating a mixture response that is distinct in strength or dynamics from the components response. Here we describe response profiles of eight classes of *Drosophila* ORNs in response to a set of ~100 odorants. We also analyzed the response dynamics from the ~800 odorant-ORN combinations in detail. For a smaller set of ORNs and odorants we studied the responses elicited by binary mixtures compared to their single components. We found breadth of tuning to be widely distributed observing both, generalist- and specialist ORNs in our set. For Or56a neurons which are known to be specifically tuned to the single odorant geosmin, we found several ligands besides that single odorant, even though these responses were much weaker. The majority of responses we recorded from the eight ORNs were excitatory, fewer were inhibitory or showed initial excitation followed by a post-stimulus inhibitory phase. Dynamical response-features differed across odorant-ORN combinations: Some generated strong and prolonged responses that continued beyond stimulus offset, others were short-lived (phasic), still others were complex over time, including excitatory and inhibitory bouts. Most of the binary mixtures tested produced weak or no interactions, though a few combinations lead to suppressive (response weaker than the response elicited by the stronger component alone) or synergistic (response much stronger than the response elicited by the stronger component alone) mixture responses. Across ORNs, about half of the mixture responses followed one of the two odorant components, while the other half had distinct activity patterns. These measurements are relevant for understanding how odor information is coded in combinatorial activity patterns.

Disclosures: D. Münch: None. **J.S. Ignatious Raja:** None. **T. Laudes:** None. **C.G. Galizia:** None. **A. Nissler:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.07/BB5

Topic: D.01. Chemical Senses

Support: DFG Schwerpunktprogramm/ SPP 1392: Integrative Analysis of Olfaction

Title: Ancestral amphibian V2Rs are expressed in the main olfactory epithelium

Authors: ***A. S. SYED**, S. I. KORSCHING;
Inst. for Genetics, Univ. of Cologne, Cologne, Germany

Abstracts: The sense of smell helps animal species to evade predators, localize prey and recognize viable mates. In mammals olfactory receptor families are segregated into different olfactory organs, main olfactory epithelium (MOE) and vomeronasal organ (VNO). In contrast, teleost fish olfactory receptor families are intermingled in a single sensory surface. To what extent such differences influence the coding and discrimination abilities of the respective olfactory systems is unclear, and the evolutionary path toward such segregation is unknown. The analysis of amphibians, which are early diverging tetrapods compared with mammals, may shed light on this transition from shared sensory surface to segregated subsystems. For this study, we focused on the V2R (vomeronasal type 2 receptor) gene family, by thorough datamining we showed the Xenopus V2R family to encompass nearly 500 genes in total. A phylogenetic analysis led to the identification of three distinct subdivisions in the largest group of V2R genes (A1, A2, A3). We used this sequence information to clone several Xenopus laevis V2R gene representatives of above mentioned subdivisions. Xenopus laevis (African clawed frog), is the system of choice for physiological studies of the amphibian olfactory system and furthermore, a close relative of Xenopus tropicalis. We report here that to our surprise, several V2R genes were expressed exclusively in the MOE, and not in the VNO. These genes occupied basal positions in the phylogenetic tree, whereas late diverging V2R genes were exclusively expressed in the VNO. Moreover, within the MOE V2R genes are expressed in a basal zone, partially overlapping, but clearly distinct from an apical zone of OMP and odorant receptor-expressing cells. The unique bimodal V2R expression pattern in main and accessory olfactory system of amphibians presents an excellent opportunity to study the transition of V2R gene expression during evolution of higher vertebrates. References: Syed AS, Sansone A, Nadler W, Manzini I, Korsching SI. Ancestral amphibian v2rs are expressed in the main olfactory epithelium. PNAS 2013 Sansone A, Syed AS, Tantalaki E, Korsching SI, Manzini I. Trpc2 is expressed in two olfactory subsystems, the main and the vomeronasal system of larval Xenopus laevis. J Exp Biol. 2014

Disclosures: **A.S. Syed:** None. **S.I. Korsching:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.08/BB6

Topic: D.01. Chemical Senses

Support: NSF-IOS-0641433

Title: Compensatory plasticity in the olfactory periphery: Timing and reversibility

Authors: C. N. BARBER[1], *D. M. COPPOLA[2];
[1]Biol., [2]Randolph-Macon Col., Ashland, VA

Abstracts: Like other biological systems, olfaction responds homeostatically to perturbations, including chronic fluctuations in the stimulus milieu. This phenomenon, referred to as compensatory plasticity, appears to be implemented at multiple levels of the olfactory system; however, it has been most thoroughly studied at the periphery. In the olfactory epithelium, chronic stimulus deprivation or enrichment trigger opposite changes in the transcription/translation of proteins involved in odor transduction. These responses, at the molecular level, to odor environment manipulation are thought to underlie the modulation of receptor neuron gain observed physiologically and, at least in part, odor threshold modulation observed behaviorally. Until now, olfactory compensatory plasticity has been studied, almost exclusively, in developing animals. It is unknown whether, like in other sensory systems, this plasticity has a 'critical' period and is irreversible. Here we study unilateral odor deprivation in adult mice using nasal plugs to eliminate nasal airflow unilaterally. Plugs were placed in one nostril for two to six weeks after which electroolfactograms (EOGs) were recorded from the olfactory epithelium of the occluded and open sides of the nasal cavity. The stimuli were isoamy acetate, carvone, and eucalyptol, delivered at 1 part per thousand. Untreated animals served as negative controls and adult mice that had undergone standard unilateral naris cautery as neonates served as positive controls. Mean EOG amplitudes from plugged mice were significantly greater on the occluded side than the open side of the nasal cavity. The duration of plugging did not affect the results, suggesting that plasticity occurs within two weeks. Moreover, EOG differences between the open and occluded sides of the nasal cavity in plugged animals were comparable to those seen in positive controls. In another group of adult mice, plugs were allowed to stay in place for four weeks and were then removed for two weeks. After this recovery period, EOG mean amplitudes were not significantly different between the always-open and previously-plugged sides of the nasal cavity suggesting that compensatory plasticity in the olfactory mucosa is reversible.

Disclosures: C.N. Barber: None. **D.M. Coppola:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.09/BB7

Topic: D.01. Chemical Senses

Support: NIH DC007395

Title: Novel protein Rook modulates olfactory transduction kinetics and contributes to proper olfactory behavior

Authors: *A. K. TALAGA[1], O. MAYBERRY, III[1], C. SIMBOLON[1], J. REISERT[2], H. ZHAO[1];
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Abstracts: The cilia of olfactory sensory neurons (OSNs) are specialized for transducing and encoding odor information. Various ciliary proteins mediate and/or modulate olfactory signal transduction. We detected a novel protein, which we named Rook (Regulator of olfactory kinetics), from a proteomic screen of murine OSN cilial membrane preparations. Rook is conserved among eukaryotes and is a unique protein, as no paralogs exist in the mouse genome. Bioinformatic analysis suggested that the majority of the Rook sequence is composed of ARM domains, which mediate protein-protein interactions. Knocking out Rook specifically in OSNs alters kinetics of the olfactory response, as assayed by the electroolfactogram. Mice that lack Rook show a faster and more transient electroolfactograph to brief odorant stimulation, with both a faster rising phase and a faster falling phase. In single cell recordings, OSNs that lack Rook display some of the same kinetic alterations, including reduced time to peak and faster termination, upon odorant stimulation. In these recordings, Rook mutant OSNs also recover the ability to fire action potentials to a second stimulus faster. Interestingly, Rook conditional mutants perform poorly in more demanding behavioral assays, demonstrating that proper kinetics of the OSN response are critical for olfactory behavior. Together, we identify a novel protein, Rook, that functions to modulate olfactory transduction kinetics by “slowing down” the olfactory response and contributes to proper olfactory behavior.

Disclosures: A.K. Talaga: None. **O. Mayberry:** None. **C. Simbolon:** None. **J. Reisert:** None. **H. Zhao:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.10/BB8

Topic: D.01. Chemical Senses

Title: Formyl peptide receptor expressing neurons in the mouse vomeronasal organ - a comparative biophysical characterization

Authors: *T. ACKELS[1], B. VON DER WEID[2], I. RODRIGUEZ[2], M. SPEHR[1];
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Abstracts: The mouse vomeronasal organ (VNO) is a chemosensory structure that detects both hetero- and conspecific social cues. Based on largely monogenic expression of either type 1 or 2 vomeronasal receptors (V1R / V2R) or members of the formyl peptide receptor (FPR) family, the vomeronasal sensory epithelium harbors at least three neuronal subpopulations. While various neurophysiological properties of both V1R- and V2R-expressing neurons have been described using genetically engineered mouse models, the basic biophysical characteristics of the more recently identified FPR-expressing vomeronasal neurons have not been studied. Here, we employ a transgenic mouse strain that expresses an enhanced variant of yellow fluorescent protein driven by the Fpr-rs3 promoter to identify and analyze FPR-rs3 expressing neurons in acute VNO tissue slices. Single neuron electrophysiological recordings thus allow comparative characterization of the biophysical properties inherent to a prototypical member of the FPR-expressing subpopulation of VNO neurons. In this study, we provide an in-depth analysis of (a) passive membrane properties, (b) several types of voltage-activated ionic currents, and (c) action potential discharge patterns in fluorescently labeled versus unmarked vomeronasal neurons. Our results reveal striking similarities in the basic (electro)physiological architecture of transgene-expressing and nonexpressing neurons, confirming the suitability of this transgenic mouse model for future studies addressing more specialized issues in vomeronasal FPR neurobiology.

Disclosures: T. Ackels: None. **B. von der Weid:** None. **I. Rodriguez:** None. **M. Spehr:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.11/BB9

Topic: D.01. Chemical Senses

Support: Korea NRF grant WCI 2009-003

US NIH Grants DC005259

Title: Stimulus-dependent lateral interactions between olfactory glomeruli revealed via *in vivo* optogenetic analysis

Authors: Y. CHOI[1], T. TOMBAZ[1,2], R. HOMMA[2], T. BOZZA[3,4], *L. B. COHEN[1,2], O. BRAUBACH[1,2];
[1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Yale Univ., New Haven, CT; [3]Northwestern Univ., Evanston, IL; [4]Visiting Scientist Program, HHMI Janelia Farm Res. Campus, Ashburn, VA

Abstracts: Olfactory glomeruli serve as functional units of the olfactory bulb which organize and process the olfactory signals that are transduced by the olfactory receptor neurons. Multiple mechanisms of interglomerular lateral interaction have been proposed to play roles in olfactory processing, but these have not been established *in vivo*. We used optogenetics combined with calcium imaging in mice that express channelrhodopsin-2 in a single olfactory sensory neuron type. Low power laser stimulation of the olfactory epithelium activated a single target glomerulus, several hundred juxtaglomerular neurons, and, rarely, non-target glomeruli. We next examined if an increase in laser intensity to the epithelium could cause a spread of the ON responses from the target glomerulus to neighboring cells and glomeruli. Increasing the laser intensity indeed increased the numbers of ON non-target glomeruli and juxtaglomerlar cells. The new ON response cells were close to newly recruited ON glomeruli, and 70 % of these cells were GABAergic, suggesting that non-target glomeruli receive an inhibitory input from the nearby GABAergic ON cells. These data demonstrate that *in vivo* processing of olfactory information involves interglomerular lateral inhibition.

Disclosures: Y. Choi: None. **T. Tombaz:** None. **R. Homma:** None. **T. Bozza:** None. **L.B. Cohen:** None. **O. Braubach:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 620.12/BB10

Topic: D.01. Chemical Senses

Support: R03-DC11373

R01-DC1339

T32 DC000014

Title: The role of a single olfactory receptor in odor perception

Authors: *C. TRIMMER[1], J. R. WILLER[2], A. KELLER[3], L. B. VOSSHALL[3], N. KATSANIS[2], H. MATSUNAMI[2], J. D. MAINLAND[1];
[1]Monell Chem. Senses Ctr., Philadelphia, PA; [2]Duke Univ., Durham, NC; [3]The Rockefeller Univ., New York, NY

Abstracts: In color vision, genetic variation in a single type of vision receptor leads to a perceptual deficit termed red-green colorblindness. Humans have a large amount of genetic variation in odorant receptors (ORs), suggesting that there may be olfactory phenomena analogous to color vision, but it is unclear how the absence of a single odorant receptor alters perception. Only 10% of human ORs have even one published ligand, and we know of only five cases that specifically link genetic variation in an OR with alterations in perception, making it difficult to generalize to the entire 400-member OR family. The objective of this study was to determine how loss-of-function in a single odorant receptor alters an odor's perceived intensity and valence. To this end, we asked 321 human subjects to rate the intensity and valence of 68 odors. We then developed a protocol to sequence each subject's OR subgenome with high coverage. In a genotype/phenotype association analysis, we identified 31 polymorphisms significantly associated with the perception of 24 different odors ($p < 0.05$ with false discovery rate correction). These polymorphisms are found in 15 distinct gene clusters, but extensive linkage disequilibrium in these clusters precludes the identification of a single causal receptor. To address this issue, we cloned major receptor variants found in several OR gene clusters. We then tested these OR variants for their response to odors via a heterologous luciferase assay. Our results show that, under certain circumstances, receptor function *in vitro* corresponds with human intensity perception *in vivo*. For example, human subjects with genetic variants of OR411 that reduce response to 2-ethylfenchol *in vitro* rated the intensity of the odor to be lower ($F(3,325) = 13.08$, $p < 0.001$) in comparison to subjects with a functional allele. However, we find that the affinity of the OR for the odor determines the relevance of our assay to human behavior and

define a functional allele that will increase intensity perception as one that responds to odor with high affinity. For 5 ORs with segregating functional and non-functional variants, human subjects with a functional variant rate the intensity of the corresponding odor to be significantly higher than those subjects with a nonfunctional variant. These results provide a potential approach for identifying behaviorally relevant OR/odor interactions via a heterologous assay and demonstrate that, despite the combinatorial nature of the olfactory code, alterations in a single odorant receptor can have a significant effect on odor perception.

Disclosures: **C. Trimmer:** None. **J.R. Willer:** None. **A. Keller:** None. **L.B. Vosshall:** None. **N. Katsanis:** None. **H. Matsunami:** None. **J.D. Mainland:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

Location: Halls A-C

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Program#/Poster: 620.13/BB11

Topic: D.01. Chemical Senses

Support: NIH DC007395B

Title: Determining the molecular basis of olfactory adaptation: An EOG analysis of double mutant mice that lack CNG channel desensitization and PDE1C

Authors: *C. FERGUSON, H. ZHAO;
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Abstracts: Olfactory sensory neurons (OSNs) exhibit reduced sensitivity upon prolonged or repeated odor exposure--a phenomenon known as adaptation. This physiological plasticity is thought to underlie, at least in part, the perceptual desensitization to an odor over time. The precise molecular basis underlying olfactory adaptation remains unclear. Here we report investigations on mutant mice that lack multiple negative regulatory mechanisms of olfactory transduction. Specifically, we have performed electroolfactogram (EOG) analysis on mice that lack both the calcium-dependent desensitization of the olfactory cyclic nucleotide-gated (CNG) channel and the activity of phosphodiesterase 1C (PDE1C). We used two adaptation stimulation paradigms, a paired pulse paradigm and a sustained pulse paradigm, and found that these mice display deficits in both stimulation paradigms. In contrast, the lack of CNG channel desensitization or PDE1C alone resulted in a less severe deficit and in only one of the two

paradigms. These results support the notion that olfactory adaptation is derived from an integration of multiple regulatory mechanisms.

Disclosures: C. Ferguson: None. **H. Zhao:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

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Program#/Poster: 620.14/BB12

Topic: D.01. Chemical Senses

Support: SNF Grant 31003A_147081/1

Title: CNGA4 and TRPC2, two proteins involved in the detection of mice pheromones

Authors: D. BOVARD[1], J. BRECHBÜHL[1], M. NENNIGER TOSATO[1], I. RODRIGUEZ[2], *M.-C. BROILLET[1];
[1]Univ. Lausanne Dep. Pharmacol & Toxicol, CH-1005 Lausanne, Switzerland; [2]Univ. Geneva Dep. Genet. & Evolution, Geneva, Switzerland

Abstracts: Mammalian pheromones are key chemical signals in the regulation of intraspecies social behaviors. Detection of these pheromones, which takes place in sensory neurons of the vomeronasal organ (VNO), implies the activation of the transient receptor potential canonical channel 2 (TRPC2) as the final effector. Interestingly, discrepancies between TRPC2-/- mice and mice lacking a VNO suggest the implication of another protein in the pheromone signaling pathway. This protein could either form a heteromeric channel with TRPC2 or a separate homomeric ion channel. The cyclic nucleotide-gated channel subunit CNGA4 is also expressed in the rodent VNO but its role and properties in this organ remain unknown. CNGA4 belongs to the CNG channel family which is playing an important role in different sensory pathways such as in light and odorant detection. We thus decided to study the role of the CNGA4 protein in the mouse VNO. We found CNGA4 to be expressed in axons, dendrites and, together with TRPC2, on the microvilli of the vomeronasal sensory neurons. We then performed *in vitro* experiments using HEK cells as an expression system. We transfected these cells with both CNGA4 and TRPC2. We could verify that CNGA4 directly interacted with TRPC2 functioning either as a chaperon translocating TRPC2 at the plasma membrane or as a subunit of a heteromeric channel. To further investigate the vomeronasal function of CNGA4 we generated CNGA4-/- mice. We observed that these mice were impaired in some pheromone dependent behaviors such as

aggressivity or mating. Interestingly these modified behaviors were similar to the behaviors of TRPC2-/- mice and, given the in-vitro results, we looked at the expression of CNGA4 in the VNO of TRPC2-/- mice. We observed that even if the neuronal microvilli were still present in TRPC2-/- mice, CNGA4 was not expressed anymore in this cellular region. Conversely, the expression of TRPC2 in CNGA4-/- mice was observed. These results might suggest a modulatory role for CNGA4 in a heteromeric TRPC2+CNGA4 ion channel. Further experiments will give more insights on the combined role of these transduction ion channels in pheromone detection.

Disclosures: D. Bovard: None. **J. Brechbühl:** None. **M. Nenniger Tosato:** None. **I. Rodriguez:** None. **M. Broillet:** None.

Poster

620. Olfactory Sensory Neurons: Development and Signal Transduction

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Program#/Poster: 620.15/BB13

Topic: D.01. Chemical Senses

Support: NIH F32DC011242

Title: An RNA-Seq screen of the *Drosophila* antenna identifies a non-neuronal gene required for olfactory response to ammonia

Authors: K. MENUZ, N. K. LARTER, J. PARK, *J. R. CARLSON;
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Abstracts: Many insect vectors of disease detect their hosts through olfactory cues, making it imperative to better understand how odors are encoded. In insects, olfactory receptor neurons (ORNs) are compartmentalized with non-neuronal auxiliary cells in sensilla. In *Drosophila*, there are three morphological classes of olfactory sensilla, but few genes have been identified that distinguish them, other than the olfactory receptors themselves. In particular, little is known about the function of coeloconic sensilla, an evolutionarily ancient class of sensilla that detect amines and acids, including components of human odor that are cues for many insect vectors. Here, we undertook a high-throughput RNA profiling screen to identify genes that are highly enriched in coeloconic sensilla and may contribute to their unique function. The analysis revealed unanticipated chemosensory receptors in the wild type antenna, and the screen identified 250 genes whose transcripts are depleted in a mutant that lacks coeloconic sensilla.

Many of these 250 genes are predicted to function as ion channels, transporters and biotransformation enzymes; few were previously implicated in olfaction. We further investigated one of them, an ammonium transporter, to validate the utility of the screen. Electrophysiological and genetic analysis revealed that this transporter is essential for ammonia responses in a class of coeloconic ORNs, but is not required for responses to other odorants. The transporter is not expressed in ORNs, but rather in neighboring auxiliary cells. Thus, our data support the notion that auxiliary cells make an essential contribution to the coding of specific odors, and illustrate why comprehensive studies of sensory signaling must consider neural circuits in the context of their environment.

Disclosures: K. Menuz: None. **J.R. Carlson:** None. **J. Park:** None. **N.K. Larter:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.01/BB14

Topic: D.01. Chemical Senses

Support: NIDCD R01DC010389

Title: Development of an automated method for analysis of mouth movements and orofacial reactions in restrained rats

Authors: *M. P. GARDNER[1], L. HOU[2], C. SAMUELSEN[1], A. FONTANINI[1], D. SAMARAS[2];
[1]Neurobio. and Behavior, [2]Computer Sci., SUNY At Stony Brook, Stony Brook, NY

Abstracts: Animals respond to gustatory stimuli with well-defined facial expressions. Orofacial responses, such as licking and gaping, have been shown to reflect the palatability and aversiveness of tastes. These behaviors are used to measure the perceived hedonic value of different taste solutions (Grill and Norgren, 1978). The standard method used to assess orofacial responses relies on visual inspection and manual labeling of videos of rats' mouth movements. This procedure is both dependent on the reviewer and is a very time consuming process. In order to increase the efficiency and reduce the subjectivity of scoring these behaviors, we developed a method for automatic scoring of rats' orofacial behaviors using a support vector machine for classification. To optimize the classifier, different combinations of 9 independent features (such as eigenfaces, fisherfaces, optical-flow, local binary patterns, etc.) were tested to determine the

most accurate and efficient method. This classifier can detect particular orofacial movements (licks, gapes, tongue protrusions, lateral tongue protrusions, mouth openings) based on few samples defined and cross-validated by multiple users. Electromyographic recordings of the digastric muscle were also included to complement information provided by the videos. Altogether, the classifier was able to correctly predict 82.0% of user-labeled frames (chance level was 21.2%) with 93.4% of lateral tongue protrusions and 84.1% of gapes being correctly labeled (chance levels were 16.6% and 4.40% respectively). We also trained a similar classifier to detect and disambiguate conditioned mouth movements evoked by cues predicting different tastes in rats trained on a classical conditioning paradigm. By implementing features extracted during and after the predictive cue (CS), but prior to the taste (US), the classifier was able to identify the trial-type with an accuracy of 41.4% (the chance level being 20.0% for five cues). The classification of the tastes themselves reached an accuracy of 65.6%. Finally, using this automated method we were able to compare the numbers of palatable and aversive mouth movements across expected and unexpected taste deliveries within single sessions of the classical conditioning paradigm. Overall our method improves the current efficiency of labeling orofacial behaviors and also provides a novel procedure for detecting subtle distinctions between conditioned responses in a multi-cue classical paradigm.

Disclosures: **M.P. Gardner:** None. **L. Hou:** None. **D. Samaras:** None. **A. Fontanini:** None. **C. Samuelsen:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.02/BB15

Topic: D.01. Chemical Senses

Support: DC013770

Title: Layer-specific amygdalar activation of excitatory and inhibitory circuits in gustatory cortex

Authors: ***A. MAFFEI**, M. S. HALEY, A. FONTANINI;
Neurobio. and Behavior, SUNY-Stony Brook, STONY BROOK, NY

Abstracts: Projections from the basolateral amygdala (BLA) to gustatory cortex (GC) have been shown to underlie firing activity that carries information on the palatability of a taste stimulus as

well as sensory cues that predict the availability of a taste. While the behavioral and functional aspects of this projection have been the focus of many studies, the synaptic mechanisms that underlie the effects of BLA activation on GC are poorly understood. In order to study the BLA to GC synapse in greater detail, we employed an optogenetic approach to activate BLA terminal fields in an acute slice preparation of GC. Using a combination of whole-cell patch-clamp recordings and pharmacological methods, we tested the hypotheses that 1) BLA directly targets excitatory (EXC) and inhibitory (INH) neurons in GC; 2) BLA engages GC circuits in a layer-specific manner. Our results demonstrate that BLA does in fact provide monosynaptic, excitatory input to both EXC and INH neurons across GC laminae. Furthermore, we show that this input has layer-specific properties. BLA directly targets a larger percentage of EXC (64%) and INH (57%) neurons in supragranular GC, with significantly larger BLA-evoked currents than in infragranular GC. We also find that BLA input is sufficient to drive a similar fraction of EXC and INH neurons to fire action potentials in all layers. Using a brief (5ms) pulses of LED stimulation we observe a fast monosynaptic EPSC in EXC neurons, followed by a short-latency polysynaptic IPSC. Despite differences in current amplitudes, the ratio of excitation to inhibition is not significantly different between layers. Given the ability of BLA projections to drive feedforward excitation and inhibition in GC more strongly in the superficial layers, we next sought to determine how sustained BLA activation might differentially engage circuit dynamics across laminae. Ramp stimuli were used to activate terminal fields with increasing intensity over 6s, and BLA-evoked spontaneous EPSCs and IPSCs were recorded. Sustained BLA activation increased the frequency of spontaneous EPSCs and IPSCs in both supragranular and infragranular layers, however activation of supragranular GC was more robust and longer-lasting. This increase in spontaneous activity outlasted the duration of the ramp. Increases in spontaneous event frequency could also be recorded in neurons that did not receive direct BLA input, suggesting that these neurons were driven by BLA-dependent recruitment of GC recurrent circuits. Together these data indicate that BLA can persistently alter network dynamics in GC by recruiting both excitatory and inhibitory circuits in a layer-specific manner.

Disclosures: **A. Maffei:** None. **M.S. Haley:** None. **A. Fontanini:** None.

Poster

621. Taste

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.03/BB16

Topic: D.01. Chemical Senses

Support: NIH Grant R01DC010389

Title: Integration of gustatory and anticipatory signals in the gustatory thalamus (VPMpc) of behaving rats

Authors: *H. LIU[1,2], A. FONTANINI[1,2];
[1]The Dept. of Neurobio. & Behavior, SUNY, Stony Brook, Stony Brook, NY; [2]Program in Neuroscience, SUNY Stony Brook, Stony Brook, NY

Abstracts: Recent reports on the gustatory cortex (GC) have emphasized its involvement in coding both the physiochemical and the psychological aspects of gustatory experience. The chemosensory and psychological dimensions of taste are hypothesized to originate from thalamic and amygdalar (AMY) pathways, respectively. Studies focusing on AMY-GC pathway have shown its contribution to hedonic, attentional, and anticipatory signals and have emphasized the temporal dynamics of its neural coding. However, less attention has been paid to the thalamic route, which involves the parvicellular portion of the ventral posteromedial nucleus of the thalamus (VPMpc). The VPMpc has been shown to encode gustatory stimuli and to be responsible for taste responses and network states in GC. Behavioral studies with VPMpc lesion have challenged the traditional view of sensory thalamus as just an information relay station and have suggested its roles in higher-order functions, such as anticipation. Despite the importance of VPMpc in taste processing and taste-guided behavior, little is known about taste coding in VPMpc of behaving animals. Here, we present results from multielectrode recordings in VPMpc of alert rats, which were engaged in a task involving cued, self-administrations (Self) and passive, unexpected (Pass) deliveries of taste solutions. 128 single units were recorded from 11 rats. Almost half of the neurons (43.75%, 56/128) had significantly different responses across tastants with fast onsets and rich temporal dynamics. Further analyses revealed the encoding of both taste quality and palatability. Comparison of responses to expected and unexpected tastes showed a strong state-dependency, with faster (average taste-quality coding onset: 669.5 ms Pass vs 502.5 ms Self) and better (average decoding performance: 0.4 Pass vs 0.48 Self) coding in the case of cued, self-administered taste. Improvements in coding were related to a reduction of neural response variability (Fano factor mean: 0.81 Pass vs 0.58 Self) and an increase of coding density (taste number decoded/unit: 3.13 vs 2.46). Surprisingly, we found a considerable proportion of thalamic neurons (64.06%, 82/128) responding to the anticipatory cue and whose activity was correlated with the animal's decision to self-administer. Altogether our data provide the first description of how the VPMpc of behaving rats encodes gustatory and anticipatory information according to the state of the animal. The results suggest that the role of VPMpc goes beyond encoding the physiochemical properties of taste stimuli and extends to the coding of palatability and expectation.

Disclosures: H. Liu: None. **A. Fontanini:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.04/BB17

Topic: D.01. Chemical Senses

Support: NIDCD Grant R01DC010389

Title: Multimodal integration of taste and odor signals in the gustatory cortex of alert rats

Authors: *C. L. SAMUELSEN, A. FONTANINI;
Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstracts: Gustatory and olfactory signals are naturally coupled during food consumption. The simultaneous activation of taste and olfactory receptors results in robust multisensory associations. The gustatory cortex (GC) receives multiple inputs conveying gustatory and olfactory signals. Neuroimaging experiments point to GC as an integrative locus for these chemosensory signals. However, GC single neuron responses to olfactory stimulation have not been studied in alert animals. Using multielectrode recordings in behaving rats, multimodal chemosensory responses in GC were examined after intra-oral deliveries of single tastants and tasteless odorants in water. We recorded 272 single units in GC, of which 33% (89/272) responded to taste-only, 6% (16/272) to odor-only, with a substantial multimodal group of 12% (33/272) responding to both taste and odor. To determine whether odor responses in GC depend on the activation of olfactory receptors in the nasal epithelium, a nasal deciliation protocol was used. A mild detergent was applied directly into each nostril rendering the subjects anosmic. Preliminary results (49 single units) show that detergent application does not affect taste-responsive GC neurons (Pre: 122/272, 45% vs. Post: 21/49, 43%), but significantly reduces the number of odor-responsive GC neurons (Pre: 49/272, 18% vs. Post: 2/49, 4%). Additional analyses were performed to examine taste coding in the three groups of neurons recorded. Multisensory neurons, those which respond to both taste and odor, appeared to be strongly tuned to encode taste palatability. This group of neurons showed increased activity to the palatable tastes, sucrose and salt, while showing suppressed responses to aversive stimuli (citric acid and quinine). Interestingly, the response dynamics to intraoral odors were similar to those evoked by palatable tastes. Using a palatability index, we found a significantly greater number of multisensory neurons coding palatability (29/33, 88%) compared to taste-only neurons (58/89, 65%). This difference was temporally represented 2.5 to 3 seconds after stimulus onset. To

determine whether the response dynamics of multisensory neurons paralleled the behavioral consumption of chemosensory stimuli, a brief access task was employed. We found that rats avoid sampling the aversive taste stimuli, while equally sample palatable tastes and odors, supporting the suggestion that multisensory neurons represent palatability. Altogether these results provide novel evidence for GC as an area of multimodal integration of intra-oral chemosensory signals.

Disclosures: C.L. Samuelsen: None. **A. Fontanini:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.05/BB18

Topic: D.01. Chemical Senses

Support: NIDCD DC006666

Title: Pre-exposure to a diverse array of tastes enhances later conditioned taste aversion to novel sucrose

Authors: *V. FLORES[1,2], A. MORAN[2,3], D. B. KATZ[2,3];
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Abstracts: In conditioned taste aversion (CTA), an animal learns to avoid a particular taste that has been paired with malaise. Many factors influence the strength of aversion learning; notable among these is taste novelty - the animal's familiarity (or lack thereof) with the taste used as a conditioned stimulus (CS). The effect of familiarization with tastes other than the CS has received less investigation, however. The present studies examine the impact that exposure to a range of fluid stimuli (including distilled water, sodium chloride and citric acid) before or during conditioning has on an aversion learned to novel sucrose. Rats were exposed to tastes via both a bottle and intra oral cannula (the latter method facilitated exposure of diverse tastants, while the former ensured strong conditioning). Presentation of the entire taste array within the conditioning session reduced the resultant sucrose aversion, as expected. Pre- training exposure to a taste array that excluded sucrose, however, strengthened CTA, an effect that scaled with the number of tastes in the pre-exposure array and with the amount of pre-exposure; this phenomenon was observable regardless of the diversity of tastes presented during the conditioning session. These results reveal that experience with the realm of tastes changes an animal's future handling of

even novel tastes. Since CTA protocols using animals often lack stimulus exposure prior to conditioning training, these results have implications for the interpretation and development of CTA protocols investigating the complex underpinnings of learning and behavior.

Disclosures: V. Flores: None. **A. Moran:** None. **D.B. Katz:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.06/BB19

Topic: D.01. Chemical Senses

Support: CIHR 114934

Title: Pharyngeal sense organs drive robust sugar consumption in *Drosophila*

Authors: *E. E. LEDUE[1], Y.-C. CHEN[2], A. Y. JUNG[1], A. M. LOMELI[2], A. DAHANUKAR[2], M. D. GORDON[1];
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Abstracts: The fly pharyngeal sense organs lie at the transition between external and internal nutrient sensing mechanisms. Inaccessible to electrophysiology, their function has remained largely unexplored. Here, we investigate the physiology and behavioural role of pharyngeal sense organ gustatory neurons expressing sweet receptors. Using calcium imaging, we find these neurons to be broadly responsive to sweet compounds. Selective rescue of Gr64e and Gr5a mutants in just two labral sense organ (lso) neurons is sufficient to restore preference for glycerol and trehalose, respectively. We also demonstrate that pox-neuro mutants lacking peripheral taste function have intact pharyngeal sweet taste, which is both necessary and sufficient to drive robust consumption of sweet compounds. Moreover, in the absence of sweet taste, flies prefer some, but not all, nutritional sugars. Together, our data demonstrate that pharyngeal sense organs play an important role in directing consumption of sweet compounds, and that their function must be considered when distinguishing between taste and postingestive sensory inputs.

Disclosures: E.E. Ledue: None. **Y. Chen:** None. **A.Y. Jung:** None. **A.M. Lomeli:** None. **A. Dahanukar:** None. **M.D. Gordon:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.07/BB20

Topic: D.01. Chemical Senses

Title: The ventral tegmental area modulates gustatory responses of the neurons in the parabrachial nuclei

Authors: *C.-S. LI[1], Y. K. CHO[2];
[1]Anat., Southern Illinois Univ. Sch. Med., CARBONDALE, IL; [2]Physiol. & Neurosci., Kangnung Natl. Univ. Col. of Dent., Kangnung, Korea, Republic of

Abstracts: The nucleus of the solitary tract (NST) and the parabrachial nuclei (PbN) are the first and second central relays in the central taste pathway of the rodent, respectively. Taste information is further transmitted to the various forebrain nuclei from the PbN, including the thalamus, the lateral hypothalamus, the central nucleus of the amygdala, the bed nucleus of the stria terminalis, the nucleus of accumbens, and to the insular cortex. These forebrain gustatory nuclei not only influence activity of gustatory cells in both the NST and PbN but are also known to modulate gustatory responses of the cells in the NST and PbN. The ventral tegmental area (VTA) in the midbrain is the site of the origin of dopaminergic cells bodies of the mesolimbic reward circuitry. Although there is no anatomical data to support the direct neuronal connectivity between the gustatory pathway and the mesolimbic reward pathway, recent behavioral and electrophysiological studies provided strong evidence that indicate the interaction between these two pathways. It has been reported that alcohol taste selectively activates sucrose cells in both the NST and PbN. Furthermore, ethanol increases firing activity of dopamine cells in the VTA, and sham feeding of sucrose increases dopamine concentration in the nucleus acumens which is a component of the mesolimbic reward circuitry. In the present study, we investigated whether activity of gustatory neurons in the PbN is influenced by activation of the VTA, and that whether activation of the VTA modulates taste responses of the PbN cells. Extracellular single-unit activity was recorded from the urethane anaesthetized hamster PbN and taste responses were confirmed by delivery of 32 mM sucrose, NaCl, quinine hydrochloride, and 3.2 mM citric acid to the anterior tongue. After confirming the responsiveness of a PbN cell to taste stimulation, the VTA was stimulated (0.5 ms, ≤ 100 μA, 1/3 Hz) using a concentric bipolar stimulating electrode. We recorded a total of 32 taste-responsive cells from the PbN; 11 being NaCl-best, 7 being sucrose-best, 6-being citric acid-best, and 8 being quinine hydrochloride (QHCl)-best neurons. Electrical stimulation of the VTA activated 20 of 32 PbN neurons (62.5%) orthodromically (15

cells were inhibited and 5 cells were excited). We further examined whether electrical stimulation of the VTA alters taste responses of the PbN cells. High frequency stimulation (100 Hz, 0.2 ms) of the VTA activated or suppressed taste responses in 6 cells tested which is parallel to the type of the input of the PbN cells receive from the VTA. These results suggest that the mesolimbic reward pathway influences taste information processing in the PbN.

Disclosures: C. Li: None. **Y.K. Cho:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.08/BB21

Topic: D.01. Chemical Senses

Title: Toll-like receptor 4 is involved in spontaneous fat and sugar preference

Authors: *S. CAMANDOLA, R. G. CUTLER, M. P. MATTSON;
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Abstracts: The gustatory system allows animals to discriminate among foods in order to select nutritious diets and maintain energy balance. Although a broad range of economic, social and behavioral factors influences food choices, the immediate pleasantness generated by taste is still for most individuals the driving force behind food consumption. Most animals, including humans, display an innate attraction for lipid-rich foods. In a typical Western diet fats account for almost 40% of the daily energy content. The hedonic response to palatable macronutrients, and consequent over-consumption of tasty high calorie foods, has been suggested to play a role in the increasing prevalence of obesity world wide. However, the mechanisms underlying such eating behavior are largely unclear. Toll-like receptor 4 (TLR4) is a transmembrane protein involved in the detection of lipopolysaccharide in gram negative bacteria. In addition to its well characterized role in innate immune responses, it was recently shown that TLR4 plays a role in central nervous system plasticity, learning and memory, and cognition. Since the discovery that obese, type 2 diabetic, and metabolic syndrome subjects have increased levels of TLR4 expression in various tissues, many studies have been conducted to elucidate its function in the metabolic consequences of diet-induced obesity. In the present study we provide evidence that TLR4 is involved in orosensory detection of fat and sugar. TLR4 knock mice displayed decreased spontaneous preference for a high fat, high sugar diet, resulting in reduced food consumption and caloric intake, and less weight gain. Compared to wild type animals TLR4

deficient mice showed reduced preference for lipids (i.e. linoleic acid), as well as sugars (i.e. sucrose, fructose, saccharin) and umami (i.e. inosine-5'-monophosphate) in two bottle preference tests. The altered gustatory preferences of TLR4 knock mice were associated with decreased expression of key regulatory molecules for the detection of sweet, umami and fat taste in the tongue epithelium. Experiments are currently under way to determine the cellular and molecular mechanism by which TLR4 impacts taste perception and eating behavior.

Disclosures: S. Camandola: None. **R.G. Cutler:** None. **M.P. Mattson:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.09/BB22

Topic: D.01. Chemical Senses

Title: Decision making in *C. elegans* chemotaxis to alkaline pH

Authors: *I. MARUYAMA, T. MURAYAMA;
Okinawa Inst. of Sci. & Technol. Grad. Univ., Okinawa, Japan

Abstracts: Monitoring of environmental and tissue pH is crucial for the survival of animals. The nematode *C. elegans* is a model organism suitable for the analysis of neural circuits that regulate animal behaviors. The animal is attracted to mildly alkaline pH, and avoids strongly alkaline pH. Our genetic dissection and Ca2+ imaging demonstrate that ASEL and ASH are the major sensory neurons responsible for the attraction to mildly alkaline pH and repulsion from strongly alkaline pH, respectively. In ASEL, a transmembrane guanylyl cyclase, GCY-14, is activated by environmental alkalinization, and in turn, a cGMP-gated channel serves for Ca2+ influx into the sensory neuron. In ASH, TRPV channels are found to be required for the neural activation upon stimulation with strongly alkaline pH. To understand the animal's behavioral switch at molecular and cellular levels, we have also analyzed behaviors of mutants defective in ASEL and/or ASH under various alkaline pH, and have found that activities of ASEL and ASH compete each other for the behavioral switch. While mildly alkaline pH preferentially activates ASEL, strongly alkaline pH activates both ASEL and ASH, and ASH activity overrides the activity of ASEL. Neural circuits responsible for this decision making will be discussed.

Disclosures: I. Maruyama: None. **T. Murayama:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.10/BB23

Topic: D.01. Chemical Senses

Title: Aftertaste sans taste: ageusia with palinageusia

Authors: *A. R. HIRSCH[1], K. V. GAFTANYUK[2];
[1]Smell & Taste Treatment and Res. Fndn., Chicago, IL; [2]Intl. Univ. of the Hlth. Sci. Sch. of Med., Winnipeg, MB, Canada

Abstracts: Introduction: A case study with vivid and perceptually correct aftertaste in the absence of taste is described. Case Study: A 55 y/o male two years prior to presentation was exposed to Scott's Crabgrass Preventer herbicide. Within one week there was a gradual decline in sweet and salt taste perception and dysgeusia where potato chips taste like potatoes, apple pie tastes like apples, Pepsi tastes like chemicals, chocolate tastes bitter and corn flakes have a salty taste. For the last one year, he has intermittent salty phantageusia of his entire mouth, whereby regular water tastes like seawater. Six months ago he noted onset of palinageusia, a strong aftertaste of food that was consumed earlier in the day, the way it should taste, but of 50% intensity. Cheese, has no taste, but 8 hours later he can taste cheese flavor. Cinnamon bun is flavorless, but half-hour after eating, the taste appears and lasts for 5 minutes. Sausage has a slight taste at the time of consumption, but two hours later he experiences a much more flavorful and savory taste of sausage, which lasts for half an hour. The aftertaste is always of the last food he has eaten. Rather than being repulsed by these aftertastes, he "savors them" because it provides he only taste experience. Since onset, he has lost of pleasure in eating and has lost 60 pounds. He complains of mild dysphagia, halitosis and omeprazole responsive reflux. Neuropsychiatric exam: decreased short-term memory, sadness, and irrational fears of others out to do him harm. Beck Depression Inventory and Zung Anxiety Scale: normal. Neurologic exam: decreased blink frequency, bilateral palmar erythema and 1+ bilateral pedal edema. Motor: R pronator drift. Reflexes: absent. Candidiasis cultures - negative. Chemosensory tests: see table. Discussion: Potential causes for his condition include: Zenker's diverticulum, esophageal dysmotility, Gastroesophageal Reflux, herbicide (Pendimethalin) toxicity with phantom aftertaste, prolonged chemosensory deprivation phantageusia and palinageusia (chemosensory equivalent of Phantom Eye Syndrome), illusion of taste due to autosuggestion, somatoform

delusion or somatic manifestation of depression or psychosis. Aftertaste warrants exploration in those with chemosensory dysfunction.

Olfactory Functions

Name of Test	Right	Left	Dirhinous	Interpretation
Sniffin' Sticks Threshold Test	<1	<1	<1	Hyposmia
Sniffin' Sticks Discrimination Test	7	4	7	Hyposmia
Sniffin' Sticks Identification Test	11	10	11	Hyposmia

Name of Test	Right	Left	Interpretation
Olfactometer Threshold Test	6	7	Hyposmia
Olfactometer Identification Test	Mild	Moderate	Hyposmia

Name of Test	Dirhinous	Interpretation
Quick Smell Identification Test	3/3	Normal
Sniff Magnitude Test	Ratio 1.27	Anosmia
Alcohol Sniff Test	8 cm	Hyposmia
Odor Memory Test	¾ at 10 seconds, ¾ at 30 seconds, ¾ at 60 seconds	Hyposmia
Suprathreshold Amyl Acetate Odor Intensity Taste	✓	Normal
Suprathreshold Amyl Acetate Odor Hedonic Taste	✓	Crossed pattern
Jelly Bean Retronasal Smell	2/10	Markedly impaired

Test

Gustatory Functions

Name of Test	Interpretation
Taste Threshold Test	Normogeusia to sucroseAgeusia to sodium chloride (NaCl), hydrochloric acid, urea, and phenylthiocarbamide
Quadrant Taste Test	No L to R and front to back differences in taste to NaCl, sucrose, citric acid, quinine, and alcohol. Taste weakness to all modalities
Propylthiouracil	Absent
Piesesthesiometry Test	Normal on anterior and posterior top, and the undersurface of the tongue

Name of Test	Location						Interpretation
Electrogustometry Test	Palate		Tongue				
	R	L	Anterior		Posterior		
	28	24	R	L	R	L	Markedly abnormal
			>34	>34	>34	>34	

Name of Test	Result	Interpretation
Saxon Test	12 grams	Normal

Disclosures: **A.R. Hirsch:** A. Employment/Salary (full or part-time):; Smell & Taste Treatment and Research Foundation. **K.V. Gaftanyuk:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.11/BB24

Topic: D.01. Chemical Senses

Support: NIDCD R01-DC006914

Title: Temporal coding of foods in the parabrachial nucleus of the awake, freely licking rat

Authors: *M. S. WEISS[1], P. M. DI LORENZO[1], J. D. VICTOR[2];
[1]Psychology, Binghamton Univ., Binghamton, NY; [2]Weill Cornell Med. Col., New York, NY

Abstracts: The parabrachial nucleus of the pons (PbN) is the obligatory second synapse in the central gustatory pathway of the rodent. Analyses of taste-evoked spike trains in single neurons in the PbN of awake animals have shown that these neurons can use temporal coding to convey information about taste qualities (Weiss et al., J. Neurophysiol., 111(8):1655-70, 2013). However, the amount of information that these cells convey about taste qualities is far less than they convey when recorded from anesthetized rats. We hypothesized that the PbN, as part of the feeding system, might convey more information about actual foods than prototypical taste stimuli often tested in gustatory research. To test this idea, temporal coding of PbN responses to real foods (Clam juice [0.12M NaCl], 25% cream, grape juice [0.12M sucrose], lemon juice [0.016M citric acid], and coffee [0.019M caffeine]) vs. prototypical tastants (0.1M NaCl, 0.1M MSG, 0.1M sucrose, 0.01M citric acid, 0.0001M quinine-HCl) was assessed in freely licking rats. An 8-channel tungsten microwire assembly was implanted into the PbN of Sprague-Dawley rats. Following recovery, animals were water-deprived (22 hours/day) and placed into a testing chamber with free access to a lick-spout that delivered 12±1 µl of fluid per lick. Taste stimuli were presented as a 5-lick stimulus block, and five artificial saliva licks were each presented on a VR5 schedule as a rinse between stimulus blocks. An information theoretic metric was used to determine the amount of information conveyed by firing rate and spike timing. Preliminary results show that PbN neurons can respond to all of the food stimuli. Of particular interest, a subgroup of neurons (n = 4 of 10) responded to cream (representing a “fat taste”) within 100ms of a lick. Additionally, ~30% of neurons that responded to a food stimuli do not respond to their prototypical counterparts or vice versa. Finally, in neurons where temporal coding contributes significantly to the amount of taste quality information, PbN cells convey more information about food stimuli than prototypical tastants. These results underscore the idea that the PbN is a node in the feeding circuit, as well as the taste pathway.

Disclosures: **M.S. Weiss:** None. **P.M. Di Lorenzo:** None. **J.D. Victor:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.12/BB25

Topic: D.01. Chemical Senses

Support: NSF IOS-0951016

Title: L-amino acid taste: Receptor system and transduction mechanisms

Authors: *S. PAL CHOUDHURI, R. J. DELAY, E. R. DELAY;
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Abstracts: Receptor heterodimer T1R1+T1R3 has been shown to be involved in detection of prototypical umami compound L-glutamate. Research with L-glutamate also suggests two other G-protein coupled receptors, taste-mGluR4 and taste-mGluR1, are important in umami taste. Umami taste transduction is mediated by PLC-β2 dependent rise of IP3 followed by release of intracellular calcium. Umami research also suggests a Gα-dependent pathway that down-regulates cAMP. 5' inosine monophosphate (IMP) is another umami taste stimulus and a potent flavor enhancer that synergistically enhances umami taste of L-glutamate. HEK cell expression data show that IMP can also potentiate the response to other L-amino acids (Nelson et al., 2002). However, the transduction mechanism for IMP and its synergy mechanisms are largely unknown. Further, while T1R1+T1R3 receptors appear to detect L- amino acids, contribution of other receptors have just begun to be studied. We used calcium imaging of isolated taste sensory cells (TSCs) and taste buds of mice to determine if: (1) receptors other than T1R1+T1R3 detect L-amino acids detection with or without synergistic responses, (2) transduction of L-amino acids other than glutamate also utilize the PLC-β2pathway, and (3) L-amino acids also use a cAMP-dependent pathway. Our calcium imaging data show that response patterns elicited by L-amino acids and IMP vary across TSCs. Further, TSCs also show synergy for different L-amino acids when mixed with IMP. We also found that TSCs from T1R3-/- mice can respond to various L-amino acids and IMP. Our data suggest that receptors or possibly receptor complex other than T1R1+T1R3 may be involved in detection of L-amino acids. Currently we are using pharmacological approach to elucidate the downstream signaling pathways for L-amino acids transduction.

Disclosures: S. Pal Choudhuri: None. **R.J. Delay:** None. **E.R. Delay:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.13/BB26

Topic: D.01. Chemical Senses

Support: F32 DC0128980

T32 DC000014

Title: Anion effect and osmotic sensitivity of salt-responsive taste bud cells isolated from mouse circumvallate papillae

Authors: *B. C. LEWANDOWSKI, S. K. SUKUMARAN, R. F. MARGOLSKEE, A. A. BACHMANOV;
Monell Chem. Senses Ctr., Philadelphia, PA

Abstracts: At least two distinct pathways transduce salty taste in rodents: a low threshold, amiloride-sensitive, Na+ (and Li+) selective pathway mediated by epithelial sodium channels, and a high threshold, amiloride-insensitive (AI), cation non-selective pathway whose mechanism is currently unknown. In both pathways, a salt's cation appears to be the proximal stimulus necessary to initiate cellular responses. In the AI pathway, however, responses are significantly modulated by a salt's anion, with gustatory nerve responses growing smaller as anion size increases. One popular model to explain this 'anion effect' phenomenon is built upon evidence that tight junctions at the taste pore are differentially permeable to cations vs anions. As cations diffuse through these tight junctions, larger and less permeable anions accumulate at the taste pore resulting in a negative transepithelial potential (TP) that, among other effects, inhibits diffusion of cations to the basolateral surface of taste cells where AI salt sensitive channels may exist. We tested the importance of TPs for the anion effect by measuring Fura2 calcium responses to Na+ salts in isolated taste cells from mouse circumvallate taste papillae. Using isolated taste cells removes any potential influence of TPs on salt responses. AI salt-sensitive type III taste cells were identified by responses to NaCl, NaCl + amiloride, and KCl. The anion effect was measured in physiologically identified AI type III taste cells by comparing responses to 250 mM NaCl and 250 mM NaGluconate. A cell was defined as exhibiting an anion effect if its response to NaGluconate was <70% of its response to NaCl. Of 44 AI type III cells, 30 exhibited an anion effect (average normalized (to 250 mM NaCl) response = 0.43 +/- 0.13) and 14 did not (1.05 +/- 0.26). To ensure that responses were salt related and not purely osmotic

effects, cells were tested with cellobiose stimuli osmotically matched to 250 mM NaCl. Osmotic responses were strongly dependent on the presence of Na+ (norm. resp. = 0.20 +/- 0.08 with 30 mM NaCl + 110 mM NMDG.Cl vs 0.61 +/- 0.36 with 140 mM NaCl; $p<0.05$). Furthermore, cells exhibiting the anion effect had significantly smaller osmotic responses than cells without an anion effect (0.43 +/- 0.29 vs 0.80 +/- 0.34; $p<0.05$). These data suggest that the lack of an anion effect in some putative AI taste cells may be attributed to the presence of osmotically sensitive channels permeable to Na+ but not larger cations (NMDG+). Current models of the anion effect may need revision because the observation of an anion effect in isolated taste cells indicates that larger anions can directly inhibit the AI salt transduction pathway in taste cells.

Disclosures: B.C. Lewandowski: None. **S.K. Sukumaran:** None. **R.F. Margolskee:** None. **A.A. Bachmanov:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.14/BB27

Topic: D.01. Chemical Senses

Support: NIH Grant AG004085-26

Title: Altered brain response to sweet taste in older adults with metabolic syndrome: An fMRI study

Authors: *C. MURPHY[1], A. JACOBSON[2], E. GREEN[3], L. HAASE[3], A. BUNCIC[2], E. MCINTOSH[2];
[1]San Diego State Univ/Univ of California, San Diego, San Diego, CA; [2]San Diego State Univ., San Diego, CA; [3]SDSU/UCSD Joint Doctoral Program, San Diego, CA

Abstracts: Metabolic syndrome involves a constellation of risk factors (obesity, large waist circumference, high blood pressure, elevated triglycerides, low HDL) for cardiac and vascular disease that are also associated with obesity, diabetes and dementia in later life. Obesity has increased to epidemic proportions, with significant health consequences. The abundance of highly palatable food may contribute to obesity, yet not everyone becomes obese. Physiological state (i.e., hunger and satiety) has been shown to affect the processing of appetitive stimuli in young adults, and age differences in hormones that influence hunger and satiety suggest differences in brain response to nutrients. We investigated brain activation with fMRI, recorded

at 3T, in older adults (65+ yrs), with and without metabolic syndrome, and compared their activation to those of young adults (18-26), while they rated the pleasantness of sweet taste, under the conditions of hunger and satiety. Results in the hunger condition are reported here. We conducted both whole brain activity and region of interest analyses, focusing on regions involved in taste and reward processing. FMRI revealed that older adults showed effects of metabolic syndrome, with group differences in activation in the hunger condition in brain areas involved in both gustatory and reward processing (e.g., OFC, Nucleus Accumbens). Reduced activation may reflect reduced dopaminergic function and a blunted response to appetitive, rewarding taste stimuli in older adults with metabolic syndrome. Further research with neuroimaging of human response and in animal models is needed to understand the mechanisms that drive brain response in those with metabolic syndrome. We hypothesize that altered brain response to chemosensory stimuli is one underlying mechanism that contributes to the development and maintenance of obesity in metabolic syndrome. We gratefully acknowledge the assistance of Stephanie Oleson, Lisa Graves, Laura Gramling, Jacqueline Szajer, Jean-Loup Bitterlin, Chelsea French, the SDSU Lifespan Human Senses Laboratory, and the UCSD Keck Center for fMRI.

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Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.15/BB28

Topic: D.01. Chemical Senses

Support: NIDCD grant RO1DC006914

Title: Effects of selective gastric vagotomy on sucrose consumption in obese and lean rats

Authors: *A. DENMAN-BRICE[1], P. DI LORENZO[1], K. CZAJA[2];
[1]Psychology, Binghamton Univ., Vestal, NY; [2]Washington State Univ., Pullman, WA

Abstracts: Obese humans exhibit differences from lean humans in taste preference for sucrose, which may play a role in sustaining disordered eating behavior. Roux-en-Y gastric bypass surgery (a weight loss surgery which damages the vagus nerve) has been reported to cause changes in sucrose taste perception in both humans and rats. Additionally, evidence suggests that the gut contains taste-receptive cells which may project to taste areas of the brain (particularly

the nucleus of the solitary tract, or NTS) via the vagus nerve. We hypothesize that taste changes in obesity are mediated by changes in vagal post-ingestive feedback. In the present experiment we tested sucrose preference and sucrose reward value in diet induced obese (DIO, induced by feeding a high-fat chow for at least 4 wks) and lean rats before and after selective gastric vagotomy (Vx) or sham surgery. Sucrose preference was examined by presenting food-deprived rats with brief access (10 sec, signaled by a house light and counted from the first lick) via a computer-controlled lick spout to 6 sucrose solutions of different concentrations (0.005M, 0.015M, 0.05, 0.15M, 0.5M, 1.5M) presented in random order. The number of licks to each sucrose solution as well as the proportion of total licks for each concentration was calculated. Sucrose reward value was tested with a progressive ratio (PR) schedule with licks as the operant response. This procedure begins at 1 dry (unreinforced) lick between "reinforcement blocks" consisting of 5 consecutive licks, each reinforced with 12 µl of 0.1M sucrose. The number of dry licks required for each subsequent reinforcement block increments by 1 dry lick. Subjects remain in the apparatus until at least 5 min have passed without licking. The breakpoint is the number of dry licks that the rat emits to get its last reinforcement. Results showed that prior to surgery, obese rats exhibited a steeper preference curve for varying concentrations of sucrose and a lower PR breakpoint compared to lean rats. After surgery, lean Vx rats exhibited lower PR breakpoints, resembling the performance of DIO subjects. These results imply that altered sucrose taste preferences in obese animals may be mediated by hypoactivity of vagal post-ingestive feedback signals.

Disclosures: **A. Denman-Brice:** None. **P. Di Lorenzo:** None. **K. Czaja:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.16/BB29

Topic: D.01. Chemical Senses

Support: NIH/NIDCD Grant R21DC012746

Title: *In vivo* confocal Ca2+ imaging to study salt taste in the geniculate ganglion

Authors: ***A. WU**[1], G. DVORIANTCHIKOV[2], E. PEREIRA[2], N. CHAUDHARI[1], S. ROPER[1]; [1]Dept. of Neurosci., [2]Dept. of Physiol. and Biophysics, Univ. of Miami, Miller Sch. of Med., MIAMI, FL

Abstracts: NaCl elicits contrasting gustatory responses in mammals: dilute NaCl solutions (<~100 mM) are preferred over water while high concentrations (>~150 mM) are aversive. Evidence exists for two taste transduction pathways for sodium salts. One, an amiloride-sensitive pathway, is dependent on epithelial sodium channels (ENaC) expressed in a subset of taste bud cells. An alternative, amiloride-insensitive pathway may be specialized for aversive salt taste. This latter pathway is ENaC-independent and appears to be mediated by a different population of taste bud cells. We are interested in identifying salt-sensing gustatory neurons in the geniculate ganglion. These neurons innervate taste buds in the anterior tongue, a region that is highly sensitive to salt taste. To study how individual neurons respond to oral stimulation with salty stimuli, we have used confocal Ca^{2+} imaging of geniculate ganglion neurons from *Pirt*-GCaMP3 mice (Kim *et al. Neuron* 2014). We first confirmed that the genetically-encoded Ca^{2+} reporter in these mice is expressed in all geniculate ganglion neurons and that in acutely isolated ganglia, all neurons respond with a robust increase in fluorescence ($\Delta F/F_0$) when depolarized with 50 mM KCl. We developed an *in vivo* preparation with a surgically exposed geniculate ganglion, imaged confocally while the tongue and palate were stimulated with NaCl (30 to 250 mM). The preparation permits us to e visualize individual neurons with good resolution and obtain highly reproducible responses ($\Delta F/F_0$) simultaneously from many neurons. Individual geniculate ganglion neurons varied widely in their sensitivity to NaCl, with EC_{50} ranging from 50 to 500 mM. We have not observed discrete classes of low- *vs.* high Na-sensitive cells. In one series, responses in ≈ half the geniculate neurons were completely blocked by adding benzyl amiloride (benzamil, 1 μM) to the NaCl stimuli. In the remaining half, NaCl-evoked responses were insensitive to benzyl amiloride. Our investigations of salt taste are designed to extract a new and more precise view of how salt taste is represented in the peripheral gustatory system.

Disclosures: A. Wu: None. **G. Dvoriantchikov:** None. **E. Pereira:** None. **N. Chaudhari:** None. **S. Roper:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.17/BB30

Topic: D.01. Chemical Senses

Support: KAKENHI 11004102

Title: Design of voltage sensitive dye imaging for analysis of taste-recognition neural network in *Aplysia* buccal ganglion

Authors: *Y. MIYAKE[1], Y. YOSHIMI[1], T. NAGAHAMA[2];
[1]Dept. Appl. Chem., Shibaura Inst. Technol., Koto-Ku, Japan; [2]Dept. Biophysics, Fac. Phar. Sci., Toho Univ., Funabashi, Japan

Abstracts: Taste is sensed by small number of chemoreceptor types but identified by a sophisticated neural network. Analysis of the neural network concerning taste will contribute to further understanding of informational processing in the central nervous system. A marine gastropod *Aplysia* shows clear food preferences. We attempted to analyze the neural network which generates ingestive or rejective response by using voltage sensitive dye (VSD) imaging. Buccal mass and buccal ganglia of *Aplysia* californica were isolated with keeping their connection by buccal nerves. The neurons in the ganglia were stained with VSD (Di-4-ANEPPS) in the presence of tetraethylammonium (TEA) chloride. Then the responses of the stained neurons were explored after administration of 1 mM solution of L-asparagine (L-Asn) or L-aspartic acid (L-Asp) to radula inside the buccal mass. L-Asn induces ingestive response of the jaws and radula, while L-Asp induces rejective response of them. The administration of L-Asp induced the firing in S1 and S2 clusters with shorter time lag than that of L-Asn as shown in Table 1. This tendency was also observed by electrophysiological experiments using a glass microelectrode. Therefore, the staining and TEA treatment of neurons which are necessary to VSD imaging would not affect seriously neural response because the time lag of firing from the administration of amino acids were almost same whether these treatments were performed or not. The results suggest that VSD imaging is useful for an analysis of neural network for taste recognition because this method can detect neural signals in response to taste stimulation in large numbers of neurons simultaneously. A clarification of the neural network for taste will be achieved by the further analysis of spike activity in these clusters using VSD imaging.

The time lag of firing from administration of amino acids in S clusters neurons (n=6)

	S1 cluster	S2 cluster
L-Asn	4.52 ± 1.40 s (4.57 s)	4.47 ± 1.37 s
L-Asp	2.16 ± 0.31 s (2.02 s)	2.15 ± 0.31 s

Values in parenthesis are the time lags measured electrophysiologically. (n=1)

Disclosures: **Y. Miyake:** None. **Y. Yoshimi:** None. **T. Nagahama:** None.

Poster

621. Taste

Location: Halls A-C

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Program#/Poster: 621.18/BB31

Topic: D.01. Chemical Senses

Support: NIH Grant P41EB015903

NIH Grant U54CA143837

NRF-2013R1A6A3A03060958

Title: Optical interrogation of peripheral taste sensation *in vivo*

Authors: *M. M. CHOI, W. LEE, S.-H. YUN;
Harvard Med. Sch., Cambridge, MA

Abstracts: Intravital microscopic imaging of the sensory system could be a powerful experimental tool, but this has been unprecedented for the taste sensory organ due to the anatomical barrier. Here, we report cellular imaging of the dorsal surface of the tongue in live mice. This was made possible by developing a suction holder that externalizes the tongue from the oral cavity noninvasively and a tongue stabilizer that suppresses the tissue motion while allowing optical and chemical access simultaneously. Using the tongue imaging window in combination with a video-rate two-photon microscope, we visualized the three-dimensional microanatomy and physiological dynamics, such as blood perfusion, molecular diffusion, and functional activity of taste cells. Moreover, we revealed that the so-called intravascular taste sensing takes place in the taste cells, presumably at the microvilli, by small tastant molecules diffused out from pericellular blood vessels and permeated through tight junctions. Intravital tongue imaging would open a new avenue towards comprehensive understanding of peripheral taste sensation.

Disclosures: **M.M. Choi:** None. **S. Yun:** None. **W. Lee:** None.

Poster

621. Taste

Location: Halls A-C

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Program#/Poster: 621.19/BB32

Topic: D.01. Chemical Senses

Support: NIH Grant DC006456

Title: Effects of temporal inactivation of gustatory insular cortex and gustatory thalamus on taste neophobia

Authors: J. ARTHURS, S. REILLY, *J.-Y. LIN;
UIC PSYCHOLOGY, CHICAGO, IL

Abstracts: Permanent lesions of the gustatory insular cortex (GIC) or gustatory thalamus (GT) attenuate taste neophobia (TN). That is, rats with either type of lesion consume more of a novel, fear-inducing taste than neurologically intact subjects; with repeated benign exposures all rats recover from neophobia and reach the same intake asymptote. In the present study we attempted to dissect the temporal aspects of the permanent lesion effect by inactivating the GIC (Experiment 1) or GT (Experiment 2) before or after the first taste neophobia trial. In Experiment 1a, water deprived male Sprague Dawley rats received 15-min access to 0.5% saccharin once every third day for a total of 5 trials. Twenty min before Trial 1, rats in the control group were handled or given intra-GIC infusions of the saline vehicle; the experimental rats were infused with a baclofen/muscimol (BM) cocktail; all rats were allowed to freely consume saccharin without handling or infusions on Trials 2-5. The results show that GIC inactivation elevated intake on Trial 1, decreased intake on Trial 2, and that saccharin intake was not different from control subjects on Trials 3-5. In Experiment 1b, GIC inactivation occurred after the first exposure to a novel 0.0001 M quinine solution, in a procedure that was otherwise identical to that of Experiment 1a. The results reveal no influence of GIC inactivation on the recovery from TN. In a second series of experiments, the same procedures were used to investigate the involvement of the GT in TN. In Experiment 2a, GT inactivation had no influence on Trial 1 intake. However, whereas control subjects increased intake on Trial 2 relative to Trial 1, the GT rats showed no such between-trial change, an effect that served to delay their recovery from TN over the remaining taste trials. In Experiment 2b, post-Trial 1 GT inactivation significantly suppressed intake on Trial 2, which delayed the across-trial recovery from TN relative to the control subjects. This series of experiments has benefitted understanding of the roles of the GIC and GT in TN. We propose that the GIC is critically involved in the perception of taste novelty and that a disruption of this process results in a genuinely novel taste being treated as if it were familiar and safe (which explains why lesions of the GIC retard but do not eliminate conditioned taste aversion learning). Importantly, the absence of a Trial 1 disruption clearly indicates that the GT has a different role in TN than the GIC. We propose that the GT is involved in processing "safety" feedback from a novel taste stimulus, the nature of which is under investigation.

Disclosures: **J. Arthurs:** None. **J. Lin:** None. **S. Reilly:** None.

Poster

621. Taste

Location: Halls A-C

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Program#/Poster: 621.20/BB33

Topic: D.01. Chemical Senses

Support: TM received Japan Society for the Promotion of Science Research Fellowship for Japanese Biomedical and Behavioral Researchers at NIH (2011-2013).

MS receives an intramural grant from NIH-NICHD.

Title: Candidate second-order gustatory neurons that connect the primary gustatory center to other regions in the gnathal (subesophageal) ganglia in the *Drosophila* brain

Authors: ***T. MIYAZAKI**[1], T.-Y. LIN[1], K. ITO[2], C.-H. LEE[1], M. STOPFER[1];
[1]Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD; [2]Inst. of Mol. and Cell. Biosci., The Univ. of Tokyo, Bunkyo-ku, Tokyo, Japan

Abstracts: With a relatively simple nervous system and a plethora of genetic tools, *Drosophila* affords an excellent model for mapping neural circuits and testing their functions. Previous studies identified various classes of gustatory sensory neurons (GSNs) which respond to different tastants and relay information to distinct subregions of the primary gustatory center (PGC) in the gnathal (subesophageal) ganglia. However, little is known about the identities of the neurons that receive synaptic inputs in the PGC, or how these follower neurons process taste information. Here we used a combination of anatomical and functional methods to identify candidate second-order gustatory neurons from a pool of genetically-defined subsets of cells. After screening ~5,000 GAL4 lines, we identified 32 lines that label neurons whose dendrites innervate the PGC. As a secondary screen, we used the GRASP (GFP reconstitution across synaptic partners) technique to visualize potential contacts between the candidate neuron dendrites and axonal terminals of Gr5a-expressing GSNs, which have been shown to respond to sucrose. To differentiate simple membrane contacts from true synapses, we incorporated an active zone marker (Brp-mCherry) to label presynaptic sites of Gr5a-expressing GSNs and checked whether it was co-localized with GRASP. Finally, by expressing a genetically-encoded calcium indicator (G-CaMP6m) in candidate neurons and by using a novel tastant-delivery system we determined whether the neurons increased their activity when sucrose solution was delivered to the

proboscis. Two types of candidate second-order neurons met these criteria and showed stimulus-elicited increases in fluorescence. Together, our results suggest these neurons receive excitatory input from sucrose-responsive Gr5a-expressing GSNs. In addition, we analyzed distributions of input and output sites of one of the candidate neurons using GFP/RFP-tagged acetylcholine receptor subunit (Dα7) and active-zone marker (Brp), respectively. Whereas postsynaptic sites were almost coincident with the synaptic contacts to Gr5a-expressing neurons, presynaptic sites were distributed in distinct regions, suggesting that the labeled neurons transmit information from the PGC to distinct third-order neurons. Further, we are using our tastant delivery apparatus to present multiple taste solutions to animals to analyze responses of the labeled neurons. Our results will provide new information about how the gustatory system encodes tastants.

Disclosures: **T. Miyazaki:** None. **T. Lin:** None. **K. Ito:** None. **C. Lee:** None. **M. Stopfer:** None.

Poster

621. Taste

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 621.21/CC1

Topic: D.01. Chemical Senses

Title: Chemical neural processing and behaviour in zebrafish larvae

Authors: *R. CANDELIER[1], M. S. MURMU[2], G. SUMBRE[2], G. DEBREGEAS[1];
[1]Lab. Jean Perrin (UMR 8237), CNRS / UPMC, Paris, France; [2]Inst. de Biologie de l'École Normale Supérieure, Paris, France

Abstracts: Vertebrates use two chemosensory systems, taste and olfaction to detect and distinguish chemicals from their surroundings. How these chemical sensory stimulus are detected by the brain, processed and transformed into adequate motor pattern remains a central question. However, experimental investigation of the precise mechanisms underlying this sensory modality is extremely difficult since the delivered stimuli have very often poor spatial and temporal resolution, owing to the complex nature of molecule transport and diffusion in fluids. To study the neural responses and motor behaviour associated with chemical stimulation, we have developed an innovative microfluidic chip that enables the presentation of different chemical stimuli (e.g. gustatory, olfactory or nocioceptive) with unprecedented spatio-temporal accuracy. We combined this microfluidic chip with a two-photon microscope to monitor with single-cell resolution the dynamics of neural circuits induced by gustatory stimuli in intact,

optionally behaving, transgenic zebrafish larva expressing a genetically encoded calcium indicator (GCaMP). Optical imaging of neural responses reveals that sour (aversive) and umami (appetitive) stimuli activate non-overlapping neural circuits in the primary gustatory centre, the vagal lobe. The spatial segregation of sour and umami tastes is also maintained in the upstream taste processing centre, the telencephalon where both tastes activate non-overlapping neural circuits in Dm (dorsomedial), Vi (area ventralis pars intermedia) and Dp (area dorsalis pars posterior) telencephalic nuclei. This segregation between aversive and appetitive stimuli evoked responses may play a role in the generation of gustatory-induce motor behaviours, where aversive stimuli evokes a startle response away from the source while appetitive stimuli induces forward swim (towards the source) or no behaviour at all. The effect of stimulus duration has also been investigated: for an increasing presentation time, the number of responding neurons in the vagal lobe and in the telencephalon increase but the average activity of the responding neurons remains constant. Altogether, this evokes a spatial coding scheme in both the primary gustatory center and the telencephalon.

Disclosures: **R. Candelier:** None. **G. Debregeas:** None. **M.S. Murmu:** None. **G. Sumbre:** None.

Poster

621. Taste

Location: Halls A-C

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Program#/Poster: 621.22/CC2

Topic: D.01. Chemical Senses

Support: Giract Bursary award 2013/14 PhD Flavor Research Programme

German Research Foundation Me 1024/8-1

Title: The neural processing of gustatory information in the mouse

Authors: ***S. M. TYREE**, J. TÖLE, W. MEYERHOF;
Mol. Genet., German Inst. of Human Nutr. (dife) Potsdam, Nuthetal, Germany

Abstracts: Taste receptors mediate the detection of tens of thousands of bitter compounds. Research in our group has revealed bitter receptor cell populations on the tongue expressing different subsets of receptors. Results from the Nucleus of the Solitary Tract (NTS) indicate that different bitter stimuli activate distinct, yet overlapping neuron populations. These results

suggest possible discrimination between bitter tastants occurring at the level of the taste receptors and the brain stem. The NTS projects to the Parabrachial Nucleus (PbN), a crucial structure for learned taste aversion, which projects via the Thalamus to the Gustatory Cortex. Integration with other sensory information produces the complex sensation of flavour. Being crucial for avoidance behaviour, the PbN is an interesting structure in which to study the neural response to bitter taste stimulation. We aim to characterise the cellular processing of gustatory information in the PbN to investigate mechanisms underlying discrimination between bitter substances. The Arc catFISH method (cellular compartment analysis of temporal activity by fluorescent *in situ* hybridization) relies on the immediate early gene Arc (activity-regulated cytoskeleton-associated protein) as a neuronal activity marker. Arc expression is induced by neuronal activity and its mRNA follows a strict temporal pattern of intracellular distribution. A protocol was designed in which animals were stimulated twice, allowing the intracellular localization of the Arc mRNA to provide information about which stimulation activated which neurons. C57BL/6 mice were stimulated orally with bitter tastants. Animals showed an increase in Arc expression in the PbN compared to controls, in a similar ratio to results from the NTS, however, the number of PbN cells activated by both stimulations was lower.

Disclosures: **S.M. Tyree:** None. **J. Töle:** None. **W. Meyerhof:** None.

Poster

621. Taste

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Program#/Poster: 621.23/CC3

Topic: D.01. Chemical Senses

Support: A Grant-in-Aid for Young Scientists (B) (22790134) from the ministry of Education, Science and Culture of Japan.

Title: Expression profile of ecto-nucleotidases and equilibrative nucleoside transporter in the rat circumvallate papillae

Authors: *K. NISHIDA, A. OHISHI, K. NAGASAWA;
Kyoto Pharmaceut. Univ., Kyoto, Japan

Abstracts: Taste cells in taste buds have been classified into four cell types on the basis of their cytological and ultrastructural characteristics, and their communication plays crucial roles in gustatory function. ATP is one of the intercellular signaling molecules in taste buds, and

extracellular ATP fate is tightly regulated by its cellular clearance. As for ATP metabolism, ectonucleoside triphosphate diphosphohydrolase 2 is expressed by type I glial-like cells, but little is known about expression of other ecto-nucleotidases, such as ecto-5'-nucleotidase (NT5E), ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) 1, 2 and 3. Equilibrative nucleoside transporters (ENTs) are major molecules that regulate extracellular levels of adenosine as a metabolite of ATP and adenosine also works as a signaling molecule in taste buds. However, there is no information on their expression in taste cells. Therefore, we examined expression profiles of ecto-nucleotidases and ENTs in rat circumvallate by real-time PCR and immunohistochemistry. mRNAs for Nt5e and Enpp1, 2 and 3 were detected in the rat circumvallate papillae, and NT5E immunoreactivity was detected in a region of non-taste bud cells of the circumvallate papillae, while neither PLC-β2-positive type II, chromogranine-A-positive type III nor SNAP25-positive type III cells were immunoreactive for NT5E. ENT1 immunoreactivity was detected in the PLC-β2-positive type II, chromogranine-A-positive type III and SNAP25-positive type III cells, but not in the non-taste bud cells. These results indicate that there is an ATP clearance system mediated by ecto-nucleotidases and ENTs, their coordination being considered to be important for gustatory function.

Disclosures: **K. Nishida:** A. Employment/Salary (full or part-time):; Kyoto Pharmaceutical 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.; A Grant-in-Aid for Young Scientists (B) (22790134) from the ministry of Education, Science and Culture of Japan.. **A. Ohishi:** None. **K. Nagasawa:** A. Employment/Salary (full or part-time):; Kyoto Pharmaceutical University.

Poster

621. Taste

Location: Halls A-C

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Program#/Poster: 621.24/CC4

Topic: D.01. Chemical Senses

Support: NIH R01 Grant DK-091946

NIH F32 Grant DK-094704

Title: A neuropod in enteroendocrine cells is nurtured by enteric glia

Authors: *D. V. BOHORQUEZ, R. A. LIDDLE;
Medicine/GI, Duke Univ. Med. Ctr., Durham, NC

Abstracts: Enteroendocrine cells are epithelial biosensors of the gastrointestinal tract. They sense nutrients, tastants, and bacterial byproducts in the lumen of the gut and when stimulated, they respond by secreting neuropeptides, like peptide YY or cholecystokinin, to modulate appetite and food intake. Although enteroendocrine cells in the intestine are largely regarded as flask-shaped, we recently developed transgenic Pyy-GFP and Cck-GFP mice and found in these cells a prominent axon-like process of unknown function. We call this process a neuropod. Here, we combined confocal microscopy with serial block face scanning electron microscopy to study the three-dimensional ultrastructure of a specific enteroendocrine cell and its neuropod. This approach consists of the following three steps: First, tissue blocks 300 microns wide by 50 microns thick are obtained from a transgenic mouse model in which a specific subset of enteroendocrine cells expresses green fluorescent protein. Second, laser scanning confocal microscopy is used to identify and obtain z-stack images of tissue blocks with cells of interest. Third, selected tissue blocks are processed for 3D Electron Microscopy by using a Serial-Block Face Scanning Electron Microscope. We cut through an entire tissue block containing an enteroendocrine cell to generate a stack of 700 micrographs spaced every 70 nm at a resolution of 7 nm per pixel. Manual 3D rendering of the micrographs series revealed that the neuropod is tightly packed with secretory vesicles of various sizes, contains numerous mitochondria clustered at its tip, and has visible filaments, which we confirmed to be neurofilaments medium and light. The neuropod is also escorted by enteric glia, which are the cells that nurture enteric axons. And, using an *in vitro* minigut organoid model, we found that neurotrophins like NGF-B, artemin or S100B, augment the number and length of neuropods in enteroendocrine cells. These findings open a new field of exploration in the mechanisms of gastrointestinal chemosensation by revealing an unforeseen enteroendocrine cell-entric glia connection.

Disclosures: D.V. Bohorquez: None. **R.A. Liddle:** None.

Poster

621. Taste

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Program#/Poster: 621.25/CC5

Topic: D.01. Chemical Senses

Support: DC006666

DC007703

Charles King Trust Grant

Title: Cortical responses to monosodium glutamate suggest umami is a combination of sweet and salty tastes

Authors: E. E. REID[1], M. A. BAEZ- SANTIAGO[1], J. X. MAIER[1], *D. B. KATZ[2];
[1]Brandeis Univ., Waltham, MA; [2]Dept Psychol, Brandeis Univ., WALTHAM, MA

Abstracts: Gustatory cortex, GC, has been shown to process sweet, salty, sour, and bitter tastes in a dynamic manner, with select epochs of responses containing specific types of taste information (i.e. their presence, identity, and palatability). However, responses to monosodium glutamate, MSG, an exemplar of the umami taste, have not been studied in this way. Here, GC responses were analyzed to better understand the relationship between MSG and the other basic tastes, as well as to further assess the nature of taste dynamics. We recorded single-unit gustatory cortical (GC) responses from awake behaving female Long Evans rats to a battery of five tastants: 0.2 M sucrose, 0.2 M NaCl, 0.1 M MSG, a mixture of 0.2 M sucrose and 0.2 M NaCl, and 0.0001 M quinine, all delivered in 40 uL aliquots through intraoral cannulae. Midway through tasting sessions, tastants were mixed with amiloride (40 uM), a selective sodium channel blocker, which aided in parsing the possible sodium contribution to the MSG responses. GC responses to MSG showed similar temporal structures found in responses to other basic tastes. Earlier periods of the MSG responses (corresponding to the previously mentioned "identity" epoch) were most similar to the sucrose and NaCl mixture responses, while later periods of the MSG responses were more similar to those of NaCl. After adding amiloride, NaCl responses not only became more similar to water responses, but MSG responses became more similar to sucrose responses. These findings are consistent with behavioral tests suggesting that MSG is a combination of sweet and salty tastes. Further analysis of these taste responses should reveal more specific information regarding the dynamics of the MSG response in GC, as well as the relationship between umami and sweet and salty tastes.

Disclosures: E.E. Reid: None. **D.B. Katz:** None. **M.A. Baez- Santiago:** None. **J.X. Maier:** None.

Poster

621. Taste

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Topic: D.01. Chemical Senses

Support: NIH Grant DC010012

NIH Grant DC011735

NSF grant DBJ-0216310.

Title: Expression of taste signaling molecules in immune organs

Authors: *P. FENG, H. WANG;
Monell Chem. Senses Ctr., Philadelphia, PA

Abstracts: Interactions between the immune and chemosensory systems and, especially, the roles of taste receptors in immune defenses have been increasingly appreciated. Recent research has found high levels of expression of multiple key immune-related molecules in specific types of taste cells in taste buds and the expression of bitter and sweet taste receptors in various "extra-oral" tissues. Bitter receptors expressed in the airway epithelial cells can detect and respond to bacterial products, and regulate innate immunity. These studies greatly support the hypothesis that taste receptors and their downstream signaling components play an important role in innate immunity. However, the cellular and molecular connections between taste and immune signaling pathways are unclear. Here, we investigated the expression of α-gustducin in immune cells of spleen, thymus and GI tract using immunohistochemistry and RT-PCR. Differential α-gustducin immunoreactivities were found in these immune tissues. The presence of α-gustducin mRNA was further verified by real-time RT-PCR analysis. Specific antibodies against α-gustducin and immune cell-type markers (CD3 for T cells, B220 for B cells, and CD11b for macrophages) revealed that the α-gustducin immunoreactivity is primarily in T cells, less extensively in macrophages, and few in B cells.. These preliminary results indicate that taste signaling pathways may engage in some innate immune processes and GI tract homeostasis.

Disclosures: P. Feng: None. **H. Wang:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.01/CC7

Topic: D.02. Auditory

Support: NIH NIBIB Grant 5T32EB006350-05

Renz Foundation

Title: A wearable vibrotactile sensory substitution device for the deaf and severely hearing impaired

Authors: *S. D. NOVICH[1,2], D. M. EAGLEMAN[1,2];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Electrical & Computer Engin., Rice Univ., Houston, TX

Abstracts: There are at least 2 million functionally deaf individuals in the United States alone and an estimated 53 million worldwide. The cochlear implant is an effective solution for regaining hearing; however, such implants are expensive, require invasive surgery, and have low efficacy in early-onset deaf adults. Given this, many deaf individuals would benefit from a hearing replacement that is low cost, does not involve an invasive procedure, and may have a higher efficacy for early-onset deaf adults. To this end, we have developed a "vibratory vest" by which auditory information is captured, digitally processed, and delivered to the skin of the torso via an array of small vibratory motors. Such sensory substitution approaches have previously been shown to allow congenitally blind individuals to have visual experience through the tongue or skin. We here present the current development status of our device and results of a speech perception experiments. Participants trained on the Vest by engaging in an identification task: on each trial, the participant was presented with a vibration-mapped stimulus of a spoken word from a training set of 50 phonetically balanced words. The participant was then presented with a set of options displayed on a screen from which they selected the word thought to have been felt, and they were given feedback on their choice. After 12 days of training, participants then ran the same procedure on a novel set of 50 words. Performance reached proficiency in 8 days, on average, from participants running the training task once per day (30-minutes to 1-hour). We performed this experiment across several different candidate audio-processing algorithms, based on (1) linear predictive coding, (2) cosine transforms, or (3) cepstral analysis. Our results demonstrate evidence of learning and transfer of knowledge: participants perform significantly better on their first day with the novel test set than their first day on the training set. Further, participants perform at or near chance the first time identifying a word from the training set, and significantly above chance the first time identifying a word from the test set. Funding for this research is supported by the Renz foundation and a training fellowship from the Keck Center of Interdisciplinary Bioscience Training of the Gulf Coast Consortia (NIBIB Grant No. 5T32EB006350-05).

Disclosures: S.D. Novich: None. **D.M. Eagleman:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.02/CC8

Topic: D.02. Auditory

Title: Deconstructing the sensory and cognitive components of hearing loss

Authors: K. GRODNER, B. VIPARINA, W. M. COLLINS, *L. BOUCHER;
Nova Southeastern Univ., Davie, FL

Abstracts: Hearing loss is commonly thought of as a sensory problem; not a cognitive one. According to the effortfulness effect, working memory in elderly adults with hearing loss is impaired due to the increased cognitive effort hearing loss places on the central executive. This increased need for attentional control makes it difficult to properly encode to-be-remembered information. We replicated hearing loss in the lab by presenting college-aged, normal hearing participants with white noise concurrent with to-be-remembered words. Specifically, participants were instructed to complete an auditory working memory load task in which they heard five to-be-remembered words, followed by a distractor task before they had to free recall the list of words. White noise was presented either during encoding of the words, during retrieval of the words, during both encoding and retrieval, or not at all. We also measured working memory capacity. We hypothesized that when white noise is presented during encoding, participants would recall significantly fewer words. We also hypothesized that if cognitive load is increased when white noise is present during encoding, then working memory capacity should predict performance in this task. We found support for both of these hypotheses strengthening the idea that white noise (as a proxy for hearing loss) adds strain to working memory. It is clear then that hearing loss should be thought of as not just as a sensory issue, but a cognitive one as well.

Disclosures: K. Grodner: None. **B. Viparina:** None. **W.M. Collins:** None. **L. Boucher:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Program#/Poster: 622.03/CC9

Topic: D.02. Auditory

Support: Wellcome Trust: WT074414MA

Title: Compensatory mechanisms for processing speech in noise in older adults

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Abstracts: Adults often report that they find listening to speech in the presence of background noise more effortful as they get older. Indeed, studies have shown that older listeners sometimes perform more poorly in speech in noise tasks than would be predicted by their pure-tone thresholds. Whilst a small number of studies have examined the neural basis of perception in noise in older adults, these studies have tended to examine neural responses to a single type of noise background. However, in our everyday life we encounter many different kinds of background noise, for example noise from machinery and the speech of others, and these different kinds of masking sounds have been shown to draw upon different cognitive and neural mechanisms. Here we compared neural responses between younger and older adults with normal hearing using functional Magnetic Resonance Imaging. In the scanner participants listened passively to short spoken narratives presented either without noise or in the presence of different masking sounds that had been equated for intelligibility but differed “parametrically” in their similarity to speech: speech modulated noise (SMN), rotated speech (Rot) and intelligible speech (Sp). Whilst the groups performed similarly on speech in noise tasks in post-scanner testing, their neural responses were shown to differ within the scanner. Older individuals exhibited reduced responses in the thalamus, putamen and left superior temporal gyrus, regions associated with the encoding of masking sounds. Whilst they showed a compensatory increase in activation in in the bilateral middle frontal gyrus, a region associated with cognitive control. Furthermore, the response in a number of frontal and parietal regions indicated that older adults processed non-speech maskers in a manner that was associated with responding to speech maskers in the younger group.

Disclosures: S. Evans: None. **D. Boebinger:** None. **C. Lima:** None. **S. Rosen:** None. **M. Ostarek:** None. **A. Richards:** None. **C. McGettigan:** None. **Z. Agnew:** None. **S. Scott:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.04/CC10

Topic: D.02. Auditory

Support: Hearing Health Foundation, emerging research grant

Title: Multiple deficits impact auditory stream segregation in aging

Authors: *E. DINCES, E. SUSSMAN;
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Abstracts: Aging adults often have difficulty communicating in everyday situations when there are multiple competing sound sources. However, for aging individuals without sensorineural hearing loss, it is not well understood what type of impairments contribute to the inability to listen to one sound stream amongst many. This study investigated the level (e.g., automatic or attention-based processes) of difficulty for hearing a single sound stream amongst competing sounds in aging individuals (ages 61-79 years) with normal hearing. Electrophysiological and behavioral measures were obtained to assess the ability to segregate sounds, and to compare passive and active listening situations. Results were also compared with those from normal hearing young adults to assess the level of impairment and determine differences that contributed to identification of targets within a sound stream embedded in background noise. Overall, the results indicate that multiple levels of processing impairments, those associated with automatic and attention-related auditory processing, contribute to difficulty in segregating sounds in aging individuals.

Disclosures: E. Dinces: None. **E. sussman:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.05/CC11

Topic: D.02. Auditory

Support: Max Planck Society grant to Jonas Obleser

Title: Neural entrainment is less responsive to attentional demands in older listeners

Authors: ***M. J. HENRY**[1], B. HERRMANN[2], J. OBLESER[2];
[1]Max Planck Res. Group Auditory Cognition, Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstracts: Increasing age is accompanied by decreasing speech comprehension performance in the presence of background noise that cannot be fully explained by peripheral hearing loss. For young, normal hearing listeners, separating speakers in a "cocktail party" capitalizes on neural synchronization with (i.e., entrainment by) attended speech. Thus, an intriguing but as of yet untested possibility is that age-related speech-comprehension deficits might be attributable to changes in the fidelity or flexibility of neural entrainment. The current electroencephalography study characterized differences in entrainment capacities between younger and older adults under varying attentional demands. Younger (age 18-35 years) and older (age 65+ years) participant groups listened to 10-s narrow-band frequency-modulated sounds (FM = 2.8 Hz). Participants completed two sessions in counterbalanced order; in one session they passively listened to the sounds while fixating (passive), while in the other they detected the presence of near-threshold gaps (active); gaps were present in both stimulation blocks, although their presence was only relevant during active task performance. In order to characterize differences between onset-evoked neural responses between age groups, the same participants also completed a passive-listening block in which they were presented with an 8-minute tone sequence in which each tone randomly took on one of five frequencies. This block of stimulation was presented between the active and passive blocks of the frequency-modulated stimuli. Overall, entrained neural responses were larger when participants attended to the stimuli compared to during passive stimulation. However, the difference between entrainment strength for active versus passive stimulation was more pronounced in young than in older participants. This suggests that older participants' entrained neural responses are less flexible under changing attentional demands. With respect to evoked responses, older adults exhibited a larger N1 component than older adults during passive tone stimulation, but a significantly smaller P2. Gap-detection hit rates were modulated by FM stimulus phase, but the degree of performance modulation was similar across age groups. These results reveal critical age-related changes in the way that entrained neural responses adapt to changing attentional demands. Behavioral performance did not differ between groups in the current paradigm. However, we suggest that age-related deficits in perceiving speech against a noisy background might stem from decreasing flexibility to modulate entrainment strength based on top-down attention.

Disclosures: **M.J. Henry:** None. **B. Herrmann:** None. **J. Obleser:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.06/CC12

Topic: D.02. Auditory

Title: Low-delta phase coherence reflects implicit temporal anticipation for supra-threshold stimuli

Authors: ***A. WILSCH**, M. J. HENRY, B. HERRMANN, B. MAESS, J. OBLESER;
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Abstracts: Temporal anticipation enhances encoding precision and facilitates subsequent processing of near-threshold stimuli. With respect to neural oscillations, anticipation is thought to affect the organization of phase in the delta (0.5-4 Hz) frequency band, presumably representing the alignment of an optimized brain state with the expected time of occurrence of an upcoming stimulus. For a situation in which an event is certain to occur, but the exact time of occurrence is not known, temporal anticipation of the event increases with passage of time. Thus, we expected to observe delta phase organization to be sensitive to temporal anticipation of supra-threshold stimuli in the context of variable event occurrence times. In a magnetoencephalography experiment using an auditory delayed-matching-to-sample task on clear speech tokens (N = 10), we serially presented two syllables (S1 and S2) following a temporal cue (analogous to Wilsch et al., 2014). Critically, the interval between cue offset and S1-onset ("foreperiod") was varied parametrically (0.5-1.8 s). Data were analyzed by means of time-frequency analyses (0.5-20 Hz; -4-7 s, time-locked to S1-onset) and event related fields (ERFs). With respect to S1, there were no differences in power, ITPC, and ERFs between cueing conditions for these supra-threshold stimuli. Next, in order to examine the neural correlates of temporal anticipation, single trials from all cueing conditions were binned (7 bins) according to pre-S1 interval (i.e., foreperiod), and inter-trial phase coherence (ITPC), power, and ERFs (-1-1 s time-locked to S1) were calculated for each bin. The relation between foreperiod and each dependent measure was assessed using a cluster-based permutation approach on correlations calculated across bins. Low-delta (i.e., 0.5-0.8 Hz) ITCP correlated positively with foreperiod at right-frontal sensors centered on S1-onset. Longest foreperiods yielded strongest phase concentration. Additionally, longer foreperiods were associated with lower alpha power (8-13 Hz) during the ensuing memory retention (i.e., post-S1) period. Higher-frequency ITCP, delta power, and ERFs were insensitive to foreperiod. Increasing low-delta ITCP with foreperiod most

likely reflects the alignment of an optimized brain state with the occurrence of a temporally anticipated stimulus. In turn, decreased alpha power during memory retention is a marker of reduced cognitive load due to longer foreperiods. Altogether, these results indicate that the neural system relies on temporal anticipation to optimize stimulus processing.

Disclosures: **A. Wilsch:** None. **M.J. Henry:** None. **B. Herrmann:** None. **B. Maess:** None. **J. Obleser:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

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Program#/Poster: 622.07/CC13

Topic: D.02. Auditory

Title: Human sensing inaudible infrasound

Authors: ***T. IIJIMA**, Y. NAKASHIMA, Y. SUGITA;
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Abstracts: We seldom notice the existence of infrasound, although infrasound is generated by air turbulence, ocean waves, traffic and other machinery and is also produced by our own respiration and heartbeat. We can perceive only infrasound of sufficiently high intensity, since our hearing sensitivity drops off markedly in the low frequencies. Here we show human subjects with normal hearing are sensing infrasound and able to learn appropriate response to the sound, even though the intensity of the infrasound is far below the hearing threshold. Human subjects received differential eyelid conditioning for two auditory conditioned stimuli (CSs): a 523.25 Hz tone and a complex tone consisted of the same 523.25 Hz tone and a 16 Hz inaudible tone, one of which was always followed by an airpuff (CS+), whereas the other was not (CS-). After the conditioning trials, the subjects began to respond to CS+ but not to CS-. However, the subjects could not discriminate these two tones at all. We also tested whether the infrasound alone can elicit the eye blink response in a single cue paradigm where the infrasound was presented alone and followed by the airpuff. However, the response was not observed even after extensive training. These results indicate that, when presented in conjunction with the other audible sounds, our brain detects the existence of inaudible infrasound and learns to respond appropriately.

Disclosures: **T. Iijima:** None. **Y. Nakashima:** None. **Y. Sugita:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Program#/Poster: 622.08/CC14

Topic: D.02. Auditory

Support: NIH

Boucai Foundation

Title: Neural oscillatory correlates of detected concurrent spectrotemporal regularities in acoustic stimuli

Authors: *A. M. GIFFORD[1], M. J. KAHANA[2], Y. E. COHEN[3];
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Abstracts: A fundamental goal of the auditory system is to transform acoustic waveforms from low-level sensory representations into perceptual representations. These perceptual representations are the computational result of the auditory system's ability to detect, segregate, and group spectrotemporal regularities in the acoustic environment. Therefore, our perception of a sound is dependent on the regularities that exist in an acoustic stimulus. To understand the neural computations that transform sensations into perceptions, it is fundamentally important to determine how neural activity reflects both sensory- and perceptual-related representations. In addition to the functions of spiking activity in mediating auditory perception, several findings suggest that neural oscillations, which reflect large-scale, coherent activity in neural populations, may also contribute to sensory and perceptual representations. However, whereas the contribution of spiking activity to auditory perception has been extensively studied, the potential roles of neural oscillations have not. Thus, there are critical gaps in our knowledge of the relations between neural oscillations and auditory perception. For instance, it is unknown whether or if neural oscillations are modulated by (1) auditory stimuli that have spectrotemporal regularities occurring concurrently over multiple timescales and (2) a listener's perceptual judgments of these stimuli. Thus, the goal of this study was to test these relationships by analyzing neural oscillations in human subjects while they participate in an auditory deviance-detection task. Stimuli were sequences of tone bursts that contained regularities over different timescales. Each sequence consisted of 3 tone-burst triplets. In this sequence, a local regularity governed the frequency of the tone bursts in a triplet and a more global regularity governed the frequency change across each triplet. A sequence could maintain this pattern throughout its duration or deviate from either regularity with a frequency increase. Subjects listened to the

stimuli and reported whether or not they detected a deviation in one of the regularities by pressing one of two buttons. We found that the sequences elicited significant power and phase modulations in the oscillatory frequencies that corresponded to the timescales of the spectrotemporal regularities in the stimulus. These modulations occurred in auditory-related cortical regions including non-primary auditory, prefrontal, and parietal cortices. These findings support a potential role for neural oscillations in tracking spectrotemporal regularity and organizing the auditory scene.

Disclosures: **A.M. Gifford:** None. **M.J. Kahana:** None. **Y.E. Cohen:** None.

Poster

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Topic: D.02. Auditory

Support: NIH Grant DC004263

Title: The role of attention in the buildup to stream segregation

Authors: *J. SUSSMAN-FORT, E. SUSSMAN;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstracts: The ability to process an auditory scene is impacted by both the dynamics of the environment and focused attention. The auditory system separates out the mixture of sounds that enters our ears into distinct sound sources and groups them together across time via a process termed stream segregation. The time required to perceive this mixture as segregated is known as the “buildup” period. The goal of this study was to examine the effect of focused attention on the timing of the buildup under different conditions of stimulus context to determine whether attention can speed up the buildup period. To do this we presented a pattern of repeating low and high frequency tones (L-L-H) in trains of stimuli separated by silence and recorded event-related potentials (ERPs). The stimulus context was manipulated by presenting two conditions in which frequencies roved or did not rove across stimulus trains. Subjects were instructed to press a button to identify higher intensity target tones randomly positioned at the end of each stimulus train, past what would be defined as the buildup period. Non-target higher intensity tones were randomly embedded in the early part of the stimulus train that would be defined as within the buildup period. ERP responses to the non-target tones indicated when the sounds were

segregated. This allowed us to observe the timing of the buildup. No responses were obtained to the non-target tones when the stimulus trains roved in frequency indicating attention did not impact the timing of the buildup. In contrast, responses were obtained to the non-target tones when the stimulus trains did not rove in frequency, providing evidence that in addition to effects of stimulus context on the buildup period, attention influenced how quickly sounds were segregated.

Disclosures: J. Sussman-Fort: None. **E. Sussman:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Topic: D.02. Auditory

Support: NIDCD R03 DC011304

Title: The mismatched negativity as a marker for acoustic and phonological distinctions between vowel sounds

Authors: J. BURNISON[1], *J. S. BRUMBERG[2,1];
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Abstracts: Speech perception of similarly sounding vowels is difficult despite clear phonological differences between perceptual neighbors. This effect can be characterized by the perceptual magnet effect, which states that discrimination of vowels similar to a good example of a referent vowel is more difficult than to a poor referent. In this study, we examine the neural processes that underlie perception of neighboring vowels, focusing on the influence of acoustic and phonological factors. We explore these effects in a mismatch negativity (MMN) event-related potential protocol using an auditory oddball paradigm. Prior MMN studies investigating auditory and phonological neural processing of vowels have lead to inconclusive results, with some studies indicating an auditory-only mechanism while others suggest linguistic factors enhance the neural response. Some of the ambiguity may be due to individual differences in vowel identification; therefore, the present studies used vowel sounds tailored to each participant. Methods: One standard and four deviant vowels were presented acoustically in a traditional MMN oddball paradigm. All five stimuli were synthesized according to prototypical and vowel transition boundary examples obtained from participants' perceptual identification

task results. We compare two experiments, one in which the standard stimulus is a prototypical vowel sound and deviants occur on the boundary transition with a neighboring vowel. Specifically, the four deviants are: within category, transition midpoint, across boundary and the prototypical neighboring vowel. The first three deviants are all poor examples of the standard stimulus. The second experiment utilizes the boundary midpoint condition as the standard to eliminate priming asymmetries. Results: In our first experiment, an MMN was elicited generally for each of the deviant stimuli relative to a good example of a referent vowel sound. We found linear trends indicating an increase in MMN peak amplitude and area under the curve and a decrease in peak amplitude latency as a function of perceptual distance from the standard, but there are no statistical differences between the four deviant stimuli. We therefore infer that preattentive neural processing is neither sensitive to acoustic nor phonological differences between vowels along the border transition from one vowel to its neighbor. This result is somewhat predicted the perceptual magnet effect; therefore, our second experiment will clarify whether differences in MMN amplitudes and latencies are found when discriminating border transition vowels relative to a poor referent, specifically the transition midpoint vowel.

Disclosures: J. Burnison: None. **J.S. Brumberg:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.11/CC17

Topic: D.02. Auditory

Title: Relative magnitude of loudness determines pitch accents in sequencially presented noise-vocoded Japanese vowels

Authors: *M. TAKABAYASHI[1], K. I. KOBAYASI[2,3], H. RIQUIMAROUX[1,2,3];
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Abstracts: We often perceive pitch changes created by temporal change in the fundamental frequency when we listen to somebody talks or sings. Noise-vocoded speech sound (NVSS) is a synthesized sound whose frequency information is greatly reduced while the amplitude envelope information remains preserved. In NVSS the fundamental frequency does not exist. However, changes in amplitude envelope, intensity cause changes in pitch. The “intensity” is physical characteristic and variations of intensity and loudness are not identical. The purpose of this study

is to investigate whether loudness of each vowel and that of noise- vocoded vowel are identical or not, and to examine whether loudness creates pitch change in NVSS. Japanese language has 5 vowels. Therefore, 25 combinations can be made when two vowels are paired. Noise-vocoded vowel pairs were also prepared. They were used for stimuli in this experiment. Intensity of the first sound was fixed at 60 dB SPL (re: 20μPa), while intensity of the second sound was varied between -9 and 9 dB SPL. Subjects listened to stimuli 4 times in random order. They evaluated whether loudness of the second sound was louder or softer than the first one. Loudness of each vowel in original speech was similar to that in NVSS. Results suggest that sequences of vowels act on whether the second sound was perceived louder or softer. On the other hand, differences between the first and the second sound when subjects evaluated two magnitudes of loudness as identical were measured. The extremely negative difference was shown in [a-e] pair, while extremely positive difference was shown in [u-u]. The smallest difference was found in [i-o] pair. There 3 noise-vocoded vowel pairs were used in the experiment where pitch change in NVSS was investigated. Subjects listened to these 3 combinations whose sound pressure level of the second sound was varied in a similar way used in the first experiment. Subjects evaluated the second sound whether pitch rises or falls from the first sound. Then results showed that pitch changes in NVSS were affected by spectral information of each noise-vocoded vowel. However, as 3 combinations, change in pitch could be perceived easier as rising as loudness of the second sound increased. In conclusion, loudness change creates pitch change in NVSS. However, it does not appear to be the only factor. Hence, vowels that tend to be overestimated or underestimated in loudness and its reason were investigated. Relationships between loudness and pitch change in NVSS were discussed.

Disclosures: **M. Takabayashi:** None. **K.I. Kobayasi:** None. **H. Riquimaroux:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Topic: D.02. Auditory

Support: NIH/NIDCD R01 DC006287

Title: Differential neural adaptation of spectral transition and steady-state features in speech and non-speech

Authors: *M. SABRI, K. LEWIS, C. J. HUMPHRIES, E. LIEBENTHAL;
Neurol., Med. Col. of Wisconsin, MILWAUKEE, WI

Abstracts: We investigated feature-specific adaptation of speech and non-speech sounds during a repetition-suppression paradigm using auditory event-related potentials (ERPs). Stimuli were synthesized CV syllables consisting of two formants (F1, F2) representing an initial spectral transition (T) period followed by a spectral flat steady-state (SS) period. Three sets of stimuli were compared. Speech stimuli were generated with F1 and F2 in their canonical form. Non-speech stimuli were generated by spectrally rotating the F1 formant. Single-formant stimuli included only either the F1 or F2 formant. ERPs were recorded while subjects (n=15, 2 sessions) listened to trains of six sounds (inter-stimulus interval=200 ms), in which the last two sounds either: (1) matched the first four (i.e., full adaptation), (2) differed in the T period, (3) differed in the SS period, or (4) differed in both the T and SS periods. Subjects were instructed to identify catch trials (p=.1) that were missing one of the six sounds. These trials were discarded from the analysis. A moving window analysis based on an average of frontal electrodes was performed on the evoked response of sound 5 in the train (when a change from the adapting sound occurs). Differences between adaptation conditions were observed in the N1-P2 complex, showing a larger amplitude for SS and TSS compared to T ($p<.05$). Within the speech and non-speech conditions, N1 was greater for SS than T. Further, there was no difference within the speech condition between TSS and SS. Taken together these results demonstrate greater release from adaptation of steady-state (important for vowel identity) compared to transition (important for consonant identity) features of speech and acoustically-matched non-speech analogs, possibly reflecting the greater vulnerability to repetition of the longer and steady-state spectral segment. Differential neural adaptation may be a mechanism by which phonemic brain regions attain finer tuning to spectral transition information coding consonant identity relative to spectral steady-state information coding vowel identity, possibly resulting in more categorical perception of consonants relative to vowels.

Disclosures: **M. Sabri:** None. **K. Lewis:** None. **C.J. Humphries:** None. **E. Liebenthal:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Program#/Poster: 622.13/CC19

Topic: D.02. Auditory

Support: R01 AG 038490-01

Title: Neural correlates of acoustic and linguistic contributions to listening effort during speech comprehension

Authors: *Y. .-S. LEE[1], J. E. PEELLE[2], C. ROGERS[3], N. E. MIN[1], A. WINGFIELD[1], M. GROSSMAN[1];
[1]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [2]Dept. of Otolaryngology,, Washington Univ. in St. Louis, St. Louis, MO; [3]Volen Natl. Ctr. for Complex Systems, Brandeis Univ., Waltham, MA

Abstracts: Understanding connected speech requires coordination between sensory and cognitive processes within a core speech system centered in bilateral temporal cortex and left inferior frontal gyrus. Although it has been shown that additional resources are recruited when dealing with syntactically complex speech, less is known about how the brain can successfully cope with acoustically challenging speech. We hypothesized that similar compensation mechanisms may be at play when processing degraded speech signals. Here we used interleaved silent steady-state (ISSS) functional magnetic resonance imaging (fMRI) to examine compensatory neural mechanisms that support processing of acoustically-degraded sentences that vary in their syntactic demands. Twelve healthy young adults (mean age=24 yrs) were presented with a series of spoken sentences. Each sentence comprised of six words (e.g., “Boys that kiss girls are happy”); subjects indicated the gender of the character performing the action via button press. Sentences were presented in the absence of acoustic scanner noise, after which we collected 10 seconds of data. The sentences were constructed in a 2 (subject-relative vs. object-relative embedded clause) x 2 (clear vs. degraded speech) factorial design. To degrade the speech we used a noise-vocoding algorithm with 24 channels that reduced spectral detail but preserved the overall amplitude envelope of the signal. Behavioral testing in a separate group of 20 young adults confirmed that this manipulation preserved intelligibility, but reduced perceptual clarity, $t(19) = 5.84$, $p<.001$. In the fMRI study, accuracy was high (mean > 94%) and did not differ as a function of acoustic clarity. Our results revealed that the left frontotemporal areas were more activated by increasing the syntactic demand. Importantly, this network also showed increased activity to successfully process acoustically degraded speech. Lastly, a part of the left inferior frontal area yielded activity when processing sentences requiring most listening effort (e.g., degraded speech with object-relative embedded clause). Together, our data suggest that compensation mechanisms are required when processing spoken language signals for both linguistic and acoustic challenges along the frontotemporal speech network.

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Poster

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Program#/Poster: 622.14/CC20

Topic: D.02. Auditory

Title: Neural mechanisms for the recognition of the noise-vocoded speech sounds: An fMRI study based on individual differences

Authors: *S. MURAI[1], K. I. KOBAYASI[2,3], H. RIQUIMAROUX[1,2,3];
[1]Grad. Sch. of Life and Med. Sci., [2]Dept. of Biomed. Information, Fac. of Life and Med. Sci., [3]Neurosensing and Bionavigation Res. Ctr., Doshisha Univ., Kyoto, Japan

Abstracts: Human speech perception is robust. Even when frequency information of sounds is greatly reduced, a listener uses amplitude envelope information to comprehend speech. The neural basis for individual differences in the robustness, however, is still largely unknown. In the present experiment, the noise-vocoded speech sounds (NVSS), where frequency information was greatly reduced, were used to reveal neural circuits for speech perception. We tried to identify brain regions for contributing to individual differences in NVSS comprehension by using the functional magnetic resonance imaging (fMRI) with a sparse sampling paradigm. The original speech sounds were Japanese sentences consisted of 13-16 morae (2-3 s) recorded at 8 kHz sampling rate with 16 bits and reduced noise below 60 Hz using high-pass filter. NVSS was created by dividing the original speech sounds into 3 bands (60-600, 600-1500, 1500-4000 Hz). The amplitude envelope for each band was extracted by half-wave rectification after low-pass filtering at 32 Hz. In addition, the amplitude envelope in each frequency band was multiplied with a band noise of the same frequency bandwidth. In test sessions, comprehension of NVSS was behaviorally evaluated before the MRI session. Adult native Japanese speakers listened to NVSS sentences and reported how they perceived NVSS without feedback in a soundproof chamber. Answers were written down on paper. In the MRI session, participants listened to new sets of NVSS sentences through headphones. Then, the same NVSS sentence was repeated while the original sentence was shown on a screen in text. As a result, average percentage of correct morae was 15.0%, and standard deviation of correct percentage was 16.9%. The individual differences were quite variable. To identify regions contributing to the NVSS comprehension, a multiple regression analysis for the individual scores from pre-scanning test (percent of correct morae) was performed on individual contrast images while listening to NVSS in each subject. The individual variation in the NVSS comprehension score was significantly correlated to BOLD

signal of the left inferior frontal gyrus (IFG). Data showed that brain activities in the left IFG were reflected subjects' ability to comprehend NVSS sentences, and the region may be related to the individual variation in robustness of speech perception.

Disclosures: S. Murai: None. **K.I. Kobayasi:** None. **H. Riquimaroux:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Hearing Health Foundation

Hoover Fund

Title: Network analysis of human speech perception: An intracranial recording perspective

Authors: *M. STEINSCHNEIDER[1], K. NOURSKI[2], A. RHONE[2], H. OYA[2], H. KAWASAKI[2], M. HOWARD, III[2];
[1]Albert Einstein Med. Col., BRONX, NY; [2]Univ. of Iowa Med. Ctr., Iowa City, IA

Abstracts: Speech perception engages a large network of cortical regions that begins in Heschl's gyrus (HG) and later activates many areas, including the temporal and frontal lobes. In order to clarify the sequence and roles of these regions, we recorded the electrocorticogram of subjects who were neurosurgical patients undergoing chronic invasive monitoring for medically refractory epilepsy. All research protocols were approved by the NIH and The University of Iowa IRB, and subjects could rescind consent at any time without detriment to their surgical evaluation. Stimuli were words and non-words from TIMIT or LibriVox or complex tones. Each word/non-word was spoken by different speakers (14 male, 6 female) and edited to be 300 ms in duration. There were three categories of words: animals (cat, dog), colors (red, white), numbers (five, ten) and two sets of non-words (res, tem). Subjects were instructed to press a button

whenever they heard a target (e.g., female voices, animals) embedded in a stream of all other stimuli. Locations of recording sites were confirmed by co-registration of pre- and post-implantation structural imaging. Event-related band power in the high gamma frequency range (70-150 Hz) was examined. Responses recorded directly from posteromedial HG (i.e., core auditory cortex) primarily reflected the acoustic attributes of the sounds (e.g., male vs. female voice, voice onset time) and were not strongly modulated by the behavioral tasks. A similar pattern was observed in the early activity recorded from posterolateral superior temporal gyrus (PLST). In contrast, later activity on PLST was strongly modulated by task requirements and was enhanced to targets and, to a lesser degree, non-targets whose perception was relevant for the task. Later activity elicited by targets, however, was not modulated by behavioral performance. Activity overlying the inferior frontal gyrus (IFG) was also enhanced to targets and relevant non-targets, but the activity was strongly modulated by behavioral performance. Finally, activity overlying the middle frontal gyrus (MFG) was only elicited by targets. We conclude that differential patterns of responses to speech are distributed across HG, PLST, IFG, and MFG. HG and early activity on PLST primarily reflect acoustic attributes of speech, whereas later activity on PLST and that elicited in all other regions studied are strongly modulated by task requirements. The finding that later activity on PLST is not modulated by behavioral performance, contrasting with activity on IFG, suggests that object formation of speech (e.g., semantic meaning) only begins to emerge on PLST and becomes complete in regions of frontal cortex.

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Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.16/CC22

Topic: D.02. Auditory

Support: Studienstiftung des deutschen Volkes (German National Academic Foundation) scholarship to BZ

EURYI Award to RV

Title: EEG oscillations entrain their phase to high-level features of speech sound

Authors: *B. ZOEFEL, R. VANRULLEN;
Ctr. De Recherche Cerveau Et Cognition (cerco), Toulouse, France

Abstracts: Neural oscillations adjust to rhythmic patterns in the environment, a mechanism called phase entrainment. Phase entrainment has encountered wide interest in speech research, as aligning neural oscillations and speech sound can improve speech intelligibility. However, the precise nature of phase entrainment is still debated, as, in everyday speech sound, low-level (e.g., fluctuations in sound amplitude) and high-level features (e.g., fluctuations in speech information) co-vary. Thus, on the one hand, brain oscillations could merely passively follow the rhythmic changes in amplitude or spectral content of speech sound. On the other hand, phase entrainment might also include a high-level process: an active adjustment to the co-varying speech information. Obviously, these alternatives could not be disentangled by the use of common speech sound as experimental stimuli. Thus, we constructed novel speech/noise stimuli without systematic fluctuations in sound amplitude or spectral content, while keeping both fluctuations in speech information and intelligibility. This construction made it possible to disentangle, for the first time, passive (low-level) and active (high-level) components of phase entrainment. Recently, by using those stimuli in a psychophysical experiment, we showed that perception indeed entrains to high-level features of speech sound (Zoefel and VanRullen, Society for Neuroscience Abstract 2013). Here, we ask whether this perceptual entrainment to high-level features of speech goes along with neural entrainment as well. We presented subjects (N = 12) with our constructed speech/noise stimuli while recording electroencephalography (EEG). Indeed, we found that neural oscillations entrain to speech rhythm (as assessed by speech-EEG phase-locking) even when speech information is not accompanied by changes in sound amplitude or spectral content. This effect was abolished when intelligibility was disrupted by presenting snippets in reverse, indicating that entrainment was indeed due to rhythmic changes in speech information. Additionally, by comparing original (without noise) and constructed (with noise) speech snippets, we were able to dissociate sensory (low-level) and high-level components of phase entrainment: Cross-correlation revealed two prominent time lags of coherence between original speech and neural signal at ~110 ms and 190 ms, whereas only the later component was present for constructed speech/noise snippets. The latter effect was abolished as well when constructed snippets were time-reversed, indicating again a high-level component that reflects entrainment to rhythmic changes in speech information.

Disclosures: B. Zoefel: None. **R. VanRullen:** None.

Poster

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Program#/Poster: 622.17/CC23

Topic: D.02. Auditory

Title: Alpha oscillations index differential distractor-speech interference

Authors: *M. WÖSTMANN, B. HERRMANN, B. MAESS, J. OBLESER;
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Abstracts: Speech processing can be demanding *in situ*ations where listening to a particular speaker is obstructed by distracting speech from other speakers. When confusion between target speech and distractors is high, listening success depends on selective attention to the speech signal at the expense of distractors. Cortical alpha (~10 Hz) oscillations are argued to play a role in attention by inhibiting task-irrelevant processing. However, the role of alpha oscillations in selective attention during effortful listening to auditory stimuli, for example speech, is less well investigated. In a combined magneto-/electroencephalography (MEEG) study, we tested the hypothesis that alpha oscillations contribute to auditory selective attention and that alpha oscillations allow predicting the participant's degree of distractor-speech interference during selective listening. In a dichotic listening paradigm, human participants were cued to attend to and maintain a stream of four spoken digits presented to one ear, while ignoring a distracting (same-talker) stream of digits presented to the other ear. Following acoustic stimulation, participants selected the digits in the to-be-attended stream from a visually presented array of twelve digits. Behaviorally, participants correctly recalled the majority of digits (~80 %) from the to-be-attended stream. Critically, participants also showed specific interference by the distractor stream. That is, they showed confusions between the two streams as indicated by reports of digits from the to-be-ignored stream. Furthermore, stream confusions occurred more frequently than reports of digits that were not presented in any of the two streams (false alarms). On the neural level, alpha power showed significant modulations and generally decreased during the time course of the trial. These findings suggest an important role of alpha oscillations during on-line speech processing in effortful multi-talker listening situations. In particular, fluctuations in alpha power might indicate the differential inhibition of distracting information to support auditory selective attention to the task-relevant speech signal.

Disclosures: **M. Wöstmann:** None. **B. Herrmann:** None. **B. Maess:** None. **J. Obleser:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Program#/Poster: 622.18/CC24

Topic: D.02. Auditory

Title: Neural amplitude fluctuations in multiple frequency bands predict auditory perception in a rhythmically variable context

Authors: *B. HERRMANN, M. J. HENRY, J. OBLESER;
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Abstracts: Neural oscillations are thought to provide listeners with a mechanism for making and evaluating temporal predictions. When acoustic stimulation is temporally regular, low-frequency neural oscillations become aligned with (i.e., entrained by) the event structure. In turn, listening behavior is optimized, as expected events coincide with the "excitable" phase of the entrained neural oscillation. However, it is less clear how amplitude-envelope fluctuations influence listening behavior, in particular in contexts with variable degrees of temporal regularity in which listeners might use either a "rhythmic" or a "continuous" attentional processing mode. To that end, the current magnetoencephalography study investigated the relation between neural amplitude-envelope fluctuations in auditory cortex and human listening behavior in a rhythmically-variable listening situation. Tone sequences varied in temporal regularity (i.e., mean rate 2 Hz ± 0.14 Hz standard deviation), and participants (N=20) indicated the presence of difficult-to-detect intensity changes. An oscillator model was used to estimate the degree to which each target tone was expected based on the preceding temporal context. Time-frequency analyses quantified the degree to which the amplitude of pre-target neural oscillations predicted behavioral performance. First, we confirmed that intensity changes were better detected when their occurrence was more predictable based on the timing of the preceding sequence. With respect to neural oscillations, we observed interactive effects of pre-target neural amplitude at three distinct frequencies on perception. First, amplitude of the 2-Hz neural oscillation differentially predicted target-detection performance - expected targets were best detected when 2-Hz neural amplitude was high, whereas unexpected targets were best detected when amplitude was low. Second, we observed modulations of target-detection performance by alpha-frequency amplitude (~8 Hz and ~13 Hz, respectively), which, third, depended on low-frequency (2 Hz) amplitude. In detail, hit rates increased linearly with increasing 8-Hz amplitude, but this effect was strongest when 2-Hz amplitude took on intermediate values. Hit rates were further increased for either high or low 13-Hz alpha amplitude, but this quadratic trend was strongest for high and low, but not intermediate, 2-Hz amplitude values. The current results demonstrate that, in temporally-variable contexts, auditory perception depends on complex interactions of neural amplitude fluctuations that might reflect an instable neural state between "rhythmic" and "continuous" processing modes.

Disclosures: **B. Herrmann:** None. **M.J. Henry:** None. **J. Obleser:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.19/CC25

Topic: D.02. Auditory

Title: Training induced change in categorical boundaries of speech sounds

Authors: E. STIPES[1], P. MASON[2], M. PIERCE[1], *C.-L. TENG[3];
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Abstracts: Humans and animals perceive the world categorically. Our abilities to categorize, simplify and streamline information are fundamental to the development of cognition. Take color perception for example: although the changing wavelengths are continuous, color perception is categorical. Another example is speech sound perception: /ba/-/da/-/ga/ varies in a continuous dimension but human perception separates the physical attributes into perceptual categories. The phenomenon categorical perception (CP) is found in many sensory and cognitive dimensions. However, to what degree it is learned rather than innate remains unclear. We hypothesize that a categorical boundary is a by-product in optimizing discrimination and that its location varies with the training stimuli. Using a linear decoding model, we show that optimal weights assigned to neurons tuned between the training stimuli changes sign. Assuming a perceptual decision is based on the weighted sum of the sensory inputs, an optimized discriminator has the same characteristics as in categorical perception. They are (1) stimuli appear similarly within a category, (2) stimuli appear dissimilarly between categories, and (3) of the same change of physical attributes, stimuli appear most differently at the categorical boundary. We developed an awake-behaving animal experiment, instructing a rat to either initiate and sustain or withhold from licking to indicate a perceptual decision. We found that by training an rat to discriminate two speech sounds /ba/-/pa/, a categorical boundary emerged naturally in between. There was a sigmoidal but not a linear change in percent licking as voice-onset-time varies. Further, discrimination was the highest at the boundary. The location of the boundary, however, was not in the middle of the training stimuli, suggesting an interaction with innate neuronal processing.

Disclosures: **E. Stipes:** None. **P. Mason:** None. **M. Pierce:** None. **C. Teng:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

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Title: The influence of hearing acuity and perceptual effort on recall for word-lists

Authors: *K. COUSINS, A. WINGFIELD;
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Abstracts: Everyday listening conditions are rarely ideal, as ambient noise or hearing loss can interfere with the auditory signal, and can make speech difficult to hear. Studies of noise-masking have shown that even when the distracting noise is mild, and auditory stimuli are identifiable, memory for the auditory information suffers. In previous studies, we have demonstrated that this negative effect of noise-masking extends beyond the masked material, and recall for nearby non-masked words suffers as well. It is assumed that this adverse effect of masking on recall is due to the perceptual effort expended to correctly identify the masked word. Here, we test if this recall deficit for masked and non-masked words is a unique effect of background noise, or also extends to other situations of strained perception. Participants were older adults (aged 65 or older) who ranged in hearing acuity, measured by their speech reception threshold (SRT). Word-lists were 6 words long. Instead of using noise-masking, all words in the list are presented in quiet. To test the effect of difficult perception, one word in the list was made challenging to perceive by presenting it just above listening threshold, adjusted according to each individual's SRT (7dB sensation level; SL). All other words in the list, and words in the control list, were played at 15dB SL. Participants showed a depressed recall for the critical-quiet word, as well for neighboring, appreciably louder words. These results indicate that straining to hear a soft word can impoverish the ability to also encode other words presented, even when they are clearly audible.

Disclosures: K. Cousins: None. **A. Wingfield:** None.

Poster

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Title: The Oxford Vocal (OxVoc) Sounds Database: A validated set of non-acted affective sounds from human infants, adults and domestic animals

Authors: *C. PARSONS[1], K. YOUNG[1,3], M. CRASKE[4], A. STEIN[1], M. KRINGELBACH[2]; [1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. of Oxford, oxford, United Kingdom; [3]UCLA, La, CA; [4]UCLA, LA, CA

Abstracts: Sound moves us. Nowhere is this more apparent than in our responses to genuine emotional vocalisations, be they heartfelt distress cries or raucous laughter. Here, we present perceptual ratings and a description of a freely available, large database of natural affective vocal sounds from human infants, adults and domestic animals, the Oxford Vocal (OxVoc) Sounds database. This database consists of 173 non-verbal sounds expressing a range of happy, sad and neutral emotional states. Ratings are presented for the sounds on a range of dimensions from a number of independent participant samples. Perceptions related to valence, including distress, vocaliser mood, and listener mood are presented in Study 1. Perceptions of the arousal of the sound, listener motivation to respond and valence (positive, negative) are presented in Study 2. Perceptions of the emotional content of the stimuli in both Study 1 and Study 2 were consistent with the predefined categories (e.g., laugh stimuli perceived as positive). While the adult vocalisations received more extreme valence ratings, rated motivation to respond to the sounds was highest for the infant sounds. The major advantages of this database are the inclusion of vocalisations from naturalistic situations, which represent genuine expressions of emotion, and the inclusion of vocalisations from animals and infants, providing comparison stimuli for use in cross-species and developmental studies. The associated website provides a detailed description of the physical properties of the each sound stimulus along with cross-category descriptions.

Disclosures: C. Parsons: None. **K. Young:** None. **M. Kringelbach:** None. **A. Stein:** None. **M. Craske:** None.

Poster

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KAKENHI(21120009)

Title: Neural substrates representing temporal and motor sequences of rhythm

Authors: *N. KONOIKE[1], Y. KOTOZAKI[2], J. HYEONJEONG[2], A. MIYAZAKI[2], K. SAKAKI[2], T. SHINADA[2], M. SUGIURA[2], R. KAWASHIMA[2], K. NAKAMURA[1];
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Abstracts: When separate sounds occur with temporally structured patterns, we can feel a 'rhythm'. Perception of rhythm often makes us move involuntarily or intentionally. We have previously reported that the fronto-parieto-cerebellar system showed a dynamic change in activity during the encoding and retrieving of rhythm information. Most of these regions have been thought to play an important role in motor control. Based on our results and those of previous studies, we hypothesized that rhythm information is represented in two different domains; temporal and motor sequences. The motor sequences should depend on effectors, such as, finger, foot, or mouth. On the other hands, the temporal sequence would be effector-independent. So far, little is known about neuronal substrates representing temporal or motor sequences of rhythm. To address this issue, we measured brain activity by functional magnetic resonance imaging while 29 healthy right-handed subjects memorized auditory rhythm and reproduced it by tapping using a right index finger, a left index finger, or a right foot, or by articulation. Conjunction analysis revealed that the right inferior frontal gyrus (IFG) and inferior parietal lobule (IPL) exhibited significant effector-independent activations during both encoding and retrieving of rhythm information. On the other hand, the left IFG exhibited effector-dependent activation during encoding, and the left IPL and supplementary motor area (SMA) showed effector-dependent activations during the retrieving of rhythm information. These results suggest that temporal sequences of rhythm may be represented in the right fronto-parietal

system, whereas the motor sequences of rhythm may be represented in the left fronto-parietal system.

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Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

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Topic: D.02. Auditory

Title: Dynamic affective and neural responses to expressive timing fluctuations in music

Authors: ***N. K. FLAIG**[1], T. P. ZANTO[2], H. L. CHAPIN[3], E. W. LARGE[1];
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Abstracts: Research has shown that the brain synchronizes to musical events. The perception of rhythm involves entrainment of endogenous cortical oscillations to low frequency periodicities in many spatially distinct neural regions, and rhythmic entrainment is thought to underlie temporal expectancy. In expressive musical performances tempo varies, creating violations of temporal expectancy. A recent study has shown activation of motor-related areas, emotion-related limbic and paralimbic structures, and reward centers when listening to expressive, but not mechanical, music. However, it is not known whether expectancy violations themselves are responsible for increased limbic and paralimbic activations. We asked whether the fluctuations in tempo and intensity of an expressive musical performance would lead to emotion and reward related neural activations, and whether musical experience would modulate these responses. Musicians and non-musicians listened to a skilled music performance that included the natural expressive variations that musicians use to evoke emotional responses. We compared the time derivative of performed tempo and performed intensity to both real-time ratings of arousal and valence and to BOLD signal changes. Results showed that fluctuations of expressive timing (changes in tempo) are correlated with listener ratings of affect as well as BOLD signal changes in cortical and subcortical motor areas. Our findings provide evidence that expressive timing fluctuations and resulting expectancy violations are integral to affective musical responses.

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Poster

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Fondazione Neurone

Title: Pitch direction modulates theta activity in classical music - an ECoG study

Authors: C. A. MIKUTTA[1], S. DUERSCHMID[2,3,4], M. LEHNE[5], A. ALTORFER[6], W. K. STRIK[6], J. PARVIZI[7,8], H.-J. HEINZE[9,3,4], H. HINRICHS[10,3,4], P. BRUNNER[11,13,14], G. SCHALK[12,13,14], S. KOELSCH[5], *R. T. KNIGHT[15];
[1]Dept. of Psychology, Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA; [2]Otto-von-Guericke Univ., Dept. of Neurol., Magdeburg, Germany; [3]Leibniz Inst. of Neurobio. (LIN), Magdeburg, Germany; [4]German Ctr. for Neurodegenerative Dis. (DZNE), Magdeburg, Germany; [5]Dept. of Educational Sci. and Psychology Cluster "Languages of Emotion", Freie Univ. Berlin, Berlin, Germany; [6]Dept. of Psychiatric Neurophysiology, Univ. Hosp. of Psychiatry,, Univ. of Bern, Bern, Switzerland; [7]Dept. of Neurol. and Neurolog. Sci., [8]Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP), Stanford Univ., Palo Alto, CA; [9]Dept. of Neurol., Otto-von-Guericke University,, Magdeburg, Germany; [10]Dept. of Neurol., Otto-von-Guericke Univ., Magdeburg, Germany; [11]Div. of Translational Med., [12]Div. of Translational Med. Albany,, Wadsworth Center, New York State Dept. of Hlth., Albany, NY;

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Abstracts: Melodies are regarded as pitch sequences integrated over time, thus pitch direction is a main feature of melodic information. Contemporary western music is usually organized as a melodic stream with rhythmic/ harmonic background accompaniment. In this case the melodic stream carries the main musical interest. Conversely, in chorales 4 simultaneous melodic streams are interwoven into a complex musical score. Pitch processing is felt to take place in the primary and secondary right hemispheric auditory cortices. However, the spatial distribution and temporal dynamics of melodic pitch processing within a complex musical piece, like a choral, are largely unknown. Utilizing the temporal and spectral resolution of direct cortical recordings from subdural electrodes (electrocorticography; ECoG), we examined cortical representations of pitch direction and melodies of complex auditory stimuli. Two subjects with right temporo-parietal grids listened to a piano version of a J. S. Bach choral (BWV 406). Each of the 4 melodic lines consisted of 28 single events, appearing in a regular beat (600 msec.). Melodies occur simultaneously but in different pitches (top, upper middle, lower middle, low). For each melodic stream we identified the pitch direction of each event and categorized these events in pitch direction 'up' and 'down'. We then computed the amplitude of the recorded local field potentials by bandpass filtering in the 'delta (1-4 Hz)', 'theta (4-8 Hz)', 'alpha (8-12 Hz)', 'beta (12-30 Hz)', and 'high gamma (80-150 Hz)' frequency range for categories 'up' and 'down' were computed for each melodic stream. An ANOVA was performed comparing 'up' and 'down' in each melodic stream. F values were corrected via permutation testing. We found a significant difference in theta power between 'up' and 'down' only in the top melodic stream (mean $F = 20$, $p < .0001$). We identified differences between 150 and 450 msec. after each event. Those differences were most prominent over auditory areas. Pitch direction 'up' showed a significant increase in theta power as compared to pitch direction 'down' ($p=.005$). There were no significant results for the other frequency bands. In summary, our ongoing study provides initial evidence that theta power tracks pitch direction in auditory areas and similar to modern Western music the top melodic stream in classical choral music captures the main musical attention.

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Poster

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Title: Hemodynamic phase synchronization analysis reveals functional network dynamics with novel and familiar musical sequences

Authors: *B. M. GREEN[1], E. GLEREAN[2], M. SAMS[2], J. P. RAUSCHECKER[1], I. P. JÄÄSKELÄINEN[2];
[1]Dept. of Neurosci., Georgetown Univ. Med. Ctr., Washington, DC; [2]Biomed. Engin. and Computat. Sci., Aalto Univ. Sch. of Sci., Espoo, Finland

Abstracts: We have previously demonstrated that dorsal auditory stream areas are implicated in the processing of novel musical sequences, with both dorsal and ventral stream areas associated with the processing of familiar ones (Green et al., SFN Annual Meeting, 2012; 2013). This segregation is consistent with the current understanding of the functions of the dorsal stream (Rauschecker, HearRes, 2011) and the close relationship between the auditory and motor systems in the perception of music and speech (Zatorre, et al, NRneuroscience, 2007). However, how these areas are interconnected is not well known. In the present study we used a phase synchronization analysis (http://becs.aalto.fi/bml/software.html) with functional magnetic imaging to investigate how novel and familiar musical sequences modulate inter-subject hemodynamic similarity and dynamic functional connectivity. We computed voxel-wise time-varying inter-subject phase-synchronization and time-varying functional connectivity to identify regions and putative connections influenced by i) novel sequences, ii) familiar sequences, iii) novel sequences preceded by short familiar sequences, iv) novel sequences preceded by long familiar sequences. Increased PS across subjects during novel sequences was seen in bilateral posterior superior temporal gyrus (pSTG), Heschl's gyrus, Planum Temporale (PT), insula and operculum; left hippocampus, parahippocampal gyrus, precuneus, superior parietal lobule (SPL), and supramarginal gyrus (SMG); posterior and anterior cingulate gyrus; frontal pole, primary visual cortex, and cerebellum. Familiar sequences showed a negative effect within the same areas. Novel sequences preceded by short familiar sequences resulted in increased inter-subject synchronization and functional connectivity between bilateral pSTG, HG, PT; left SMG, SPL, insula, operculum, and inferior frontal gyrus pars triangularis. Novel sequences preceded by long familiar sequences showed increased functional similarity between right caudate, putamen, nucleus accumbens, and cerebellum; and bilateral primary visual cortex. These results are

consistent with our previous findings showing increased activity in dorsal stream, basal ganglia, and premotor areas during the processing of novel sequences. We have hypothesized that the recruitment of these areas during the perception of sound sequences reflects a broadening of their function from simply motor areas to domain-general sequencing areas. The current results show an increase in synchronization and functional connectivity in these areas during novel sequence processing in support of this hypothesized function.

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Poster

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Title: Mismatch negativity (MMN) used for analyzing the recognition of sounds in professional musicians using hearing protectors

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Abstracts: Use of hearing protectors in professional musicians performing classical music is a controversial issue. These musicians often claim that hearing protectors impair their ability to perform or hamper the recognition of errors. In order to provide objective evidence, we compared tune recognition and elicited MMN's. We investigated 10 normal hearing professional musicians (age 25-38 yrs.) Stimuli were presented as series of in-tune synthetic C-major chords intermitted by either slightly or markedly mistuned chords (altered note G). Stimuli were presented in a free field mode, in each series we randomly presented 200 stimuli according to the oddball paradigm (in-tune to mistuned = 4:1). Intensities were 65 dB SPL, 45 dB SPL, and 65 dB SPL but wearing a custom-made hearing protector (attenuation 18-43 dB). The musicians were asked to push a button when recognizing a mistuned chord. The EEG was recorded from 31 electrodes with the BrainVision™ system. We analyzed the error recognition rates by counting hits, and comparing the components of the auditory evoked potentials (AEP), focusing on MMN.

In the slightly distorted condition, the musicians recognized at average correctly 88.6 % of the chords as being whether in-tune or out-of-tune, when using hearing protectors 86.2 %. The AEPs in these series had clearly distinguishable P50, N100, and P200 components, however, no MMN or P300 was observed. In the markedly distorted condition, 98.2 % of all chords were recognized correctly, regardless whether sound intensity was reduced or hearing protectors were worn, AEPs showed clearly elicited MMN and P300 components. The onset of the MMN was slightly delayed and area under the curve was reduced by 19.3 % or 21.4 %, respectively, when the presentation intensity was reduced or hearing protectors were worn. In the latter series, we observed also significantly increased amplitudes of the N2b component which may indicate unintended increased attention or overlap with MMN component. Our results show that the use of these hearing protectors worsens error recognition significantly but not more than lowering the sound intensity by 20 dB SPL. The MMN provides objective evidence for recognition of distinctions, however, subtle distinctions between stimuli not necessarily induce the MMN.

Disclosures: **E. Emmerich:** None. **J. Günther:** None. **A. Lehmenkuhler:** None. **F. Richter:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.27/CC33

Topic: D.02. Auditory

Support: Swartz Foundation Postdoc Grant

NIH grant K18 DC011602

Title: The disctinction between stimulus strength manipulations and volitional control in bistable perception

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Abstracts: Bistable perception has been widely studied in the visual system where ambiguity in sensory information coming from, for example, binocular, depth or motion cues leads to spontaneous shifts in perception. Indeed, Moreno-Bote et al. (2010) investigated rivalrous

dynamics induced by ambiguity in these sensory cues and generalized Levelt's proposition II (Levelt, 1968) that describes the effect of stimulus strength manipulations around equidominance: ``the mean dominance duration of the stronger percept changes more than that of the weaker percept''. Bistability also occurs in auditory streaming experiments for stimuli with alternating high- A and low-frequency tones B appearing in a repeating ABA- pattern. Pressnitzer and Hupé (2006) showed that auditory and visual bistability share the common traits of perceptual bistability using such ABA- sequences and visual motion plaids. In each modality there are alternations between a grouped percept (a galloping ABA-ABA- stream; coherent pattern motion) and a split percept (segregated streams A-A-A-A- and -B---B--; drifting transpent motion). They further investigated the effect of volitional control at equidominance and found that attending to one percept (grouped or split) reduced mean dominance durations of the unattended (weaker) percept. These findings are incompatible with the generalized Levelt's Proposition II if one assumes that volition increases the strength of the targeted percept. Starting with a canonical rivalry model we propose a new volitional mechanism with state-dependent inputs that resolves this apparent conflict by accounting for differences between direct input strength manipulations and top-down attention. We further present a new three-population model with periodic inputs for the auditory case to which our general result extends. Our modeling results can explain important differences between input strength and attention that generalize across sensory modalities.

Disclosures: J.A. Rankin: None. **J. Rinzel:** None.

Poster

622. Auditory Processing: Human Studies of Perception, Cognition, and Action

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 622.28/CC34

Topic: F.01. Human Cognition and Behavior

Title: Dichotic listening while walking: Age effects on auditory control and spatio-temporal parameters of gait

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Abstracts: Dual-task paradigms are experimental procedures to study the simultaneous performance of two different tasks. Recently, dual-tasks involving motor and cognitive execution

have been used to understand how cognitive demands, especially those related to attention, affect gait in elderly populations. However, cognitive tests so far employed rely upon simple tasks like counting or standardized tests lacking ecological validity. In this study, we use a task concomitant to walking that resembles a daily situation and assesses divided attention and hemispheric lateralization (i.e. dichotic listening (DL)). **Objectives.** a) Determine degree of cognitive-motor interference of the dual-task paradigm in young and older adults. b) Evaluate possible lateralized effects on gait. **Method.** 32 young adults (19 males; age: *M* =24.9 years) and 36 healthy elderly over 65 years of age (17 males; M =68.5 years,) participated in the study. All participants were right-handed. Audiometry test was applied. The Bergen DL test was used concomitant to walking. The dual-task situation followed the 3 standard conditions of DL: Non-forced, Forced-Right and Forced-Left. Correct answers, laterality indexes, and errors were registered. Gait was evaluated over ground with the GaitRITE system. Speed, step length, and swing were quantified. The mean (*M*), standard deviation (*SD*) and the coefficient of variation (CV) were used for group comparisons. Factorial analyses of variance with repeated measures were conducted. **Results** DL: Significant main effects of condition and ear were observed for correct responses. Significant interactions DL x group and DL x ear x group were found. Followed-up tests showed significant group differences on FR condition for both ears ($p < .01$) and on FL on left ear ($p < .0001$). Gait: Speed decreased in all DL conditions ($p<.0001$) from 1.2 m/s to 1.17 m/s for young and to 1.13 m/s for elderly. Step length decreased from 67.9±1.05 cm to 63.9±1.05 cm for young and 66.5±0.9cm to 62.6±1.06 cm for elderly. Also *SD* and CV for step length changed in all conditions. Analyses of step length on each separate foot showed significantly decrements all along conditions and interactions were found CVs x group. Further analyses revealed increased CV for the elderly group on the right foot during NF condition and on left foot on FR condition. Similar results were also found for swing in older adults. **Conclusion**. Execution of DL while walking impairs particularly the control of focus of auditory attention on left ear and increases gait variability asymmetrically in the elderly. Use of DL in dual-task methods is a convenient approach to understand modulation of attention during walking in older adults.

Disclosures: C. Rodriguez-Aranda: None. **M.M. Gorecka:** None. **K. Waterloo:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.01/CC35

Topic: D.03. Multisensory

Support: TUBITAK Grant 112C010

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Title: Audiovisual associations alter low-level motion perception

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Abstracts: Perception is affected by immediate pattern of sensory inputs and prior experiences acquired through multisensory associations. Recently, several studies reported that a quick association can be formed between directions of visual motion and static sounds with distinct frequencies (Hidaka et al., 2011; Teramoto et al., 2010). After the association is formed, sounds are able to change the perceived direction of visual motion. In these studies, the moving stimulus was localizable in space. Such stimuli can activate both low-level motion energy mechanisms (Anstis, 1980; Braddick, 1980) and high-level positional tracking mechanisms (Cavanagh, 1992; Lu & Sperling, 1995). To determine whether this rapid audiovisual association and its influence on visual motion perception are dependent on the involvement of higher-order attentive tracking mechanisms, we designed psychophysical experiments using regular and reverse-phi random dot motions that isolated low-level pre-attentive visual motion processing (Anstis, 1970). In our experiments, either regular random dot or reverse-phi random dot motion was used during each association phase lasting around 8 minutes. Human observers viewed these random dot displays moving either clockwise (CW) or counterclockwise (CCW) direction with 100% coherency. CW and CCW motion directions were synchronized with a static tone of either high (2000 Hz) or low (500 Hz) frequency. To assess the influence of audiovisual associations, before and after each association phase, we measured direction discrimination thresholds by varying the coherency of regular and reverse-phi motion presented with static tones. After the regular random dot association phase, the static tones significantly biased the perceived direction of both regular and reverse-phi motion in favor of the exposed audiovisual association. However, reverse-phi motion direction and static tone association only changed the perceived direction of reverse-phi motion. Overall, our results show that an association between the directions of low-level visual motion and static sounds can be formed and this association alters the subsequent perception of low-level visual motion. Based on these findings, we conclude that multisensory associations are not restricted to high-level attention based motion system and early level visual motion processing has some role.

Disclosures: H. Kafaligonul: None. **C. Oluk:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.02/CC36

Topic: D.03. Multisensory

Title: The development of audiovisual crossmodal attentional cueing

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Abstracts: Many studies have examined the interaction between attention and multisensory integration in adults; however, little is known about how this complex relationship develops during childhood. In the current study, we investigated the development of multisensory attention using a crossmodal orthogonal attentional cueing paradigm. Adult studies of this task have shown that a stimulus in one modality can shift the spatial attention of another modality. For example, an auditory cue presented in the left periphery will speed the detection of a visual target presented on the left but slow the detection of the target on the right. We first adapted and verified an audiovisual child-themed version of the attentional cuing task. Visual targets were jellyfish presented in one of the four corners of a monitor. Exogenous auditory cues were buzzes which were presented from the left, right, or both speakers. Endogenous cues were the words “left,” “right,” or “jelly” spoken in a cartoon voice presented from both speakers. Cues could be valid (correctly identifying the location of the upcoming target), invalid (incorrectly identifying the location of an upcoming target), or neutral (giving no spatial information). Additionally, the stimulus onset asynchrony (SOA) was varied between the cues and targets in 150ms increments from 150ms to 900s for the endogenous and in 200ms increments from 200ms to 1200ms for the endogenous. The age range included in the study spanned from 4-17 years old for the child participants and from 18-40 years old for the adult participants. Preliminary results showed that global response times (RT’s) decreased with age until approximately age ten. All age ranges showed the expected “U-Shaped” relationship between SOA and RT, indicating an optimal delay between cue and target. Interestingly, the youngest children exhibited the greatest improvements in RT’s for valid cues as compared to invalid cues. In fact, the maximum difference (largest difference in RT between valid and invalid cues regardless of SOA) decreased with age until approximately age 15. Additionally, developmental changes in crossmodal attentional cueing did not differ by cue type (i.e. endogenous versus exogenous). The preliminary results of this study indicate that in children as young as four, stimuli presented in one modality may be able to shift the spatial attention of another modality, indicating an early development of this aspect of

multisensory attention. Given the complex relationship between attention and multisensory integration, many additional studies are needed to further characterize the developmental trajectory of other aspects of multisensory attention.

Disclosures: K. Schmittgen: None. **C. Vilter:** None. **L.D. Kwakye:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.03/DD1

Topic: D.03. Multisensory

Title: The neural correlates of multisensory temporal processing in an audiovisual steady-state electroencephalogram task

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Abstracts: Many studies have investigated the behavioral consequences and neural correlates of temporal multisensory processing in humans as well as animal models. Collectively, these studies demonstrate the necessity of the precise temporal alignment of multisensory stimuli for multisensory integration. These studies have also revealed a fundamental network for assessing the relative timing of audiovisual stimuli that includes the visual cortex, auditory cortex, and superior temporal sulcus. One caveat is that almost all of these studies have utilized discreet auditory and visual stimuli. Many naturalistic audiovisual objects produce somewhat continuous stimuli in the auditory and visual modalities that feature regularly repeating elements. The synchrony of these stimuli may not be judged by differences in onset as is the case with discrete audiovisual stimuli. Instead, individuals may rely on the overall temporal pattern produced in each modality. In the current study, we took advantage of the ability of sensory neurons to entrain to regularly repeating elements of external stimuli to produce steady state visual and auditory potentials. Visual stimuli consisted of a white square that flickered at a rate of either 10Hz or 30Hz. Auditory stimuli consisted of amplitude-modulated white noise at frequencies between 4Hz and 48Hz. The audiovisual stimuli were synchronous (both visual and auditory frequencies at 10Hz or 30Hz), or asynchronous, with the auditory being faster or slower (20% or 60% increase/decrease). Auditory and visual-only stimuli were presented with static stimuli in the other modality. Participants were asked to report whether the visual and auditory stimuli were synchronous while continuous electroencephalograms were recorded using a 64-channel

ActiChamp system. Preliminary behavioral data indicate that participants were generally more accurate at identifying the synchrony of audiovisual stimuli centered at 10Hz as compared to 30Hz. Additionally, participants were more accurate at correctly identifying asynchronous audiovisual presentations when the auditory stimulus was presented at a slower rate than the visual. Future analyses will investigate changes in oscillatory amplitude and coherence for trials in which participants reported the audiovisual stimuli to be synchronous as compared to asynchronous. Additionally, we will investigate individual differences in the mechanism for multisensory pattern discrimination that may be applicable to the study of individuals with known alterations of multisensory temporal processing (e.g. in autism spectrum disorders or in musicians).

Disclosures: **S. Russ:** None. **E. Aligbe:** None. **L.D. Kwakye:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.04/DD2

Topic: D.03. Multisensory

Title: Auditory-visual interactions in brain and behaviour using modulated stimuli

Authors: Q. VUONG, M. LAING, *A. REES;
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Abstracts: Outside the laboratory natural acoustic events are often accompanied by information from other modalities such as vision or touch. Compared with our understanding of individual senses we know much less about the mechanisms and brain areas that underpin such interactions. To address this, we developed a paradigm that uses sinusoidally amplitude-modulated tones and visual shapes (a cuboid which expanded and contracted at the modulation rate) that enabled us to manipulate the extent to which listeners fuse the auditory and visual components of our stimuli. Listeners heard pairs of modulated tones (1.5 s duration, 250 Hz carrier frequency, 2 Hz modulation rate) and judged whether their modulation depth (range 20-52% in 8% steps) was the same or different (Audio Only). On bimodal trials, we paired the shape (modulation depth 70%) with the tone with the greatest (AV >) or least (AV <) modulation depth. We found that the modulated shape influenced how listeners perceived the modulation depth of the tones. We found that this auditory-visual interaction was nearly eliminated when the tone and shape differed in their modulation frequency (e.g., 1 Hz shape modulation and 2 Hz tone modulation).

Furthermore the listeners report that when the modulation frequency in both modalities was the same, they perceive the shape to be the source of the tone (i.e., bimodal integration) whereas this merged percept is strongly weakened or non-existent when the frequency did not match. In a preliminary functional magnetic resonance imaging study using sparse imaging data acquisition, we found that superior-temporal, intraparietal and frontal areas responded more when the tone and shape had the same rather than different modulation frequencies. Our findings are important because they provide evidence (1) that auditory-visual interactions observed with speech and faces generalise to simpler stimuli occurring over a similar time scale to speech; and (2) that temporal information, such as modulation frequency, is important for fusing sight and sound in both brain and behaviour.

Disclosures: Q. Vuong: None. **M. Laing:** None. **A. Rees:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

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Topic: D.03. Multisensory

Support: European Community's Seventh Framework Programme FP7/2007-2013 Grant PITN-GA-2008-290011

Title: Association between concurrently recorded fNIRS and EEG signals for low-level visual and auditory stimuli

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Abstracts: Unlike other functional neuroimaging techniques functional near-infrared spectroscopy (fNIRS) does not interfere with concurrently recorded electroencephalogram (EEG) data, enabling the investigation of neuronal activity and hemodynamic responses without artifact contamination. Here we tested the feasibility of fNIRS to image sensory modality-specific cortical activation and explored the relationship between hemoglobin concentration changes and sensory-specific neuronal activity. fNIRS was recorded over visual and auditory brain areas with an 8 (sources) by 12 (detectors) system and combined with concurrent EEG

recordings from 96 scalp sites. Visual and auditory stimuli were presented in a block design for the complementary analysis of evoked EEG activity. Data from 24 normal hearing participants were collected. The fNIRS results showed a clear distinction between visual and auditory sensory modalities. Specifically, the results demonstrated significant area specificity, that is, maximal fNIRS responses in visual and auditory areas for the visual and auditory stimuli respectively, and significant stimulus selectivity, whereby the visual and auditory areas responded mainly toward their respective stimuli. In addition, a stimulus-dependent modulation of the fNIRS signal was observed in visual areas, confirming that fNIRS is sensitive to physical properties of visual stimuli. To investigate the relationship between fNIRS and EEG, features from the EEG were correlated with fNIRS data. This included sensory-related features from visual-evoked potentials (VEPs) and auditory-evoked potentials (AEPs), and components extracted from principal component analysis performed on time-frequency transformed evoked EEG signals. Both block-by-block correlation and across-subject correlation analyses were performed. Significant correlations between simultaneously recorded VEPs and deoxygenated hemoglobin (DxyHb) concentration, and between AEPs and oxygenated hemoglobin (OxyHb) concentration were found. In sum, these results suggest good sensitivity of fNIRS to basic sensory processing in both the visual and the auditory domain. The data provide further evidence for the neurovascular coupling between hemoglobin concentration changes and neuronal activities.

Disclosures: **L. Chen:** None. **P. Sandmann:** None. **J.D. Thorne:** None. **S. Debener:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

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Title: Phase tracking of visual speech in the human auditory cortex revealed by intracranial EEG

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Abstracts: People comprehend speech better when they watch the face of their speaker. Previous studies showed that speech-reading activates hierarchically early auditory cortex with short latencies, consistent with a feed-forward influence of visual cortex on auditory processing. Recent intracortical recordings in monkeys established that the phase of ongoing oscillations in sensory cortices determines their excitability, and that cross-modal sensory stimulation resets the phase of these ongoing oscillations. Based on these findings, it has been proposed that visual speech cues facilitate the comprehension of auditory speech by resetting the phase of oscillations in auditory cortex, thus placing it in a state of optimal excitability (Schroeder et al., Trends Cogn Sci 2008). To test this hypothesis, we recorded the responses of human auditory cortex to continuous auditory and visual speech using stereo-EEG electrodes in patients with drug-resistant epilepsy considered for surgery. Electrodes were localized by co-registering pre- and post-implantation high-resolution MRI and CT scans. The video stimuli consisted of 8 short stories (8-12 s) told by 2 narrators whose whole face was visible. The last word of each story was cut off, and patients had to decide whether a written word provided an adequate ending. Each video was shown 8 times in each of 3 modalities: audiovisual, auditory and visual. Time-frequency analysis of the intracranial EEG was performed on bipolar channels using wavelet transform. We focused on changes in high-gamma power (HGP; 70-170 Hz), thought to index neuronal firing, and in inter-trial coherence (ITC) in lower frequencies (1-50 Hz). Auditory-responsive channels were identified by their expected strong HGP and ITC increases to auditory speech signals as well as their anatomical location in or bordering the transverse temporal gyrus. Phase tracking of auditory speech was significant for all frequencies examined, most prominently in the delta and theta bands (1-8 Hz). Crucially, we found that the auditory cortex also displayed significant phase tracking of visual-only speech, in the delta to alpha bands (1-13 Hz). Additionally, we observed small HGP increases in response to visual speech signals in those sites where ITC increases were the strongest. Our results support the notion that visual speech cues are able to impact the early cortical processing of auditory speech through phase reset of ongoing cortical oscillations. More generally, they refine our understanding of the neural underpinnings of multisensory integration in the human brain.

Disclosures: P. Megevand: None. **D.M. Groppe:** None. **A.D. Mehta:** None. **C.E. Schroeder:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

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Topic: D.03. Multisensory

Support: NIH R01NS065395

Title: Electrocorticographic responses to synchronous and asynchronous audiovisual speech

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Abstracts: Humans readily integrate auditory and visual speech information (talker's voice and face) even when there is a substantial temporal offset (asynchrony) between the modalities. Tolerance for asynchrony is asymmetric: we more readily integrate speech in which the talker's voice begins after the talker's face moves than when the talker's face moves after the voice is heard is (Conrey and Pisoni, 2006). This asymmetry corresponds to the physical properties of naturalistic speech: talkers prepare their facial musculature before emitting a vocalization, resulting in a natural visual-before-auditory order. For optimal multisensory integration, the brain should only integrate auditory and visual speech information according to the likelihood that they have the same cause (Magnotti, Ma, and Beauchamp 2013). Activity in the human left superior temporal gyrus and sulcus (STG/S) is a neural measure of audiovisual integration during speech perception (Nath and Beauchamp, 2011). Therefore, we hypothesized that neural responses in the STG/S to asynchronous speech should reflect the propensity to integrate observed behaviorally. We examined 264 subdural electrodes implanted in 3 patients with intractable epilepsy and selected 24 electrodes that were nearest the STS, lying directly over the sulcus or closely adjacent to it. The stimuli consisted of 4 audiovisual words spoken by a native English talker (Conrey and Pisoni, 2004). For each word, the auditory and visual components were either unchanged from the original recording (Sync) or manipulated so that the auditory component either preceded the video component by 300 ms (A before V, A−>V) or followed it by 300 ms (V−>A). Subject accurately judged synchronous stimuli to be synchronous (mean synchrony reports 97% ± 3% SEM). Consistent with the behavioral literature, shifting the auditory component of the stimulus in different directions produced asymmetric results. A−>V stimuli were rarely perceived as synchronous (4% ± 3%), but V−>A stimuli often were (74% ± 17%). Next, we examined the response to these stimuli in the STG/S electrodes. A time-frequency analysis was used to compare responses in the high gamma band (70-110 Hz) to the words (analysis window from auditory onset to one second after onset) to baseline (one second before onset to half-second before onset.) The mean response to Sync was 57% ± 7% (SEM)

above baseline. The response to A−>V was significantly less than the response to V−>A (35% ± 7% vs. 48% ± 6%, t = 2.7, p = 0.01); the effect was observed in 18 of 24 electrodes. These results suggest a link between STS/G activity and synchrony perception, as observed with fMRI (Stevenson, James, et al., 2009).

Disclosures: D. Mohanty: None. **M. Ozker:** None. **D. Yoshor:** None. **M.S. Beauchamp:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

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Hearing Health Foundation

Hoover Fund

Title: Processing of audiovisual speech and non-speech stimuli within and beyond human auditory cortex: An intracranial electrophysiology study

Authors: *A. E. RHONE[1], B. MCMURRAY[2], K. V. NOURSKI[1], H. OYA[1], H. KAWASAKI[1], M. A. HOWARD, III[1];
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Abstracts: Audiovisual (AV) speech provides neurophysiological facilitation and behavioral advantages relative to audio-alone (A) speech. A large network of cortical areas has been implicated in AV processing; the visual-alone (V) responsiveness and speech-selectivity of these areas was the focus of this study. Areas of interest included Heschl's gyrus (HG), posterolateral superior temporal gyrus (PLST), middle temporal gyrus (MTG), inferior frontal gyrus (IFG), and precentral gyrus. The high temporal and spatial resolution of electrocorticography (ECoG) allowed us to test whether visual information activates auditory areas prior to the onset of speech, and whether responses to auditory stimuli were differentially modulated by visual speech vs. nonspeech content. We measured ECoG activity in neurosurgical patients undergoing chronic

monitoring for medically refractory epilepsy. Stimuli were A or V speech syllable /da/ combined with nonspeech stimuli /da/-shaped noise or gurning. Unimodal A and V /da/ were also presented. Subjects monitored for A, V, or AV /tu/ (button press response; all subjects > 95% correct; ECoG responses to response trials not analyzed). Trials were averaged to the onset of the talker's static face, approximately 700 ms prior to visible articulation. All sound tokens had the same onset (1168 ms). Event related band power in the high gamma band (70 -150 Hz) was measured in two time windows, 300 ms following the onset of mouth motion (PreA) and 400 ms following the onset of sound (PostA). Unimodal V stimuli activated PLST, IFG, MTG, and precentral gyrus; no high gamma activation was observed on HG. Comparisons of responses by modality and speech content revealed increased activity for visual speech stimuli in PreA time window for MTG only. Responses on IFG were not modulated by speech content in either window. In the PostA window, PLST, MTG and precentral gyrus showed increase for V speech relative to V nonspeech. On PLST, speech elicited larger high gamma power than nonspeech in both modalities, and showed the highest response when both modalities contained speech. A large network of cortical structures was activated by V alone stimuli; however, primary auditory area HG was not. Speech vs. nonspeech comparisons revealed distinct patterns of activation in different cortical regions. These effects occurred prior to acoustic onset in select sites on MTG. Following acoustic onset, the earliest auditory cortical area that was modulated by speech content was PLST, with no evidence for sensitivity to visual speech content on HG. Responses in other areas (IFG, precentral) were consistent with their hypothesized involvement in multisensory perception.

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Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

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Topic: D.03. Multisensory

Support: the Army Research Office

Title: The modality-specific spatial attention control between vision and audition under same stimulation background: A simultaneous EEG-fMRI study

Authors: *W. WANG, S. VISWANATHAN, S. T. GRAFTON;
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Abstracts: In natural settings, people are flooded by sensory inputs from multiple, concurrently occurring events in the immediate environment. These events can each occur at different spatial locations, generating information that is potentially detectable by distinct sensory modalities. People are able to bias their attention to both the sensory modality as well as the spatial location to localize events. In the current study, we identified brain states associated with sustained attending under modality- and location-dependent demands as well as switches between these states. Participants monitored a spatially localized stream of naturalistic stimuli for infrequent target objects in the presence of a complex multisensory (auditory-visual) background. We used distinctive images/sounds of common objects and domestic animals. Stimulus duration was 250ms for both the visual and auditory stimuli. Brain responses induced were measured simultaneously using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). Although behavioral accuracies were matched between modalities, the response times (RT) to detect auditory targets (~1000ms) showed a latency compared to visual targets (~600ms). The fMRI BOLD activity lateralized to sensory cortices. Monitoring the auditory stream produced a larger activation in superior temporal gyrus; while monitoring the visual stream evoked a larger activation in the occipital and superior parietal and inferior temporal gyrus, establishing that the task successfully modulated the allocation of attention. Unlike the block-level fMRI analysis, the EEG data were analyzed at the trial-level. The event related potentials (ERP) showed the earliest and the most significant difference between monitoring vision or audition, occurring at ~100ms on Pz electrode (bottom-up) and during 250ms to 600ms on Fz electrode (top-down) after stimulus onset. In the "late" phase the auditory ERPs over central-parietal cortex were significantly more negative than the corresponding visual ERPs (around ~600ms) ipsilateral with stimuli. Strikingly, in the electrodes over the frontal cortex from ~350ms to 600ms, the visual ERPs were more negative than the corresponding auditory ERPs when stimuli were displayed to the right side of fixation. The former could be related to suppress contralateral irrelevant auditory stimuli, and the latter may reflect a spatial-specific inhibition to irrelevant auditory stream. Our results identify timing and brain systems involved in both modality- and location-dependent mechanisms engaged in monitoring sensory stimuli coming from separable visual and auditory events.

Disclosures: W. Wang: None. **S.T. Grafton:** None. **S. Viswanathan:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.10/DD8

Topic: D.03. Multisensory

Title: The effect of early visual deprivation on the neural bases of multisensory processing

Authors: *M. J. GUERREIRO, L. PUTZAR, B. ROEDER;
Biol. Psychology and Neuropsychology, Univ. of Hamburg, Hamburg, Germany

Abstracts: Visual deprivation has long been known to affect processing in the remaining sensory modalities (e.g., Bavelier & Neville, 2002). Only recently, however, the recovery of visual and multisensory processing after visual restoration has been started to be investigated in humans, revealing deficits in higher-order visual and multisensory processes (e.g., Putzar et al., 2007, 2010) despite intact lower-level multisensory processing skills (Putzar et al., 2012). The goal of the present study was to investigate the effect of early visual deprivation on the neural bases of multisensory processing. A group of 9 cataract-reversal individuals (aged 18-51 years, M = 34, 7 female) and 9 normally sighted controls (aged 19-56 years, M = 35, 8 women) were tested in a series of 1-back tasks involving the presentation of visual, auditory and audiovisual speech stimuli, while underdoing functional magnetic resonance imaging. We performed a region of interest analysis, focusing on visual (i.e., BA 17 and BA 18/19), auditory (i.e., BA 41/42) and multisensory areas (BA 22). The results revealed no evidence for multisensory integration in visual areas. Interestingly, however, although there was no difference in visual cortex activation between cataract patients and sighted controls during unisensory stimulation, there were differences between groups during multisensory stimulation, such that activation in visual areas during audiovisual stimulation was lower in cataract patients than in sighted controls. In addition, there was evidence for multisensory integration in auditory and multisensory areas in sighted controls, but not in cataract patients. The present results suggest reduced multisensory integration in auditory and multisensory areas in cataract patients. In addition, the present results reveal reduced visual cortex activation during multisensory processing in cataract patients, despite equivalent levels of visual cortex activation during unisensory processing, suggesting that during multisensory processing, cataract patients process bimodal audiovisual stimuli as if they were unimodal (auditory) stimuli.

Disclosures: **M.J. Guerreiro:** None. **L. Putzar:** None. **B. Roeder:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.11/DD9

Topic: D.03. Multisensory

Support: NSF Grant SBE-1041725

Title: Enhanced functional connectivity between V1 and multimodal cortex in congenitally, profoundly deaf adults revealed by time-lagged cross-correlation of the "fast" optical signal

Authors: *J. L. SEYMOUR[1], A. CHIARELLI[1], M. FABIANI[1], G. GRATTON[1], M. A. FLETCHER[1], K. LOW[1], E. MACLIN[1], K. MATHEWSON[2], M. W. G. DYE[1];
[1]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstracts: Research has shown that early deafness positively impacts peripheral visual attention. The useful field of view (UFOV) task has previously been used to show that deaf subjects have lower presentation duration thresholds for determining the location of a peripheral target among distractors while performing a concurrent central discrimination task. These appear to be effects of deafness and not using sign language, as deaf non-signers show the effect and hearing signers do not. Here we combined structural MR with diffusive optical imaging techniques to explore how deafness alters functional connectivity between visual and auditory cortices. By combining functional optical data with co-registered structural MR data, it is possible to examine functional connectivity between cortical regions with excellent spatial and temporal resolution. This permits both functional connectivity analyses alongside an analysis of processing pathways with excellent temporal precision (c. 39 Hz). Results show that activity in BA17, primary visual cortex, predicts activity 50 ms later in the posterior STG of the right hemisphere in congenitally, profoundly deaf adults but not in hearing adults. Whilst enhanced recruitment of the posterior STG in the right hemisphere of deaf adults is well known, this is the first demonstration of enhanced functional connectivity between that region and primary visual cortex. The short time lag suggests either (a) direct functional connectivity between these regions rather than a mediated connection, or (b) a common precursor region that routes neural activity to V1 more rapidly than to the posterior STG (possibly the thalamus). Table of Contents

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Disclosures: J.L. Seymour: None. **A. Chiarelli:** None. **M. Fabiani:** None. **G. Gratton:** None. **M.A. Fletcher:** None. **K. Low:** None. **E. Maclin:** None. **K. Mathewson:** None. **M.W.G. Dye:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.12/DD10

Topic: D.03. Multisensory

Support: CIHR

NSERC

Title: Cortical coupling between occipital cortex and intraparietal sulcus predicts auditory abilities in early blind individuals

Authors: *P. VOSS, R. J. ZATORRE;
Cognitive Neurosci. Unit, Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstracts: Early blind individuals have been shown to possess thicker occipital cortex compared to sighted ones. This increase in occipital cortical thickness is also predictive of performance on several auditory discrimination tasks in the early blind, which suggests that the increase in thickness reflects crossmodal plasticity mechanisms. In light of this atypical relationship between occipital thickness and auditory function, we sought to investigate here the covariation of occipital cortical morphology in occipital areas with that of all other areas across the cortical surface, to assess whether the anatomical coupling with the occipital cortex is altered following visual deprivation. Measures of anatomical coupling were obtained by performing vertex-wise covariance analyses on cortical thickness measures derived from standard magnetic resonance imaging (3T) anatomical T1 images. We observed a reduction in anatomical coupling in a group of early blind individuals (n=14) relative to sighted controls (n=19) between the right occipital cortex and several frontal areas of the visual dorsal stream involved in oculomotor control. In a separate analysis, we asked the question whether better auditory abilities in the early blind could be linked to greater cross-cortical covariance between the occipital cortex and task-relevant brain areas. We show that the performance of the early blind in a transposed melody discrimination task was strongly predicted by the strength of the cortical coupling between the occipital cortex and intraparietal sulcus, a region for which cortical thickness in the sighted was previously shown to predict performance in the same task. These findings therefore constitute the first evidence linking altered anatomical coupling to early sensory deprivation, and also provide compelling evidence that investigating anatomical coupling can be an effective way to characterize individual (behavioral) differences and crossmodal plasticity.

Disclosures: **P. Voss:** None. **R.J. Zatorre:** None.

Poster

623. Multisensory: Cross-Modal Processing in Humans, Audio-Visual

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 623.13/DD11

Topic: D.03. Multisensory

Support: NIDCD Intramural Research Program

Title: An fmri study of a visual-auditory paired associate task

Authors: ***P. T. CORBITT**[1], J. F. SMITH[2], B. HORWITZ[2];
[1]NIH, MD; [2]Brain Imaging & Modeling Section, NIDCD-NIH, Bethesda, MD

Abstracts: Visual-auditory associations play an important role in many human cognitive functions, including naming and reading. To understand the neural basis for such associations, we performed an fMRI visual-auditory paired associate task. We collected fMRI data from eleven subjects. An initial abstract visual stimulus had an abstract visual (VV) or auditory (VA) target paired associate presented 9 s after the cue stimulus. The data were acquired after the subjects were trained and tested three times over a period of 4 weeks; the paired associates were well-learned. Three scanning sessions consisted of twenty trials equally divided between VA and VV (50% matching trials). A slow event-related paradigm was used to separate the BOLD signals associated with different task components. This design used a TR of 1.8 s, and each trial was 23.4 s long. Our finite impulse response (FIR) model used 11 time bins each 1.8 s in width. The FIR time bins can be considered as belonging to the cue, delay, and response periods. A prior MEG study (Pillai et al. 2013), using a similar design, detected power differences when comparing the VA to VV task in the right superior temporal gyrus (STG) during the equivalent cue/delay period. MEG, compared to fMRI, lacks the spatial resolution for pinpointing regions involved in the paired associate task. Due to the exploratory nature of this investigation, we used a voxelwise significance criterion of $p < 0.001$ (uncorrected) in the group level analysis. Comparing VA to baseline, we detected BOLD responses in the right STG during the late cue and early delay periods. We then directly contrasted the VA and VV conditions. Two regions that showed showed larger relative BOLD responses in VA compared to VV were putative Brodmann areas 44 and 45 in the cue, delay, and response periods. During the delay period, the relative BOLD response was greater in the insula, parietal lobe, and thalamus for VA compared to VV, and in the fusiform gyrus, hippocampus, and occipital lobe for VV compared to VA. In

the response period, relative BOLD signals for VA compared to VV were greater in the frontal lobe, whereas they were greater in the occipital lobe for VV relative to VA. Our results suggests that inferior frontal areas are differentially more involved in VA compared to VV. Moreover, consistent with the prior MEG study, we found activation in right STG occurred before onset of the auditory paired associate. Based on these and other results from the literature, we suggest that prefrontal areas are playing a central role in the retrieval of the paired associate of the initially presented stimulus.

Disclosures: **P.T. Corbitt:** None. **J.F. Smith:** None. **B. Horwitz:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 624.01/DD12

Topic: D.04. Vision

Support: McDonnell award to EMB

Title: An unbiased approach to analyzing the effect of numerosity and other visual features of dot arrays on neural and behavioral variables

Authors: ***N. K. DEWIND**, J. PARK, G. K. ADAMS, M. L. PLATT, E. M. BRANNON; Ctr. for Cognitive Neurosci., Duke Univ., Durham, NC

Abstracts: The approximate number system (ANS) subserves estimation of the number of items in a set. Typically, ANS function is assessed using dot arrays that differ in numerosity. Controlling these numerical stimuli is a well-known and difficult problem. In particular, when numerosity is varied, other visual stimulus features also necessarily vary. Here we present a novel mathematical description of dot array stimuli that specifies the three degrees of freedom available to researchers when designing these stimuli. As a result, dot array stimuli can be described by their position within a three-dimensional “stimulus space”. These dimensions are numerosity and two novel terms that describe the size and spacing of the items in the array. Numerosity, item surface area, item perimeter, total surface area, total perimeter, area encompassing the entire stimulus, array density, overall scaling, and foreground to background ratio can all be described algebraically as linear combinations of these three dimensions. Thus, this new stimulus space provides a very rich, but also parsimonious description of dot array stimuli. Because the three degrees of freedom are independent, we can design a generalized

linear model to estimate the effect of each stimulus dimension on a dependent variable. By virtue of the algebraic relationships between the three dimensions of the stimulus space and the other visual features, we can use the coefficients of the linear model to estimate which visual features of a stimulus drive changes in the dependent variable. A critical advantage of this approach over previous paradigms for controlling visual features is that the linear models are as sensitive to changes caused by other visual features as to those caused by numerosity, allowing an unbiased estimation of the parameters that affect the dependent variable. The most important advantage of the stimulus space, however, is its versatility. With minor changes we have adapted our linear models to estimate the effect of numerosity and other visual features on behavior in a discrimination task, scalp voltage from electroencephalograms, blood-oxygen-level-dependent signals from functional magnetic resonance imaging, and firing rate from single neurons. This approach allows us to demonstrate that human numerical discrimination is primarily reliant on number with some biasing effects of other features; that EEG event-related potentials triggered by dot arrays show sensitivity to number at very early time windows; and that single neurons in monkey intraparietal sulcus encode many stimulus features across a population distinguished by its heterogeneity.

Disclosures: **N.K. Dewind:** None. **J. Park:** None. **G.K. Adams:** None. **E.M. Brannon:** None. **M.L. Platt:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 624.02/DD13

Topic: D.04. Vision

Title: Dissecting the neural network of duration perception with fMRI

Authors: ***M. CAI**, D. M. EAGLEMAN;
Neurosci., Baylor Col. of Med., Houston, TX

Abstracts: Neural models of time perception often assume a dedicated amodal brain system for encoding time. In contrast, some recent models suggest that time may be intrinsically encoded in sensory cortices that also code for other aspects of stimuli. To date, few studies have attempted to decode temporal features of sensory stimuli with fMRI. To this end, we examined both intrinsic and dedicated models of time perception by evaluating whether we could decode subjective duration from activity in both sensory and other brain regions. A block-design

functional localizer contrasting a duration discrimination task against a color discrimination task identified higher activity for duration discrimination in early visual cortex, right inferior parietal sulcus, and right middle frontal gyrus. Participants next performed a duration bisection task in which they judged if the duration of a stimulus was longer or shorter than a remembered, intermediate duration. The stimuli were concentric sinusoidal gratings that were either static or expanding at different speeds (2, 6, or 18 Hz)_a manipulation that is known to bias subjective duration. We then used partial least square regression to decode subjective duration based on the pattern of local hemodynamic response amplitudes. We find that all three regions contribute to encoding subjective duration, allowing the decoding of subjective duration simply from observing neural activity.

Disclosures: M. Cai: None. **D.M. Eagleman:** None.

Poster

624. Visual Cognition: Decision-Making

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Program#/Poster: 624.03/DD14

Topic: D.04. Vision

Support: NWO Vidi Grant 452.08.008

NWO Brain & Cognition grant 433.09.233

Title: Overlapping topographic representations of numerosity and item size in the human parietal cortex

Authors: *B. M. HARVEY[1], A. FRACASSO[2], N. PETRIDOU[3], S. O. DUMOULIN[2]; [1]Exptl. Psychology, Helmholtz Inst., [2]Helmholtz Institute, Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands; [3]Radiology, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstracts: Introduction: Perception of numerosity, the set size of a group of visually presented items, is affected by the sizes of these items, particularly during development. We recently demonstrated neural population tuning for numerosity within a topographic map in human parietal cortex (Harvey et al, 2013). But we also showed that this numerosity tuning is affected by item size and speculated an interaction between the representation of numerosity and item size. Here we ask whether item size is also topographically represented in the human parietal cortex, and whether the representations of these two quantities coincide on the cortical surface?

Methods: Using high-field fMRI (7T), we acquired 2D-EPI volumes with 1.8mm isotropic resolution while showing visual stimuli varying systematically either in numerosity or the size of a single item. We developed a custom-built model-based analysis that captures numerosity or item size tuning following a population receptive field design (Dumoulin and Wandell, 2008). Using a forward model, we predict the fMRI response amplitude at each time point based on a model of neural response selectivity for numerosity or item size. This model describes population neural response selectivity at each recording site as a Gaussian function in logarithmic space, with parameters describing the preferred numerosity/item size and tuning width. Results: We describe neural populations tuned to small item sizes (up to 1.5°) in human posterior parietal cortex. These neural populations are organized topographically, forming a map of item sizes where preferred size item increases from posterior to anterior cortex. The location of this item size map coincides with that of reported numerosity maps, but its direction of response preference change differs. As with population numerosity tuning, tuning width increases with preferred item size. Conclusion: The topographic organization of both item size and numerosity tuning demonstrates that this fine scale organization of quantities is not limited to numerosity. As with numerosity tuning, the cortical organization of item size tuning shares similarities to the maps found in the primary sensory cortices. Overlapping representations of these two quantities may explain why their perceptions interact. These overlapping and interacting representations of item size and numerosity may arise because these quantities are estimated by similar neural mechanisms.

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Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 624.04/DD15

Topic: D.04. Vision

Support: INSERM

CEA

Collège de France

Université Paris Sud

Fondation Bettencourt

Title: High-level expertise for mathematical concepts recycles lateral occipito-temporal and parietal regions for number processing

Authors: *S. DEHAENE[1,2], M. AMALRIC[1], M. PIAZZA[1], B. THIRION[3];
[1]Cognitive Neuroimaging Unit, INSERM-CEA, GIF/YVETTE, France; [2]Collège de France, Paris, France; [3]Parietal team, INRIA, Gif sur Yvette, France

Abstracts: How does the brain represent and manipulate advanced mathematical concepts? Mathematics can be considered as a cultural construction process whose foundations lie in basic intuitions of space, time and number provided by innate "core knowledge" systems. Many studies have dissected the role of the intraparietal sulcus in basic number sense, its cross-talk with nearby areas for spatial coding, and its foundational role in school-based arithmetic. We hypothesized that mathematical expertise for abstract non-numerical concepts would rely on a "neuronal recycling" of this evolutionarily ancient system. We also predicted that the acquisition of expertise for mathematical expressions, much like the acquisition of literacy, would lead to a reorganization of ventral visual cortex, perhaps encroaching and expanding upon the recently discovered "number form area". High-resolution functional magnetic resonance images (fMRI) were acquired in 15 professional mathematicians and 15 researchers in humanities without mathematical training. Subjects listened to 90 high-level mathematical statements and non-mathematical sentences and decided whether they were true, false or meaningless. Statements, devoid of any numbers, covered algebra, analysis, topology and geometry (for instance "any square matrix is equivalent to a permutation matrix" is a false algebraic statement). Control sentences concerned general knowledge. In distinct blocks, we measured visual responses to mathematical expressions, words, numbers, faces, houses, tools, bodies and checkers, and also localized calculation and language areas. All meaningful mathematical statements, in mathematicians only, mainly evoked activations around the intraparietal sulcus, at a site also activated during numerical calculation. On the contrary, meaningful non-mathematical sentences only evoked classical language-related activations in the superior temporal sulcus. Listening to mathematical statements also induced top-down activations in the left fusiform gyrus, at a site activated by mathematical expressions and whose activation was highly enhanced for mathematicians compared to non-mathematicians. We also observed enhanced activation of the "visual number form area" in the lateral inferior temporal gyrus, a region previously reported only using intracranial electrophysiological recordings. Thus, the acquisition of mathematical expertise transforms both parietal and ventral visual areas to support abstract domain-general mathematical processing and the manipulation of mathematical expressions.

Disclosures: **S. Dehaene:** None. **M. Amalric:** None. **B. Thirion:** None. **M. Piazza:** None.

Poster

624. Visual Cognition: Decision-Making

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Topic: D.04. Vision

Support: NIH Grant EY16187

NIH Grant DA029330

Title: Monkey math beyond addition

Authors: *M. S. LIVINGSTONE[1], D. LEE[2];
[1]Harvard Med. Sch., BOSTON, MA; [2]Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstracts: We previously showed that monkeys can combine pairs of symbolically represented magnitudes to arrive at a value approximately equal to their sum. This result addresses the question of whether magnitudes are represented internally as a compressed scale, or as a linear scale with signal-dependent noise. Because, if the internal scale is compressed, we would expect supra-additive behavior, since the internal representation of, say, 3 would be more than half the internal representation of 6, leading the animal to estimate 3+3 as larger than 6. Therefore, our failure to find such supra-additive behavior contradicts the idea of a compressed scale. However, it is possible that our monkeys showed behaviors consistent with addition, because that is what we taught them. Namely, in our previous study, the animal always received reward proportional to the sum of the quantities represented by two symbols, so even if they had a compressed internal representation, their natural tendency to combine quantities supra-additively might have been overcome, allowing them to estimate the sum of two quantities more accurately. Instead, if we had rewarded them according to the the product of the quantities represented by two symbols, they might have learned to estimate the product, rather than, the sum of two quantities more easily. To test this, we presented symbol-literate monkeys with a singleton symbol on one side of a touchscreen and two symbols inside an oval on the other side, and rewarded them with the value represented by whichever side they chose: the singleton value, or the product of the two symbols presented in the oval. Results so far indicate that the monkeys value the two symbols additively, not multiplicatively, despite being rewarded multiplicatively. This argues against the idea of compressive internal representation of quantities.

Disclosures: M.S. Livingstone: None. **D. Lee:** None.

Poster

624. Visual Cognition: Decision-Making

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Topic: D.04. Vision

Support: Deutsche Forschungsgemeinschaft (DFG) Grant KN959/2 to André Knops in support of Curren Katz

Title: Changes in cerebellar-parietal connectivity contribute to differential anterior parietal activity for complex and simple mental calculation

Authors: *C. KATZ, A. KNOPS;
Humboldt Univ. of Berlin, Berlin, Germany

Abstracts: BACKGROUND: Animals are thought to have an innate "number sense" which supports numerical cognition (Dehaene and Cohen 1995). In addition to well-described parietal regions, numerical tasks have also been found to activate the cerebellum and it was suggested as an addition to the "triple-code model" of numerical cognition (Arsalidou and Taylor 2011). Despite extensive evidence of the cerebellum's non-motor cognitive role (Buckner 2013), little is known about its role in number processing. It remains to be seen how cerebellar and parietal regions are involved in functional networks underlying symbolic calculation. To this end, we examined the contribution of inferior parietal (IPL) and superior cerebellar regions to whole-brain activity in symbolic multiplication. METHODS: fMRI data (TR=2s, 3x3x3 mm) was collected from 17 right-handed adults performing simple (e.g. 6x3) and complex (e.g. 12x6) symbolic multiplication and analyzed using standard GLM analysis for functional localization and Psycho-physiological interactions (PPI) for connectivity. PPI seed regions were defined from cytoarchitectonically distinct IPL and superior cerebellar subdivisions. RESULTS: Consistent with previous research, complex multiplication (complex > simple) was associated with bilateral parietal as well as superior cerebellar activation. For cerebellar seed regions, connectivity was decreased to bilateral parietal regions in complex multiplication (cluster FDR $p < .05$). Bilateral cerebellar lobule VI hemisphere (h) and vermis (v) regions all increased connectivity to overlapping L anterior parietal clusters. Bilateral VI v regions increased connectivity to overlapping R anterior IPL clusters with R VI v connecting to a larger intra-parietal sulcus/SPL cluster. For IPL seed regions, connectivity to R middle frontal and R superior parietal (SPL) regions was increased in complex multiplication. Thus, while IPL regions increased coupling with R SPL and R middle frontal regions during complex calculation, cerebellum VI regions decoupled with larger bilateral parietal regions. Parts of these decoupled regions showed positive activation in GLM analysis. CONCLUSION: Changes in cerebellar-parietal connectivity

contribute to complexity related anterior parietal activity. Given the role of lobule VI in salience (Habas et al. 2009) and spatial attention shifts (Townsend et al. 1999); functional cerebellar-parietal decoupling in complex calculation might relate to increased spatial and attentional demands.

Disclosures: C. Katz: None. **A. Knops:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 624.07/DD18

Topic: D.04. Vision

Support: DFG KN 959/2-1

Title: Limitations of abstract number perception: An fMRI study on the temporal integration of auditory and visual number information

Authors: *S. CAVDAROGLU, A. KNOPS;
Humboldt Univ. zu Berlin, Berlin, Germany

Abstracts: The ability to perceive and manipulate numbers is thought to exist even in human infants and other animals which led to the idea of 'abstract number sense'. This notion refers to an innate mechanism that enables us to comprehend numerical values independent of the modality and format they are presented in. Previous studies point to the intraparietal suclus (IPS) as a key region for an abstract number representation. Yet, the modality independence of abtract number sense has not been studied exstensively in humans. The current study aimed at investigating the neural underpinnings of auditory and visual number perception. We presented participants with sequences of auditory (beeps) or visual (flicker) numerosities (5,7,11,16) while measuring brain activity with a 3T MR-system. Our slow event-related design allowed us to obtain numerosity-related BOLD signals that were not contaminated with interfering signal from adjacent stimuli or response-related processes. To maintain and control participants' attention in interspersed catch trials participants were required to numerically compare two subsequent numerosities. This way, we were able to keep subjects attentive during the experiment, assess the performance of subjects in comprehending numbers and disentangle number processing from comparison, which were mostly intermingled in previous studies. Localizer tasks were applied to independently define parietal sub-regions of interest (hAIP, hVIP, hLIP) for multivariate

analyses. Contrary to previous findings we observed no activation of IPS upon mere number perception. Rather, IPS was only activated during comparison trials. To further investigate the idea that IPS represents numbers independent of modality, we used multivariate pattern analyses to distinguish between numerosities in auditory and visual modalities, separately. In no-response trials classifier performance in IPS was at chance level, even when adopting a grid search approach for classifier parameter optimization or different classification algorithms (i.e. support vector regression). In contrast, we were able to classify numerosity during response trials in a task-based ROI in bilateral parietal cortex in both modalities. Our results suggest that integrating over time non-symbolic auditory or visual number information does not automatically activate the IPS in the absence of response preparation. We conclude that number-dependent IPS activity may be limited to task-regimes involving response preparation and/or numerical information that do not require integration over time.

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Poster

624. Visual Cognition: Decision-Making

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Topic: D.04. Vision

Support: NIH R01-MH092345

James S. McDonnell Foundation grant

Title: Value-based attentional capture induces automatic normalization during binary-choice decisions

Authors: *S. ITTHIPURIPAT, J. T. SERENCES;
UCSD, San Diego, CA

Abstracts: Traditional decision-making theories postulate that choice behavior is unaffected by the value associated with irrelevant stimuli (i.e., distractors), and it is therefore context-independent. However, cross-species studies have shown that choice behavior is context-dependent, as when more than two options are available the value of the lowest-valued option will influence decisions between the highest-valued and the second-highest-valued options. Recently, a computational model based on the divisive normalization of neural responses during decision stages has been proposed to account for this phenomenon. However, the model does not account for the possibility that contextual normalization effects during decision-making could be a result of automatic and early value-driven attentional capture. To test this account, we developed a novel probabilistic binary-choice paradigm, where human observers freely choose between one of two targets that were presented in the left and the right lower visual fields. Simultaneously, a single distractor that could never be chosen was presented between the two targets. All three stimuli were rendered in a unique color. The colors assigned to targets and to the distractor varied across trials. Over blocks of trials, the value associated with targets rendered in each color was also varied. We found that the value associated with the color stimulus presented at the distractor location, as was learned when it was a rewarded target in previous trials, reduced decision biases between the two target stimuli and also increased decision times. These data suggest that binary-choice decisions are context-dependent and that the recently proposed value normalization model for decision-making can be at least partially accounted for by value-based attentional capture.

Disclosures: S. Itthipuripat: None. **J.T. Serences:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 624.09/DD20

Topic: D.04. Vision

Support: ERP-Fellowship of the German National Academic Foundation (Studienstiftung des deutschen Volkes)

Title: Neurophysiologic correlates of the speed-accuracy trade-off in humans: Setting thresholds in time and in amplitude

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[1]Biomed. Engin., Neural Systems Lab, City Col. of New York, New York, NY; [2]Trinity Col. Inst. of Neurosci., Trinity Col. Dublin, Dublin, Ireland

Abstracts: The trade-off between speed and accuracy in decision-making has been studied through human and animal behavior as well as electrophysiology. The prevalent view from mathematical psychology has long been that speed-emphasis in decision-making tasks leads to decreased decision thresholds, but there has been little direct neurophysiological evidence for this. In the present study, behavioral and electrophysiological changes induced by the emphasis of speed or accuracy are studied in humans. We employ a novel two alternative forced-choice contrast discrimination paradigm designed to give independent, neural read-outs of the sensory evidence via steady state visually evoked potentials (SSVEP), a centro-parietal potential (CPP) previously connected to decision formation, and lateralized readiness potentials (LRP) quantifying motor preparation. Within this experimental set-up we evoke behavioral changes through the implementation of time-dependent reward functions under three distinct conditions 1) An accuracy condition in which subjects earned response time-independent rewards for correct responses and lost points for incorrect answers. 2) A deadline speed emphasis condition in which subjects received constant rewards up to a short temporal deadline and finally 3) A 'faster the better' speed-emphasis condition in which rewards decreased linearly over time. We reasoned that the two distinct types of speed emphasis (deadline vs. 'faster the better') may be accounted for differently in the decision process, with thresholds more likely being set in time in the deadline condition than in amplitude. Behavioral results indicate that subjects show similar mean reaction times in both speed conditions, but distributions are comparatively symmetric and narrower in the deadline condition while they were right-skewed in the condition of decreasing-rewards. The centro-parietal positivity, which has previously been linked to decision formation shows increased amplitudes for decisions made under either kind of speed pressure, while correlates of motor preparation, and sensory evidence traces remain unaffected by experimental condition. Recent intracranial recordings in frontal eye field showing a similar tendency of increased firing rates at a higher rate when fast reactions are stressed over accuracy. These findings contradict the prevailing assumption that decision thresholds are lowered if decisions need to be made quickly, pointing towards potential changes in overall gain in the accumulation process.

Disclosures: **N.A. Steinemann:** None. **R.G. O'Connell:** None. **S.P. Kelly:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

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Support: FRM (Fondation pour la Recherche Médicale, France) to MV and RQ

FP6 (EU VIth Frame Program) & ANR (Agence National de la Recherche Scientifique) project eraNET-NEURON BEYONDVIS to Dr. AV-C

IFRAD foundation for providing equipment funds.

Title: Rhythmic 30 Hz non invasive brain stimulation patterns entrain high-beta cortical oscillations relevant for conscious visual perception in the human right Frontal Eye Fields

Authors: *A. VALERO CABRE[1,2], R. QUENTIN[3], M. VERNET[3];
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Abstracts: Non invasive rhythmic neurostimulation techniques such as Transcranial Magnetic Stimulation (TMS) have emerged as unique approaches to explore causal relations between brain rhythms and cognitive processes in the human brain. Prior studies from our group (Chanes et al. J Neurosci 2013) have shown that 30 Hz TMS bursts delivered pre-target onset to the right frontal eye field (FEF), an area involved in spatial attentional orienting, increased perceptual visual sensitivity for near threshold lateralized visual targets. We here aim to further elucidate the neurophysiological mechanisms subtending such effects, and by using combined TMS-EEG recordings, we tested the hypothesis that rhythmic TMS patterns might increase the activity of neural oscillators and/or align their phases, hence entraining rhythmic activity at this frequency band, causally responsible for the ensuing visual performance modulations. To that end, a group of healthy human participants performed a visual task in which they were requested to signal whether or not they had seen a near-threshold target and if yes, if it had appeared to the left or right of a fixation cross. Prior to target onset, either frequency-specific sham or real 4 pulse 30 Hz TMS bursts, or random patterns of equal duration and pulse number were delivered to the right FEF. Behavioral results confirmed a previously reported facilitatory effect of the former, but not the latter activity patterns, increasing conscious visual detection performance. Moreover, during the stimulation, coupled TMS-EEG analysis revealed an entrainment of 30 Hz oscillatory activity, with increases of power and phase alignment, building-up during the course of the burst. In contrast, random TMS induced non frequency-selective power increases in several oscillation bands, un-modulated across the patterns. Our results provide preliminary evidence of local high-beta oscillation entrainment under rhythmic TMS delivered at this same frequency, as causally

related to conscious visual performance ameliorations, whereas, characterize random patterns as the injection of white noise with broadband oscillatory influences yielding no influences on visual perception.

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Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

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Program#/Poster: 624.11/DD22

Topic: D.04. Vision

Title: The evaluation of visual temporal resolution in the behaving mouse

Authors: ***S. YOKOTA**[1,2], S. IKUTA[2], J. MITA[2], T. SHINGO[2], D. UCHIDA[2,3], Y. NOMURA[2,3], T. ARIMURA[2,3], A. AMANO[4], K. SHIMONOMURA[5], C. KOIKE[2,3,6]; [1]Res. Organization of Sci. and Technol., [2]Lab. for Systems Neurosci. and Developmental Biology, Grad. Sch. of Life Sci., [3]Lab. for Systems Neurosci. and Developmental Biology, Sch. of Pharmaceut. Sci., [4]Dept. of Bioinformatics, Col. of Life Sci., [5]Dept. of Robotics, Col. of Sci. and Engin., Ritsumeikan Univ., Shiga, Japan; [6]PRESTO, Japan Sci. & Technol. Agency, Saitama, Japan

Abstracts: A fundamental principle of vertebrate visual system relies on the functional separation of neuronal signaling into the ON and OFF pathways that generate visual contrast. These two pathways originate in depolarizing ON bipolar cells and hyperpolarizing OFF bipolar cells. Critical flicker frequency (CFF) is one of the useful ways to evaluate visual temporal resolution based on functional separation of ON and OFF visual pathways. CFF threshold is defined as the frequency at which a flickering light is indistinguishable from a steady one. CFF is also informative for clinical diagnostic criteria of retinal disease. Temporal vision is related to the ON and OFF pathways which are involved in parallel processing in the visual system. However, there is little understanding of the molecular mechanisms about temporal vision we perceive. Genetic manipulation of mouse model is an effective and widely used strategy for investigating how specific molecule influence physiological processes. Methods that analyze temporal vision of mouse model have provided fundamental information about molecular mechanism of human retinal function and visual perception. The aim of the present study was to establish a method to evaluate visual temporal perception of mice. C57BL/6 mice responded to two stimuli by making nose poke toward a light emitting diode (LED) screen that was equipped

with a touchscreen attachment for detecting responses. Illumination was provided by green (508 nm) LED. Mice were trained to distinguish stationary illumination (correct response) from flickering illumination (incorrect response). We continued to the task until mice could discriminate the differences between correct and incorrect choice. Once mice recognized stationary illumination as correct stimuli, we changed temporal frequency until mice could discriminate flickering stimulation as stationary stimulation, which defined as CFF. We established the method to evaluate visual temporal perception of mice to characterize critical temporal frequencies of ON and OFF pathway-mediated vision. This behavioral assay of mouse model could contribute to development of diagnosis of human retinal disease.

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Poster

624. Visual Cognition: Decision-Making

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Support: NIH Grant EY001778

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Title: Perceptual decision related activity in the ON and OFF pathways of the lateral geniculate nucleus (LGN)

Authors: *Y. JIANG[1], D. YAMPOLSKY[2], G. PURUSHOTHAMAN[2], V. CASAGRANDE[1,2,3]; [1]Psychology, [2]Cell and Developmental Biol., [3]Ophthalmology and Visual Sci., Vanderbilt Univ., Nashville, TN

Abstracts: Previous psychophysical evidence indicated a clear functional asymmetry between the perception of light (i.e. contrast increment) and dark (i.e. contrast decrement) (Blackwell 1946; Short 1966; Krauskopf 1980; Bowen et al. 1989, 1992; Chan and Tyler 1992; Kontsevich

and Tyler 1999; but see Komban et al. 2011). Physiological recordings in the early visual system also revealed various asymmetries between the ON and OFF pathways (for example see Chichilnisky and Kalmar 2002; Armstrong-Gold and Rieke 2003; Zaghloul et al. 2003; Jin et al. 2008, 2011; Yeh et al. 2009; Pandarinath et al. 2010; Xing et al. 2010; Kremkow et al. 2014). How do ON and OFF neurons contribute to the perception of light and dark? How can the physiological asymmetry of the ON/OFF pathways be linked to the psychophysical asymmetry of light and dark perception? To answer these questions, we recorded from LGN Parvocellular (P) ON-Center and OFF-Center neurons in awake monkeys while they passively viewed (i.e. simple fixation) and actively detected (i.e. two-alternative, forced-choice detection) contrast increment and decrement changes. We found that: 1) OFF neurons were more sensitive in detecting contrast decrements than ON neurons were in detecting contrast increments during both tasks, 2) OFF neurons had lower choice probabilities than ON neurons during detection, 3) Interneuronal correlations of ON-ON, ON-OFF, and OFF-OFF neural pairs were not significantly different from 0 during detection, but they were all significantly above 0 during fixation, 4) OFF neurons also differed from ON neurons in their spontaneous firing rate, peak response amplitude, and response transiency, and these differences were consistent across the detection and the fixation tasks, and finally 5) OFF neurons had lower Fano factors (variance/mean) than ON neurons during detection, whereas ON neurons had lower Fano factors than OFF neurons during fixation. Across ON and OFF cell classes, we observed a breakdown of the previously established correlation between the neurometric sensitivity of a neuron and its choice probability (Britten et al. 1996; Purushothaman and Bradley 2005; Gu et al. 2007; Price and Born 2010; Liu et al. 2013), as in our experiments the neurons that exhibited lower thresholds, higher firing rates, and overall more robust contrast responses (i.e. OFF neurons) were less correlated with the behavioral choice of the animal. A causal, bottom-up pooling model linking fluctuations in LGN ON and OFF neural responses to the monkey's choices in the absence of interneuronal correlations accounted for these results.

Disclosures: Y. Jiang: None. **D. Yampolsky:** None. **G. Purushothaman:** None. **V. Casagrande:** None.

Poster

624. Visual Cognition: Decision-Making

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Topic: D.04. Vision

Support: F32-EY019851

R01-EY08890

P30-EY08126

P30-HD015052

E. Bronson Ingram Chair in Neuroscience

Title: Neurons that fire together select together: Reduced variability of visual search target selection times for simultaneously as compared to sequentially recorded neurons

Authors: *W. ZINKE, R. P. HEITZ, B. A. PURCELL, J. D. SCHALL;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstracts: Previous research by our and other laboratories has described the neural process of saccade target selection during visual search in the frontal eye field (FEF), posterior parietal cortex, and superior colliculus. After an initial visual response, neurons distinguish whether the target or a distractor is in the receptive field at a time that can be determined statistically, referred to as the target selection time (TST). If this selection process is a result of local interactions between neurons, then a reasonable implication would be that the TST of neurons recorded simultaneously will differ less than the TST of neurons recorded sequentially (hence impossible to interact). Multiple neurons were recorded simultaneously from FEF in two macaque monkeys (Macaca radiata) that earned fluid rewards for shifting their gaze to the singleton target during visual search tasks. We compared the absolute differences of TSTs (dTST) for pairs of neurons recorded simultaneously with a null distribution of dTST for all pairs recorded sequentially. Less variability in dTST, as indicated by a reduced mean dTST, was observed among simultaneously recorded neurons (mean dTST: 38.3 ms) as compared to sequentially recorded neurons (mean dTST: 50.2 ms). This result adds to our previous findings of neural coordination of saccade target selection in the FEF circuit (Cohen et al. 2010 J Neurosci). The reduced variance of dTST for simultaneously recorded neurons implies that accumulator models that pool data from neurons across sessions (Purcell et al. 2010 Psych Rev; Purcell et al. 2012 J Neurosci) overestimate the variability of the input. Instead, a group of neurons participating in the decision process select the target in a much more synchronized manner. Further work is needed to understand how this coordination happens between brain regions that exhibit measurably different TST values within search conditions (Buschman & Miller 2007 Nature; Cohen et al. 2007 J Neurophysiol; Zhou & Desimone 2011 Neuron; Katsuki & Constantinidis 2012 Nature Neuro; Purcell et al. 2012 J Neurophysiol).

Disclosures: **W. Zinke:** None. **R.P. Heitz:** None. **B.A. Purcell:** None. **J.D. Schall:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

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Program#/Poster: 624.14/DD25

Topic: D.04. Vision

Title: Psychophysical measurement of visual behavior in the ferret

Authors: S. U. NUMMELA[1], J. LEDLEY[2], *K. J. NIELSEN[1];
[1]Neurosci., [2]Johns Hopkins Univ., Baltimore, MD

Abstracts: The domestic ferret (Mustela putorius furo) is one of the main animal models for research of brain development, in particular the development of visual cortex. Due to a short gestation period, much of neocortical development occurs postnatally, and is thus accessible to experimental investigation and manipulation. The functional properties of neurons in primary visual cortex of the adult ferret are well characterized, including detailed measurements of orientation, direction, and spatial frequency tuning. The developmental time courses of these tuning properties have also been described. However, despite our knowledge of these neuronal response properties, relatively little is known about the ferret's visual behavioral capabilities. We set out to systematically measure ferret visual capabilities by training 5 adult female ferrets on a variety of visual tasks, including the detection and discrimination of sine-wave gratings. All ferrets trained on two-alternative forced choice visual detection and discrimination tasks learned these tasks with an average performance of 90% correct on the easiest conditions. We used both tasks to measure contrast sensitivities over a range of spatial frequencies. Psychometric curves could reliably be determined for all ferrets. Our data show highest contrast sensitivities for spatial frequencies between 0.18 and 0.25 cycles per degree of visual arc (cpd). Contrast sensitivity rapidly declined for spatial frequencies larger than 0.7 cpd, with no ferrets capable of reaching discrimination thresholds at even the highest contrasts for stimuli with spatial frequencies larger than 1.4 cpd. These measurements match published data of spatial frequency tuning in the central visual field locations in ferret primary visual area 17. These data report optimal spatial frequencies up to 0.5 cpd, with a geometric mean of 0.25 cpd (Baker et al, Eur J Neurosci 10, 1998). Our results demonstrate the feasibility of visual psychophysical measurements in the ferret. They also provide a benchmark for comparison for visual studies of developing ferrets.

Disclosures: S.U. Nummela: None. **K.J. Nielsen:** None. **J. Ledley:** None.

Poster

624. Visual Cognition: Decision-Making

Location: Halls A-C

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Topic: D.04. Vision

Support: McNair Foundation

Title: Temporal evolution of information in neural networks with feedback

Authors: A. GIAHI SARAVANI[1,3], *X. S. PITKOW[2,3];
[2]Neurosci., [1]Baylor Col. of Med., Houston, TX; [3]Electrical and Computer Engin., Rice Univ., Houston, TX

Abstracts: Recurrent neural networks are pivotal for information processing in the brain. Here we analyze how the information content of a neural population is altered by dynamic feedback of a stimulus estimate decoded from the network activity. We quantify information using the Fisher information which is directly related to discriminability between stimuli. We find that the temporal evolution of the Fisher information in the model with feedback is bounded by the Fisher information in a network of pure integrators. The available information in the feedback model saturates with a time constant and to a final level both determined by the match between the estimator weights and the feedback weights. This network then encodes signals specifically from either the beginning or the end of the stimulus presentation, depending on this match. These results offer an interpretation of recent experimental measurements of psychophysical kernels. These kernels indicate that earlier stimuli have a stronger influence on perceptual discrimination than later stimuli. In the context of our model, that finding constrains the nature of the feedback signals that are capable of generating such an effect. We also discuss consequences of this network model for choice correlations, which measure how individual neuronal responses relate to perceptual estimates.

Disclosures: A. Giahi Saravani: None. **X.S. Pitkow:** None.

Poster

624. Visual Cognition: Decision-Making

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Topic: D.04. Vision

Support: WT 086120/Z/08/Z

Title: Non-invasive measurement of population dynamics during computation of choice

Authors: *M. C. KLEIN-FLÜGGE[1], G. R. BARNES[1], M. W. WOOLRICH[2], T. E. J. BEHRENS[1];
[1]UCL, London, United Kingdom; [2]OHBA and FMRIB, Oxford Univ., Oxford, United Kingdom

Abstracts: One key advance in the way neuroscientists study neuronal computations was caused by a recent shift from studying individual neuronal responses towards studying neuronal population dynamics. Changes in firing rates of a neural population can be thought of as a trajectory through a high-dimensional state space, and this state space can often be reduced to a few meaningful dimensions that carry most of the variance for a particular task. For example, when monkeys are asked to decide about the motion direction or dominant colour of a noisy random-dot stimulus, the state space contains three orthogonal axes relating to the two inputs, colour and motion, and the choice (Mante et al., Nature, 2013). Movement along trajectories in state space can reveal the neural computations this population of neurons is performing. We developed a novel approach for similarly examining ongoing computations of neural populations using non-invasive neuroimaging in humans. The task was an adapted version of Mante et al., where decisions were made about the colour or motion of a random-dot stimulus. We used magnetoencephalography (MEG) which records the magnetic fields produced by the summed neural activity of large populations of neurons with a high temporal precision. Because these measurements do not allow us to identify the dimensions of the relevant subspace, we selectively manipulated the neural populations of interest using repetition suppression. Each random dot stimulus ('test stimulus') was preceded by another random dot stimulus ('adaptation stimulus'), which determined the population of neurons that would be 'primed' and thus the feature of the choice - colour, motion, or response - that was selectively suppressed in the neuronal population. By comparing situations where adaptation and test stimuli were the same versus different in either their inputs or their responses, we could then examine rapid transitions in neuronal representations during the decision computation. This revealed that responses in premotor cortex first suppressed to the relevant input and only later to the response. Plotting this input suppression directly against the response suppression revealed a 'suppression' trajectory that first deflected in the direction ('axis') of decision input and later response. This trajectory closely resembled the state-space population trajectories obtained from direct recordings in the frontal eye fields of monkeys performing the same task (Mante et al.). Thus, our combined repetition suppression and MEG approach allows a time-resolved study of the computations of populations of neurons using non-invasive measurements obtained in humans.

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Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

Support: DFG grant (Kr 1392/11-1)

PIFI-PROMEP-VIEP-Catedra-Moshinsky (EM)

PROMEP-VIEP-BUAP-PTC-352 MEBI-EDH-13-I (IMB)

Title: Suppression of enhanced physiological tremor via stochastic noise: Initial observations

Authors: C. TRENADO[1], F. AMTAGE[1], F. HUETHE[1], J. SCHULTE-MÖNTING[2], I. MENDEZ-BALBUENA[3], M.-C. HEPP-REYMOND[5], *E. MANJARREZ[4], R. KRISTEVA[1]; [1]Dept. of Neurol., [2]Inst. for Med. Biometry and Med. Informatics, Univ. of Freiburg, Freiburg, Germany; [3]Facultad de Psicologia, [4]Inst. of Physiol., Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [5]Inst. of Neuroinformatics, Univ. of Zürich and ETH Zürich, Zürich, Switzerland

Abstracts: Enhanced physiological tremor is a disabling condition elicited by a central tremor generator in combination with a finger mechanical component of the spinal stretch reflex. Under the light of the hypothesis “boosting the strength of the peripheral input pushes the tremor-related spinal and cortical systems closer to anti-phase firing and hence reduces tremor (Baker, personal communication)”, the present study aims at investigating whether Gaussian stochastic noise enables reduction of enhanced physiological tremor accompanied with performance improvement during a visuomotor task. Specifically, eight subjects with enhanced physiological tremor performed a visuomotor task requiring to compensate isometrically with the right index finger a static force generated by a manipulandum on which Gaussian noise (3-35 Hz) was applied. The finger position was displayed on-line on a monitor as a small white dot which the subjects had to maintain in the center of a green circle defined as the reference. EMG from the active hand muscles and finger position were recorded. The performance was measured by the mean absolute deviation of the white dot from the zero position. The tremor was identified by the acceleration in the frequency range 7-12 Hz. Two different conditions were compared: with and without optimum noise. We found that application of optimum noise reduces tremor (accelerometric amplitude and EMG activity) and improved the behavioral performance as

reflected by the improved mean absolute deviation from zero, although no significant correlation between both variables was found. Thus, we provide the first evidence of a significant reduction of enhanced physiological tremor in the human sensorimotor system due to application of external stochastic noise.

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Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

Support: HHMI

NIH Grant EY003878

Title: Relationship between neural latencies in area MT and behaviora latencies of smooth pursuit eye movements in monkeys

Authors: *J. LEE, J. YANG, S. G. LISBERGER;
Neurobio., Duke Med. Ctr., Durham, NC

Abstracts: Sensory motor reaction times of human and non-human primates depend on many factors, including internal factors such as arousal and attention and external factors such as the intensity of the sensory stimulus. In smooth pursuit eye movements, they tend to have longer latencies when the contrast of the visual target is low versus high. Our goal was to ask whether the effect of stimulus contrast on response latency depends on shifts in neural response latencies versus effects of contrast on response amplitudes. We have recorded the responses of MT neurons and smooth pursuit eye movements for moving targets with different forms and contrasts. We used three different forms of tracking targets: random dot patches, sine wave gratings, and bi-kinetic plaids, each at three levels of contrast (100, 36, and 12%). We used objective procedures to estimate the effect of stimulus form and contrast on neural and behavioral latencies, in independent sessions. For each neuron, we then plotted the latency of the neural response for each stimulus as a function of the latency of pursuit for the same stimulus

and performed regression analysis. For 31 MT neurons, the regression slopes ranged from -1 to 3 and formed a distribution that was centered at 0.92. On average, the behavioral latency for different stimulus forms and contrasts had almost a one-to-one relationship with the neural latency. We conclude that behavioral latency follows sensory neural latency loyally, at least for changes in the form and contrast of visual motion targets.

Disclosures: **J. Lee:** None. **J. Yang:** None. **S.G. Lisberger:** None.

Poster

625. Multisensory and Motor Interactions

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Program#/Poster: 625.03/DD30

Topic: D.05. Visual Sensory-motor Processing

Support: NINDS Intramural Research Program

Title: Whole brain cellular resolution mapping of circuits underlying goal-directed behavior in zebrafish

Authors: *C. A. HARRIS, K. L. BRIGGMAN;
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Abstracts: Goal-directed behaviors such as predation depend on multiple interacting brain regions and involve several neural processes including perception, recognition, motivation, action selection and motor control. To better understand the neural basis of goal-directed behavior we are developing a set of techniques that integrate information about behavior, neural circuit dynamics and synaptic connectivity from a whole-brain preparation. We have developed a virtual reality assay to elicit prey-tracking behavior in larval zebrafish. We carefully restrained one week-old zebrafish by the neck in agarose, leaving the eyes and tail free to move. The semi-restrained fish were presented with a prey-like target moving on the left or right side of the visual field. Prey-tracking behavior in the form of target-directed tail movements and a characteristic pattern of sustained eye convergence was detected in real-time and used to update the movement of the virtual prey in a way that simulates approach. Using this closed-loop behavioral assay we were able to evoke sequences of up to five target-directed swims with sustained eye convergence. We are now beginning to monitor the brain during these episodes of predation by means of light-sheet microscopy in fish expressing calcium indicators in the brain. In preliminary experiments we have imaged the brains of agarose-embedded live fish at cellular resolution and

with a frame rate of about 1 Hz. Once we have gathered detailed information about the activity and location of neurons whose activity is associated with prey-tracking we will use electron microscopy (EM) to study the synaptic connectivity of those neurons. We are particularly interested to see how known features of the behavior, such as the fine tuning of motor output to target trajectory, is reflected in the underlying circuit structure. In combination with modern methods for EM serial sectioning and dense circuit reconstruction, this work will help answer fundamental questions about the neural circuit basis of goal-directed behavior in the vertebrate brain.

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Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

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funds from McGovern Institute for Brain Research at MIT

Title: Behavioral training for oculomotor tasks in head-free non-human primates

Authors: *J. WANG[1], S. W. EGGER[1], E. D. REMINGTON[1], M. JAZAYERI[1,2];
[1]McGovern Inst. for Brain Res., [2]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstracts: The oculomotor system of the non-human primate (NHP) has advanced from a premier motor system to one of the most informative platforms for studying the neural correlates of cognitive and sensorimotor processing. In a typical experiment, an animal might be trained to respond through specific eye movements. Conventionally, to measure eye movements, the animal's head is fixed via a mechanical coupling between the chair and a headpost that is surgically attached to the skull. This procedure imposes several limitations. First, the post-surgical healing time required for the proper adherence of the headpost to the bone delays the start of training. Second, maintenance of the implant is time consuming for the experimenter. Third, the process of restraint can be stressful and may result in a negative association with the primate chair, which would further delay progress. Fourth, acclimatization of an untrained animal to head restraint could compromise the stability and reliability of the headpost. Here, we

report that NHPs can be straightforwardly trained to maintain approximate head position and perform sophisticated oculomotor tasks involving gaze control, smooth pursuit and targeted saccades without head fixation. Our approach is based on adapting a commercially available video eye tracker (Eyelink 1000, Desktop Mount, SR Research Ltd.), which is commonly used for monitoring eye movements in human psychophysics. Using simple forms of instrumental conditioning, we have successfully trained three head-free NHPs to perform multiple tasks that require eye fixation and targeted saccadic eye movements. Our analyses show that head-free NHPs can be trained to maintain fixation on a central spot within a 4-degree eye fixation window throughout several-second long trails, and that they can perform tasks that require the animal to make a saccade either reactively or proactively to visually or memory guided locations. We show that the accuracy and precision of head-free and head-fixed eye tracking is comparable and no additional training is required during the transfer from the head-free to the head-fixed system (for subsequent electrophysiology). This procedure expedites behavioral training, reduces post-surgery care, and facilitates the acclimation of headpost-mediated stabilization, which in turn increases stability and longevity of the headpost. This training method is advantageous both as an intermediate step to head fixation for traditional electrophysiology, and also for studies using tethered and wireless multi-electrode array systems. We aim to adapt this approach to train animals to perform sensorimotor tasks in their home cages.

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Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

Support: DFG GI 305/4-1

Boehringer Ingelheim Fonds PhD Fellowship

Title: Action value modulates mirror neuron activity

Authors: *D. ARNSTEIN, J. K. POMPER, P. THIER;
Hertie Inst. For Clin. Brain Res., Tuebingen, Germany

Abstracts: Mirror neurons discharge while a monkey performs a goal-directed action and when the monkey observes another human or monkey performing a similar action. Since their discovery, mirror neurons have been suggested to play a role in social interactions; for instance, helping observers choose appropriate responses to others' actions. Supporting a role of mirror neurons in response selection, a recent study found that mirror neuron activity depends on the ability of the observer to interfere with the observed action (Caggiano et al., 2009, Science, Vol. 324). In order to select an appropriate response to someone else's action, it is necessary to consider its egocentric value; namely, whether it is god, bad, or neutral for the observer. Experiments have shown that mirror neuron activity is modulated by the size of the reward associated with the observed action (Caggiano et al., 2012, PNAS, Vol. 109). However, manipulating reward size not only modifies value but also attention and arousal. In order to disambiguate potential influences of value and attention, we performed experiments in which we associated observed actions not only with rewards (=positive value) but also with punishments (=negative value) of varying size because both large rewards and large punishments capture attention (Leathers & Olson, 2012, Science, Vol. 338). We recorded from mirror neurons in premotor area F5 of two monkeys while they observed filmed actions. After observing the action, the monkey received a large reward (~0.6 mL water), small reward (~0.2 mL water), small punishment (0.5 s unrewarded eccentric fixation), or large punishment (5 s unrewarded eccentric fixation). At the beginning of each trial, a visual cue indicated which outcome the monkey would receive after the action. Many mirror neurons responded to the action differently depending on which outcome was expected. To rule out that this modulation was driven by differences in attention or alertness, we compared the spike rate associated with small vs. large outcomes. The large rewards and punishments raised the monkey's heart rate in comparison to the small rewards and punishments, but this effect was not reflected in mirror neuron activity. On the contrary, mirror neuron spike rates were a monotonic function of action value. These results clearly suggest that mirror neurons encode the egocentric value of an observed action. Encoding of egocentric value may facilitate selecting an appropriate response to the action.

Disclosures: **D. Arnstein:** None. **J.K. Pomper:** None. **P. Thier:** None.

Poster

625. Multisensory and Motor Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 625.06/EE1

Topic: D.05. Visual Sensory-motor Processing

Support: NIH Conte Center P50MH094258

Cedars-Sinai Neurosurgery startup

Title: Response error signalling by single neurons in human anterior cingulate cortex, amygdala and supplementary motor area

Authors: *Z. FU[1,2], A. N. MAMELAK[3], I. ROSS[5], J. CHUNG[4,3], R. ADOLPHS[2], U. RUTISHAUSER[3,2,4];
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Abstracts: Anterior cingulate cortex (ACC) has long been implicated in processing cognitive conflicts. However, whether ACC primarily contributes to conflict detection, conflict resolution, error monitoring, learning or any combinations of the above, with or without the interaction from other brain areas, remains a topic of debate. We investigated the response properties of neurons in ACC, supplementary motor areas (SMA), amygdala with a speeded version of color/word Stroop task. While patients performed this task we simultaneously recorded single neurons in 11 epilepsy surgery candidates with implanted intracranial depth electrodes. Specifically, we investigated i) whether there is single-neuron signature of cognitive conflict during the stimulus-response interim period , and ii) whether ACC/amygdala neurons signals after or before the commission of errors. For incongruent trials, subjects responded slower (Stroop effect on average 87ms) and made more errors (26% and 5% of incongruent and incongruent trials, respectively). We identified a subset of neurons in ACC, SMA and amygdala that significantly changed firing rate after subjects made errors. In ACC, 21% (29/137) neurons signal errors with a significant rate increase. In amygdala 19% (23/122) neurons and in SMA 14.2% (6/42) neurons signaled errors with rate decrease. Responsive neurons were selected using a 1s window starting at button press with a bootstrap procedure and only sessions with 8 or more errors were considered. Notably, these neurons responded immediately after button press before feedback was provided. They thus represent an internally generated error signal. In contrast, out data seem to suggest an absence of conflict modulation at the single unit level due to cognitive conflict following stimulus onset. Our results support the theory of a tripartite error detection and correction system consisting of ACC SMA and amygdala.

Disclosures: Z. Fu: None. **A.N. Mamelak:** None. **I. Ross:** None. **J. Chung:** None. **R. Adolphs:** None. **U. Rutishauser:** None.

Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

Support: NIH NEI EY023371

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Title: Behaviorally relevant efficient cortical coding of visual motion signals in the pursuit system of monkeys

Authors: *B. LIU[1], L. OSBORNE[2];
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Abstracts: Performance in sensorimotor behaviors guides our understanding of many of the key computational functions of the brain: the representation of sensory information, the translation of sensory signals to commands for movement, and the production of behavior. Eye movement behaviors have become a valuable testing ground for theories of neural computation because the circuitry has been well characterized and eye movements can be tightly coupled to cortical activity. Here we show that pursuit eye movements, and the cortical sensory signals that mediate them, demonstrate the hallmarks of efficient coding. Barlow proposed that neurons should adapt their sensitivity as stimulus conditions change in order to maintain efficient representation of sensory inputs. Evidence for efficient coding of temporal fluctuations in visual contrast has been observed in the retina. We asked whether adaptation to stimulus variance generalizes to higher cortical areas whose neurons respond to features of visual signals that do not drive adaptation in the periphery and whether such adaptation impacts performance of visually-driven behavior. Specifically, we studied the impact of cortical adaptation to fluctuations in motion direction on pursuit. We recorded eye movements of monkeys pursuing moving targets with an added stochastic perturbation. Using the same perturbation stimuli, we also recorded the responses of neurons in the middle temporal cortical area that provides visual motion inputs for pursuit. We find that both neural and behavioral gain -- the relationship between firing rate or eye movement and target movement -- rescales in inverse proportion to the standard deviation in target direction fluctuations, consistent with the efficient coding hypothesis. Step changes in target motion variance created a transient decrease in the information capacity of pursuit and in MT. Rescaling of the stimulus-response distributions can be detected as early as 20ms after a change in motion variance, but the detection of a step on single trials takes substantially longer. Furthermore, these

gain changes cannot be accounted for by saturation - they occur when the highest observed firing rate is below the peak firing rate of the neuron (or eye velocity), however this work does not identify a mechanism. These data suggest that feature selective cortical areas are themselves capable of efficient sensory coding and that efficiencies in cortical coding can be relevant to behavioral performance.

Disclosures: **B. Liu:** None. **L. Osborne:** None.

Poster

625. Multisensory and Motor Interactions

Location: Halls A-C

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Program#/Poster: 625.08/EE3

Topic: D.05. Visual Sensory-motor Processing

Support: MEXT

Title: Neurons in the primate central thalamus predicting the timing of periodic stimulus

Authors: ***K. MATSUYAMA**, M. TANAKA;
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Abstracts: We recently found that neurons in the cerebellar dentate nucleus carry temporally-specific signals when monkeys predicted the timing of periodic stimulus (J Neurosci 33: 15432, 2013). To understand how these signals are processed further in the downstream thalamocortical pathways, we examined neuronal activity in the anterior intralaminar and paralaminar nuclei of the thalamus. In the missing oddball task, a brief (35 ms) audiovisual stimulus was presented repeatedly at a fixed interstimulus interval (ISI, 100-600 ms). Monkeys were trained to make an immediate saccade to a visible target when one stimulus in series was omitted, so that they predicted next stimulus timing throughout the trial. So far, we have recorded from 53 task-related thalamic neurons which were classified into 3 groups; 1) Entrainment-type neurons (38%) exhibited a gradual elevation of inhibitory response to each stimulus as the repetition progressed, similarly to neurons in the cerebellum. 2) Sensory-type neurons (28%) regularly responded to each stimulus but reduced the response gain for the repeated stimuli. 3) Switch-type neurons (34%) initially showed excitatory response to each stimulus, but in trials with long ISIs, the direction of firing modulation gradually reversed, resulting in a shift of the timing of peak activity that eventually synchronized with the stimulus onset. To examine the properties of these signals, two additional trials were presented. Firstly, in the non-target trials, the saccade target

never appeared and the animals maintained fixation throughout the trial so that they did not need to predict the timing of each stimulus. Like neurons in the cerebellum, both the Entrainment-type and Switch-type neurons decreased the firing modulation in the non-target trials compared to the missing oddball trials. For the Switch-type neurons, the response reversal and synchronization of peak activity to stimulus onset disappeared in the non-target trials. Secondly, to distinguish these signals from motor preparation, we devised the explicit deviant oddball trial, in which monkeys were required to detect the deviation in color and pitch of the repetitive audiovisual stimuli. In this trial, color of the fixation point differed from the other tasks in order to inform monkeys of the trial type. The firing modulation was reduced in the deviant oddball trials compared to the missing oddball trials while both tasks required saccades. These results suggest that neurons in the central thalamus might integrate signals from sensory areas and the cerebellum, thereby generating a predictive code for the next stimulus timing that can be separated from motor preparation.

Disclosures: K. Matsuyama: None. **M. Tanaka:** None.

Poster

625. Multisensory and Motor Interactions

Location: Halls A-C

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Program#/Poster: 625.09/EE4

Topic: D.05. Visual Sensory-motor Processing

Title: Alpha neural activity in the somatosensory and visual cortices when drawing under conflicting proprioceptive and visual inputs

Authors: *N. LEBAR, J. DANNA, S. MORE, L. MOUCHNINO, J. BLOUIN;
LNC, Marseille, France

Abstracts: There is increasing evidence that the brain can dynamically change the weight of sensory inputs as a function of their relevance during the planning and control of movements. For instance, EEG event related desynchronisation (ERD) around 10Hz in the somatosensory and visual areas have been linked to the use of proprioceptive and visual feedback during movement planning and execution, as well as during motor imagery. The literature suggests that *in situ*ation where vision and somatosensory information is incongruent (mirror task), somatosensory input becomes a misleading information, degrading movement accuracy. Experimental evidence argues that in such a case, inhibiting somatosensory input could be a way for the brain to overcome the discrepancy. Here we examined neural activities (time-frequency

domain of EEG recordings) in the somatosensory and visual regions in tasks with and without visuo-somatosensory incongruency. To do so, participants (n=12) had to control a cursor on a horizontal screen with a digital pen, while their controlling hand was visually hidden. After a resting period (baseline), they had to follow accurately the outline of irregular shapes with a congruent relation between the cursor and the pen movements. After a variable period of time (between 8 and 12s), participants were submitted to either a strong (120°), weak (13°, not perceived) or absent (0°) incongruence between the cursor and the pen movements. Free non-visually guided hand movements (cursor feedback only) were also executed as control trials. We assessed drawing accuracy and performed EEG time-frequency decomposition of each condition compared to baseline. We found that participants' performance was markedly impaired in strong incongruent condition, and with no impairment in trials with the weak or absent incongruence. EEG preliminary analyses showed the known 10Hz ERD linked to movement initiation in somatosensory areas, as well as in occipital areas. However, we found a supplementary 10Hz ERD in strong incongruent condition, over both the somatosensory and occipital areas. We interpret these results in term of task-relevant weighting sensory inputs.

Disclosures: **N. Lebar:** None. **J. Danna:** None. **S. More:** None. **L. Mouchnino:** None. **J. Blouin:** None.

Poster

625. Multisensory and Motor Interactions

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Program#/Poster: 625.10/EE5

Topic: D.05. Visual Sensory-motor Processing

Support: SSHRC Insight grant 527544

Title: How specific is non-specificity? Early motor facilitation during action observation is specific to the viewed effector

Authors: ***K. R. NAISH**, S. S. OBHI;
Dept. of Psychology, Wilfrid Laurier Univ., Waterloo, ON, Canada

Abstracts: It is well established that activity in the human motor system is modulated by action observation; however, the level of muscle-specificity of these motor responses is unclear. Recently, Cavallo et al. (2013) found evidence of an early non-specific facilitation of motor excitability, followed by facilitation that was specific to the muscle involved in the action.

Specifically, motor-evoked potentials (MEPs) were facilitated similarly in two hand muscles (first dorsal interroseous, FDI; abductor digiti minimi, ADM) at an early phase of index finger observation, while only the FDI (involved in the movement) was modulated after 200ms. The current study sought to determine how 'non-specific' this initial modulation is, by investigating MEP modulation in a forearm muscle (the flexor digitorum superficialis, FDS), in addition to two hand muscles (FDI, ADM). MEPs were elicited in these three muscles as healthy adult participants watched index finger and little finger abductions. The timing of effects was tracked by eliciting MEPs at five time points (100, 150, 200, 250, 300ms). During index finger observation, MEPs were facilitated (relative to static hand viewing) in both the ADM and FDI at 150ms. Muscle-specific modulation - facilitation of the FDI only - was demonstrated at 300ms. Little finger observation evoked a similar pattern of modulation: facilitation in the ADM and FDI at 150ms, and facilitation of the ADM only (i.e., muscle-specific) at 300ms. This pattern of modulation found in the hand muscles is consistent with previous findings in terms of the early non-specific and later muscle-specific modulation, although it is not clear why MEPs were not modulated at 100ms. Interestingly, data from the forearm (FDS) showed facilitation of MEPs at one time point - 200ms - during observation of both index and little finger abductions. Although the FDS is not involved in either movement, it does control finger flexion. Therefore, one possible explanation of this facilitation is that this muscle is activated in order to maintain the posture of the hand when the index or little finger moves. In addition, MEP modulation was found to be smaller overall than modulation in either the ADM or FDI, showing that activity in this muscle was influenced to a much lesser extent during finger movement observation. Importantly, our data indicate that non-specific facilitation occurring pre-200ms is specific to the effector being viewed, rather than being a more general motor priming. Such an effector-specific response might be analogous to the 'broadly-congruent' mirror responses recorded in monkeys, and their significance for behaviour should not be dismissed.

Disclosures: **K.R. Naish:** None. **S.S. Obhi:** None.

Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

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Karolinska Institutes's research Funds

BLANCEFLOR Boncompagni-Ludovisi, née Bildt

Title: The role of pretectal microcircuitry in the control of visual motor responses

Authors: *L. CAPANTINI, A. KARDAMAKIS, B. ROBERTSON, S. GRILLNER; Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstracts: A behaviourally relevant motor action has to be spatially oriented and directed toward or away from a target, such as a prey or an obstacle. During a goal-directed behaviour, visual stimuli are responsible for driving the movements and the visuo-motor coordination is essential to guarantee the success of the action. The pretectum mediates short latency motor responses, such as escape reactions and visual reflexive behaviour (Ullén et al., 1993, 1997). The knowledge of the pretectum in lamprey, as well as in other vertebrates, is however still limited. The present study aims to investigate the role of the pretectal microciruitry in visual motor responses, using the lamprey, one of the first vertebrates to emerge, as experimental model. Anatomical tracing and immunohistochemistry defined the pretectal connectivity. Afferents from the retina and optic tectum, the superior colliculus in mammals, represent the two main excitatory inputs. An additional sensory input derives from the lateral line (electro- and mechanoreception) via the octavolateral area. Projections from the lateral pallium, the homologue of cortex, indicate that pretectum may be involved in motor control, as electrical stimulation of lateral pallium elicits eye, orienting, and body movements (Suryanarayana et al., SfN Abstract 2014). The dorsal pallidum, the basal ganglia output layer, instead provides a tonic inhibition. The main pretectal outputs are direct projections to reticulospinal neurons and the optic tectum. In particular, a subpopulation of pretecto-reticular cells have large somata and a dendritic arbour that extends into the optic tract, indicating that these cells receive direct retinal input and could thus play a role in the fast motor response. Patch clamp recordings were performed to examine the basic electrophysiological characteristics and synaptic interactions of pretectal cells. Analysis of membrane and firing properties revealed different subpopulations of neurons. Electrical stimulation of the posterior commissure gave a robust inhibition in a subpopulation of pretectal cells. This inhibition most likely derives from contralateral GABAergic neurons as combined tracing and GABA-immunohistochemistry showed that GABAergic pretectal cells cross in the commissure. We believe in the critical role of pretectum in the visuo-motor coordination, and the present findings confirm the assumptions and shed light on the role of pretectum.

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Poster

625. Multisensory and Motor Interactions

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Program#/Poster: 625.12/EE7

Topic: D.05. Visual Sensory-motor Processing

Support: NSF grant DMS 1120952

NIH grant MH065339

Title: Activating medullary neurons through optogenetics in the locust optic lobe

Authors: *H. WANG[1], R. B. DEWELL[1], M. U. EHRENGRUBER[2], F. GABBIANI[1,3]; [1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Biol., Kantonsschule Hohe Promenade, Zurich, Switzerland; [3]Computat. and applied mathematics, Rice Univ., Houston, TX

Abstracts: Locusts are widely used as a neuroethological model for *in vivo* electrophysiological recordings. Previously, we had successfully expressed Channelrhodopsin (ChR) -Venus in neurons of the locust optic lobe by injection of a temperature-sensitive Semliki Forest virus variant [SFV(A774nsp)-ChR-Venus]. The Lobula Giant Movement Detector (LGMD) is an identified, looming-sensitive neuron in the lobula of the locust optic lobe, processing and relaying information on the impending collision of approaching objects to a postsynaptic neuron, the Descending Contralateral Movement Detector (DCMD). Our previous two-photon imaging demonstrated that ChR-Venus labeled medullary (MED) neuronal terminals overlapped with the dendritic arbor of the LGMD labeled with Alexa 594. These medullary neurons are thought to be presynaptic to the LGMD. However, up to now, there is no direct electrophysiological evidence. Using paired-tungsten metal electrodes, we extracellularly recorded the spontaneous spiking and ChR-mediated spiking of medullary neurons in response to 488 nm laser illumination. In contrast to spontaneous spiking, laser illumination increased the firing rate of MED neurons. Correspondingly, intracellular recordings from the postsynaptic LGMD exhibited constant spiking due to the firing of MED neurons. Spike sorting of the laser-evoked MED neuronal spiking and comparison with the spiking of the LGMD allowed us to analyze the correlation between the activity of MED neurons and the LGMD. These data suggest that SFV-based ChR-optogenetics is effective in activating the LGMD through the firing of the MED neurons, which may help identify the local neural circuitry involved in the processing of looming stimuli and understand neuronal information processing in the locust optic lobe.

Disclosures: H. Wang: None. **R.B. Dewell:** None. **M.U. Ehrengruber:** None. **F. Gabbiani:** None.

Poster

625. Multisensory and Motor Interactions

Location: Halls A-C

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Program#/Poster: 625.13/EE8

Topic: D.05. Visual Sensory-motor Processing

Title: Development of functional connections between the superficial and intermediate layers of the rodent superior colliculus revealed by optical imaging

Authors: *M. NANA[1], R. P. HASEGAWA[2,1], K. MURASE[1], H. IKEDA[1];
[1]Grad. Sch. of Engin., Univ. of Fukui, Fukui City, Japan; [2]Natl. Inst. of Advanced Industrial Sci. and Technol. (AIST), Tsukuba, Japan

Abstracts: The mammalian superior colliculus (SC) is important for visual orientation behaviors. The SC includes two layers; the superficial layer (SGS) receives visual inputs and the intermediate layer (SGI) sends motor outputs, both of which have mutual connections. Although it is generally understood that the pathway from the SGS to SGI is involved in visuomotor transformation, there are little evidence in support of the role of this pathway. Recently interlaminar excitation was reported in the pathway from SGS to SGI as well as that form SGI to SGS using optical imaging. It is, however, still unclear how these pathways develop. We focused on the development of the functional properties of the mutual connections between SGS and SGI in mice. In this study, we examined excitability of the pathways in the SC slices by the optical imaging at two different developmental stages: “infant” (7-9 days old) and “young” (25-35 days old). We applied single-pulse electrical stimulation (500μA) to either the SGS or SGI, and observed whether the evoked response was propagated. In infant mice, whose eyes had not opened yet, the stimulation in the SGS or SGI elicited the near-by response. The response, however, remained within the stimulated layer and was not propagated to the other layers. In young mice, whose eyes have already opened, we observed the propagation by single-pulse stimulation to SGI. The SGI stimulation initially evoked the response within the SGI, and then the response tended to be propagated to the SGS. The peak response in the SGI was not changed by non-NMDA-glutamate receptor antagonist CNQX, but the peak in the SGS was almost completely abolished by CNQX, indicating that the propagation from SGI to SGS was not the artifact caused by antidromic excitation of the pathway from SGS to SGI. On the other hand, by SGS stimulation, the optical response was evoked only within the SGS, but was not propagated to the SGI although the level of the near-by SGS response was stronger than that of the near-by SGI response. These results suggest that the mutual connections between the SGS and SGI in the

SC might develop in an asymmetric fashion. It is thought that not only the pathway from SGI to SGS but also the pathway from SGS to SGI physically exists and could be activated in a similar fashion. It was, however, difficult to evoke it even with the stimulation, which elicited the response at the near-by SGS site as well as the propagation from SGI to SGS. It is possible that the excitatory pathway from SGS to SGI is normally suppressed probably because the SC can control generation of too much reflexive action, giving chance of involvement of higher cognitive areas in visual orientation behaviors.

Disclosures: **M. Nana:** None. **R.P. Hasegawa:** None. **K. Murase:** None. **H. Ikeda:** None.

Poster

625. Multisensory and Motor Interactions

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Program#/Poster: 625.14/EE9

Topic: D.05. Visual Sensory-motor Processing

Title: Unilateral strength training while mirror viewing the exercising hand, augments cross-education and reduces cortical inhibition and corticospinal excitability

Authors: ***T. ZULT**[1], S. GOODALL[2], K. THOMAS[2], T. HORTOBÁGYI[1], G. HOWATSON[2]; [1]Ctr. for Human Movement Sci., Univ. of Groningen, Groningen, Netherlands; [2]Dept. of Sport, Exercise and Rehabil., Northumbria Univ., Newcastle-upon-Tyne, United Kingdom

Abstracts: Introduction: Unilateral strength training not only strengthens muscles of the trained side, but also the contralateral homologous muscles of the non-trained side. This phenomenon (cross-education) is a promising therapeutic strategy to strengthen muscles of the affected side by training the non-affected side for patients who suffer from unilateral orthopaedic and neurological conditions. The magnitude of cross-education is modest; however it has been hypothesized that heightened sensory feedback by viewing the exercising hand in a mirror could augment cross-education by modulating intracortical, intercortical and corticospinal pathways. Consequently, we examined the hypothesis that mirror training augments the cross-education of strength by modifying motor cortical excitability and short interval intracortical inhibition (SICI) of the primary motor cortex (M1) and interhemispheric inhibition (IHI) from the left to right M1. **Methods:** Healthy, young right-handed adults, were allocated to a mirror ($N = 11$) and no-mirror training group ($N = 12$) and performed 640 shortening muscle contractions of the right wrist flexors at 80% maximal voluntary contraction (MVC) during 15 sessions over three weeks. **Results:** MVC of the trained wrist flexors improved similarly in the mirror group (75 ± 39%)

and no-mirror group (76 ± 39%; $P = 0.961$). MVC of the non-trained wrist flexors increased more in the mirror (62 ± 36%) than no-mirror training group (42 ± 33%; $P = 0.044$). The augmented strength transfer of the mirror training group was accompanied by a decrease in corticospinal excitability (19%) and SICI (9%) of the left ("trained") M1, while the no-mirror training group increased corticospinal excitability (38%) and SICI (15%) (interaction, $P \leq 0.047$). Corticospinal excitability and SICI of the right ("non-trained") M1 and IHI from left to right M1 were not different between groups ($P \geq 0.093$). **Discussion:** Augmented sensory feedback by viewing the exercising hand in the mirror augments the cross-education of strength by modulating corticospinal excitability and SICI of the trained M1. Remarkably, corticospinal excitability and SICI of the non-trained M1 and IHI from trained to non-trained M1 did not show between group differences. Previous studies showed that these pathways play a key role in evoking cross-education of strength but we found that these pathways are not additionally modified when unilateral strength training is performed with a mirror. Regardless of the underlying mechanisms, this study demonstrates that mirror-aided unilateral strength training could be an adjuvant tool for accelerating functional recovery from unilateral impairment.

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Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

Support: Grant-in-Aid for Scientific Research (S) 22220006

Title: Two-photon imaging of lateral interaction in the superficial layer of the superior colliculus

Authors: ***M. KASAI**[1], T. ISA[1,2];
[1]Natl. Inst. For Physiological Sci., Okazaki, Aichi, Japan; [2]Life Sci., The Graduated Univ. for Advanced Studies (SOKENDAI), Hayama, Japan

Abstracts: The superior colliculus (SC) is a brainstem center which plays a key role in sensory-motor translation and attention. The superficial layer of the SC (sSC) is directly innervated by the optic tract and visual space is represented in the retinotopic coordinates. In the early stage of visual processing, firing activity of neuronal population in response to the stimuli presented in

their response field is often inhibited by stimuli presented outside of their receptive field. This effect is known as “lateral inhibition” or “surround suppression”. To date, neural implementation of the “lateral inhibition” remains unclear, especially at the neuronal population level. In this study, we applied *in vivo* two-photon calcium imaging to reveal visually evoked neuronal population activities. We tested two-point stimulus, in which spatially separated two stimuli were presented simultaneously. When the two stimuli were close as respective excitatory response fields were overlapped, neuronal responses were increased. On the other hand, when the two stimuli were presented with larger separation, resulted neuronal responses were significantly decreased. These results indicated there was inhibitory interconnection from the outside of their excitatory response filed. More than half of neurons in the sSC are GABAergic inhibitory neurons and it has been debated whether horizontal inhibitory connection is responsible for such lateral inhibition. To understand the relationship between the inhibitory interconnection and underlying neural circuits, we used GAD67-GFP knock-in mice, in which GABAergic neurons are specifically labelled with GFP and examined differences between stimulus size tuning properties, another form of lateral inhibition, of the excitatory and inhibitory neurons. We found both non-GABAergic and GABAergic neurons showed similar center-suppression profiles when a large stimulus was presented. These results suggested that long-range GABAergic neurons mediate the lateral inhibition in the sSC.

Disclosures: **M. Kasai:** None. **T. Isa:** None.

Poster

625. Multisensory and Motor Interactions

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Topic: D.05. Visual Sensory-motor Processing

Support: NWO Brain & Cognition 433-09-248

Title: Distinct roles for alpha- and beta-band oscillations during construction of goal-directed action plans

Authors: ***L. BRINKMAN**[1], F. P. DE LANGE[1], A. STOLK[1], H. C. DIJKERMAN[2], F. S. S. LEIJTEN[3], I. TONI[1];
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Abstracts: Action plans can be regarded as simulations of movements which are used to issue motor commands to the muscles and predict the sensory consequences of our actions. In order to construct an action plan, specific neuronal populations controlling particular features of that movement need to be selected, while other neuronal populations need to be suppressed. Neuronal oscillations in the alpha (8 - 12 Hz) and beta (15 - 25 Hz) band frequency range provide a possible mechanism for implementing these processes of activation and suppression in the sensorimotor system. However, their relevance and specificity for selecting neuronal ensembles remain unclear. Here we use magnetoencephalography (MEG, n = 24) and electrocorticography (ECoG, n = 9) to investigate the relative contribution of these rhythms to the construction of action plans. Changes in oscillatory power were measured while participants imagined grasping a cylinder whose orientation changed from trial to trial. This paradigm allowed us to study the neuronal processes that underlie the construction of an action plan in the absence of signals related to motor execution and sensory reafference. Given human biomechanical constraints, some object orientations evoke consistent over-hand or under-hand grasping movements, whereas other orientations are compatible with both movements. These task features allowed us to experimentally manipulate movement selection demands and to behaviourally validate motor imagery performance. Source-reconstructed MEG data revealed a dissociation between the alpha and beta band activity evoked during the imagery process. Alpha-band oscillatory power increased in the ipsilateral sensorimotor cortex, whereas beta-band power concurrently decreased in the contralateral sensorimotor cortex. These findings emerged both when movement selection demands were modulated within the imagery condition and when imagery trials were compared to trials of a control condition. These observations indicate that neural oscillations in the alpha-band mediate the allocation of computational resources, disengaging task-irrelevant cortical regions. In contrast, a reduction of neural oscillations in the beta-band may be directly related to the computations of movement parameters. We are currently analyzing ECoG data of patients implanted with subdural electrodes performing the same motor imagery task, to validate and spatially define these observations.

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Poster

625. Multisensory and Motor Interactions

Location: Halls A-C

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Title: Sensory gating by the delta phase determines network and behavioral response to weak visual input in freely behaving ferrets

Authors: *F. FROHLICH, J. LU, K. K. SELLERS, C. YU, S. L. SCHMIDT;
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Abstracts: Brain networks identify and selectively process behaviorally relevant sensory input. Oscillatory activity in neuronal networks has been implicated as a mechanism for such information selection and routing. In particular, both the power and the phase of alpha oscillations (8-12 Hz) modulate perceptual responses in trained cognitive tasks that require attention [e.g. 1]. Yet, it has remained unknown how neuronal networks process and filter sensory input during untrained, naturalistic behavior. We hypothesized that the phase of ongoing cortical oscillations in primary visual cortex (V1) gates neuronal and behavioral responses to weak visual stimuli that lack behavioral consequences. To test this hypothesis, we performed wireless electrophysiological recordings of multiunit activity (MUA) and local field potential (LFP) in V1 of freely behaving ferrets. The animals were acclimated to a large recording arena that included species-appropriate toys. Behavioral monitoring by infrared videography revealed a broad set of behavioral states, including play, exploration, rest, and sleep. At random intervals, we presented brief (200 ms), dim (1-3 lumen) house-light flashes and investigated MUA, LFP, and behavioral responses. We found that during naturalistic behavior, V1 represents weak, neutral sensory input with substantial trial-to-trial variability that was explained by the instantaneous phase of the delta oscillation (< 4 Hz). Synergistic interaction between MUA and LFP network dynamics mediated this gating mechanism. We found subtle changes in the behavior of the animal in response to the stimuli as measured by the velocity of the animal. Stronger representation of the stimulus in V1 was associated with more pronounced acceleration of the animal in response to the stimulus. However, acceleration of the animal was not associated with an increase in MUA representation of the stimulus. Therefore, these data suggest that the strength of representation in V1 guided overall behavioral response. In turn, the amplitude of the MUA response was shaped by the phase of the delta oscillation in the awake, behaving animal. Such a role of the delta oscillation as the key mediator of sensory gating and information routing in V1 suggests a new mechanistic role of the delta oscillation in the awake behaving animal and highlights the benefits of studying the brain-behavior relationship in the context of naturalistic

behaviors. 1. Palva, S., and Palva, J.M. (2007). New vistas for alpha-frequency band oscillations. Trends in neurosciences 30, 150-158.

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Poster

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Research to Prevent Blindness

Title: Tackling the sensitivity of FEFsem neurons during smooth pursuit: Microstimulation of the superior colliculus and multiple linear regression

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Abstracts: Accurate smooth pursuit (SP) requires the integration of retinal and extra-retinal signals to keep the fovea on a moving target. Identifying the weight of these signals during SP through visuomotor pathways is crucial to understanding the sensorimotor transformation. Here, saccades evoked by electrical microstimulation (MS) of the deep superior colliculus (SC) are used to perturb the trajectories of eye movements during a step-ramp paradigm. This technique separates the influence of eye velocity and position by causing an offset in position while eye velocity is maintained. Recently, we showed that perturbing SP reveals two types of responses in the smooth eye movement subregion of the frontal eye field (FEFsem) (Fleuriet et al. 2013). After perturbation, some FEFsem neurons show no change in their firing rate (FR) compared to control, as long as SP eye velocity is maintained. Other neurons exhibit a decrease in FR even with maintained eye velocity, presumably due to the position offset. We recorded from 10 FEFsem neurons in 1 monkey while MS (20-40 μA, 30-50 ms, 400 Hz) was applied to the SC 100-500ms after the step. Neuronal response was measured in the 100 ms following the evoked saccade. We estimated the contribution of visual motion and eye motion to the neuronal response

using multiple linear regression modeling. Neuronal latency was estimated, and neuronal dependence on visual input was tested by extinguishing the target for 150 ms, 100-500 ms after the target step. Of 10 neurons, 5 show a decrease of 10-72% in FR after perturbation compared to control trials. Qualitative analysis shows some neurons with a decreased FR after perturbation resume their response when the eyes return to the target and active tracking continues. Multiple linear regression modeling revealed 4 neurons whose FR variance was best explained by eye velocity, 1 by eye acceleration, 1 by retinal position error, and 4 by retinal velocity error. Neuronal latencies for FEFsem neurons ranged from 64-184 ms after target step. FEFsem neurons that showed a decreased FR after perturbation did not differ from the general population as defined by multiple linear regression modeling or neuronal latency. Also, the decrease in FR after perturbation was not due to visual signals, as the neurons with the largest decreases showed only a small decrease due to the target blink (FR was 75- 120% of control trials). There is also a trend suggesting those neurons that show no decrease after perturbation are more caudal in the FEFsem. These preliminary results suggest there may be a subpopulation of FEFsem neurons whose activity is dependent on accurate SP: eye velocity must be maintained and retinal error must be minimal.

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Poster

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Support: NSERC CREATE CAN-ACT Program

Title: Decomposition of visual vs. auditory representations of gaze target location into 3-D eye and head movement commands: A neural network study

Authors: *M. DAEMI[1], D. CRAWFORD[2,3];
[1]Ctr. For Vision Res., Toronto, ON, Canada; [2]Biol., York Univ., Toronto, ON, Canada; [3]Ctr. for Vision Res., Toronto, ON, Canada

Abstracts: Coordinated gaze shifts can be aimed at both visual and auditory targets (Frens and Van Opstal, 1998). Visual and auditory responses in the gaze control system show different frames of reference (Maier and Groh, 2009), but there is no clear theoretical framework for

understanding how these signals are differentially transformed and decomposed into separate 3-D eye and head commands. To investigate this question, we have designed three feedforward neural networks and trained them by kinematically correct and physiologically inspired computational algorithms. All the networks have the initial eye-in-head and head-on-shoulder 3-D orientation signals as the input and the desired eye-in-head and head-on-shoulder 3-D displacement signals as the output. Orientation and movement signals of eye and head are represented in hybrid canal/Listing's coordinates and hybrid canal/Fick coordinates respectively. The difference between the networks is in their driving input signals. The first network has the retinal error, retinotopically encoded 2-D position of the target relative to the eye, as the input. The second network has the so-called cranial error, 2-D position of the target relative to head encoded in a manner similar to auditory representation of space in SC (or bird's optic tectum), as the input. The third network has access to both of the retinal error and cranial error although with potentially different reliabilities. We trained all the networks by a resilient backpropagation algorithm. After training, all networks produced the right, biologically comparable, behavior at the output, including eye-head coordination rules, head-free version of Listing's law, and Fick constraint for head. A preliminary analysis of the activities of the units in the hidden layer of the networks shows highly nonlinear receptive fields. Hidden layer receptive fields are all eye-centered in the first network and are all head-centered in the second network. Gain field modulation of these response fields by the initial gaze orientation have been observed for first and second networks. Our further aims are to 1) analyze the 3rd version of the network as a model of sensory integration, 2) work out the details of the eye-head decomposition process in these networks, and 3) compare our results with single unit recordings from SC and FEF collected in our lab. Frens MA, Van Opstal AJ (1998) Visual-auditory interactions modulate saccade-related activity in monkey superior colliculus. Brain Res Bull 46:211-224. Maier JX, Groh JM (2009) Multisensory guidance of orienting behavior. Hear Res 258:106-112.

Disclosures: **M. Daemi:** None. **D. Crawford:** None.

Poster

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Title: Sensory recalibration in the ventriloquism aftereffect integrates local and global stimulus history

Authors: *P. BRUNS, B. RÖDER;
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Abstracts: Vision usually provides the most accurate and reliable information about the location of objects in our environment, and thus serves as a reference for recalibrating auditory spatial maps. This can be exemplified by exposing participants to consistently misaligned audiovisual stimuli for several minutes. After such an adaptation phase, a shift in unimodal sound localization is observed that corrects for the cross-modal spatial mismatch. This so-called ventriloquism aftereffect has been associated with changes in spatial representations early in the auditory cortical processing stream. In line with an involvement of early, tonotopically organized regions of auditory cortex, some studies reported that the ventriloquism aftereffect did not occur when the sound frequency of the auditory stimuli used during adaptation differed from the sound frequency of the test stimuli. The initiation of auditory space recalibration does, however, not necessarily require accumulated evidence of cross-modal mismatch to be triggered, but occurs immediately after a single exposure to discrepant audiovisual input. By measuring the ventriloquism aftereffect on a trial-by-trial basis, we show that such an immediate recalibration is mediated by different mechanisms than recalibration to sustained cross-modal discrepancy. Participants performed a sound localization task in which they indicated the perceived location of 750 Hz and 3000 Hz tones that were presented from six different locations spanning ±22.5°. The tones were either presented alone, or together with synchronous visual stimuli displaced 13.5° to the left or to the right. Importantly, the two different sound frequencies were paired with opposite directions of audiovisual spatial mismatch: For half of the participants, the 750 Hz tone was always presented with visual stimuli to the left and the 3000 Hz tone with visual stimuli to the right, and vice versa for the other half of the participants. In accordance with this global stimulus history, localization in unimodal auditory trials was shifted in opposite directions for the two sound frequencies. This frequency-specific recalibration was, however, modulated depending on whether the directly preceding audiovisual trial featured the same sound frequency (and thus direction of spatial mismatch) or not. Thus, the local stimulus history led to an immediate recalibration of sound localization in a frequency-unspecific manner, in addition to the frequency-specific adaptation to the global stimulus history. This finding suggests that separate recalibration mechanisms operating at different timescales jointly determine sound localization behavior.

Disclosures: P. Bruns: None. **B. Röder:** None.

Poster

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Title: Use of multisensory information by flying bats

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Abstracts: Animals gather sensory information about their environment in support of diverse behavioral tasks such as navigation, foraging, and mating. Integrating information from multiple sensory modalities can facilitate the performance of these behavioral tasks by improving the detectability of a stimulus. Flying bats emit ultrasonic echolocation calls, and use spatial information obtained from echoes in order to navigate. When approaching a target, they adaptively alter the temporal, spectral, and directional parameters of their echolocation calls to enable accurate target localization. The use of visual cues by flying bats has received relatively little attention, although vision may serve as a relatively long-range orientation cue guiding navigation. This study is aimed at understanding the influence of visual cues on flight and echolocation parameters. We obtained high-speed video and audio recordings of different species of bats as they flew to a hanging food source, both in darkness (where they relied largely on acoustic cues) and in lit conditions (where multisensory information was available). Preliminary analyses indicate differences in flight and adaptive vocal parameters between lit and dark conditions. This suggests that flying bats are capable of utilizing available visual information to guide their flight.

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Poster

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Title: Characterization of parietal spike train spectra during multimodal limb position estimation

Authors: *P. VANGILDER, JR, Y. SHI, G. APKER, C. A. BUNEO;
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Abstracts: Limb position sense is crucial for the maintenance of body schema and for performing volitional movements. Visual and somatic information, such as efference copy and proprioception, are the primary cues for computing limb position estimates. In order to improve neural prosthetic design, as well as fully understand the sensory basis of limb movement, it is imperative that we understand how visual and somatosensory information interact in multimodal regions of the brain. The posterior parietal cortex has been implicated as a possible location for multisensory integration and for limb position sense. As a result we recently examined the integration of visual and proprioceptive information in area 5 as monkeys reached to and actively maintained their limb positions at multiple frontal plane locations presented in a virtual reality environment. Positions were maintained with vision (V condition) or without vision (NV condition) of the arm. A 2-factor ANOVA ($P<0.05$) identified populations of neurons that were modulated by limb position, limb vision, and/or the interaction of these factors. Both the firing rate and neural variability, the latter measured by the Fano Factor and coefficient of variation, were found to be reduced in the V condition. These changes indicated the presence of both trial-by-trial and intra-trial spiking variability. To determine whether reductions in intra-trial variability were associated with greater temporal structure of the spike trains in the V condition, rate-normalized spike train spectra were computed for each neuron in both the vision and non-vision conditions, as well as for each population of neurons. We found there to be significant differences in the gamma band power (25-100 Hz) between the V and NV conditions in all three subpopulations as well as the in the overall cell population. However, these differences in spectral power did not appear to arise from a marked increase in temporal structure of the spike trains in the presence of vision. Thus, reductions in spiking variability in area 5 appear to arise from a mechanism other than enhanced temporal structure. Future analyses will be directed at exploring alternative mechanisms that may underlie this phenomenon.

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Poster

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Title: Alpha activity indexes task-related neuronal populations on large and small scales: Evidence from ECoG in a multimodal study in humans and a non-human primate

Authors: *A. DE PESTERS[1,2], P. BRUNNER[1,3], A. GUNDUZ[4], A. L. RITACCIO[3], P. DE WEERD[5,6], M. ROBERTS[6], N. BRUNET[6], R. OOSTENVELD[6], P. FRIES[6,7], G. SCHALK[1,2,3]; [1]Neural Injury and Repair, Wadsworth Ctr., Albany, NY; [2]State Univ. of New York at Albany, Albany, NY; [3]Dept. of Neurol., Albany Med. Col., Albany, NY; [4]Univ. of Florida, Gainesville, FL; [5]Dept. of Cognitive Neuroscience, Fac. of Psychology and Neurosci., Maastricht Univ., Maastricht, Netherlands; [6]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands; [7]Ernst Strüngmann Inst. for Neurosci., Frankfurt, Germany

Abstracts: Performing different tasks involves different neuronal populations. Previous studies suggested that variations in oscillatory activity in the alpha band (8-12 Hz) may implement the selection of task-specific populations by inhibiting cortical activity in task-irrelevant areas (Klimesch 2007, Jensen and Mazaheri 2010). However, the temporal and spatial relationships between modulatory alpha activity and population-level cortical activity in the gamma range (70-170 Hz) remain undefined. In addition, while there is evidence in different modalities that alpha may constitute a coarse selection mechanism, the evidence that it could contribute to fine

selection among interwoven, and spatially restricted networks is scarce. Here, we addressed these two questions by investigating the temporal and spatial dynamics of modulations in the gamma and alpha bands during auditory and motor tasks. We recorded electrocorticographic activity from subdural electrode grids in five human subjects and one macaque monkey during the presentation of natural auditory stimuli from 6 different categories. After each presentation, the human subjects were asked to press a button. Our results confirm that gamma power accurately tracks task-related behavior. Moreover, we found that alpha activity was suppressed in task-relevant areas and increased in task-irrelevant areas. Importantly, this facilitation/inhibition mechanism not only applied to the selection of neuronal populations associated with the auditory vs. the motor system, but also to subpopulations within the auditory system. Specifically, alpha activity was suppressed in the locations within the auditory system that responded to particular sound stimuli, and increased in the remaining locations. Interestingly, this pattern of facilitation/inhibition was predicted by the initial gamma response to the stimuli. Furthermore, alpha suppression trailed gamma activity in auditory areas during auditory stimulation, but preceded it in the motor areas during the motor task. This observation prompts the hypothesis that the central nervous system can regulate cortical excitability in response to sensory input and in preparation to motor output. Finally, ongoing work provides evidence for a link between this putative selection mechanism and the communication-through-coherence hypothesis (CTC). CTC suggests that cortical communication can only be accomplished during specific temporal windows. In sum, we expect that this work will contribute to our understanding of the mechanisms underlying the selection of the specific neuronal populations required for task execution.

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Poster

625. Multisensory and Motor Interactions

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Title: Auditory modulation of wind-elicited walking behavior in the cricket

Authors: *M. FUKUTOMI[1], M. SOMEYA[1], H. OGAWA[2];
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Abstracts: To perceive environments adequately, animals combine various sensory information mediated by multiple sensory organs in different modalities. Neural process for the multisensory integration has been studied previously in mammals such as cat, macaque and human (Stein and Stanford, 2008). Multisensory integration has 'cross-modal effect' in which simultaneous presentation of two or more stimuli in different sensory modalities modulate animal behavioral performance. However, little is known about how neural circuit underlying multisensory integration modulates animal behavior. We developed a new behavioral paradigm to test cross-modal effect in the cricket, which has simple neural system consisted of much smaller number of neurons comparing to mammalian brain. Crickets have two different aero-detecting sensory organs, tympanal organ for auditory sense and cerci to detect air-flow surrounding the animal. Information of stimulus direction is extracted by both of these sensory systems to mediate distinct 'oriented behaviors'. The auditory system induces positive phonotaxis to approach a singing male, and the cercal system triggers quick walking considered as 'escape behavior' from a predator. Although local circuits and ascending interneurons involved in these behaviors have been investigated, little is unknown about the interaction between both sensory systems. To verify the cross-modal effect between the auditory and cercal sensory systems, we examined wind-elicited walking behavior combining with 10-kHz pure tone using a spherical treadmill. We found that tone sound preceding the air-current stimulus provided from lateral side alters walking direction of escape behavior backward, but does not modulate other locomotion parameter including turn angle, walking velocity, and reaction time. Auditory modulation of walking direction did not depend on the stimulus side of the preceding tone. These results demonstrated cross-modal effects between auditory and cercal sensory systems, but did not suggest multisensory integration of directional information.

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Poster

625. Multisensory and Motor Interactions

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Title: Crossmodal integration improves sensory detection thresholds in the ferret

Authors: *K. J. HOLLENSTEINER, F. PIEPER, G. ENGLER, A. K. ENGEL;
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Abstracts: Sensory evidence about the environment can trigger changes in behavior or mental states. Distinct sensory systems (i.e. visual, auditory, etc.) have evolved to continuously pick up information from different modalities. Hence, perception is always based on combining and weighing inputs in all covered modalities. Information from different sensory systems can influence behavior in synergistic or competitive ways. In four ferrets (*Mustela putorius*) we have tested and quantified the effect of unimodal vs. congruent multi-modal audio/visual stimulation in a spatial detection task. In a 2-altenative-forced-choice task the water restricted animals had to detect the spatial position of a brief (100ms) auditory (white noise) and/or visual (moving circular grating; size 22,5°, 0.2 cycl/°, 5Hz) presented at random position and time (500-1500ms after trial start) either left or right from their midline. During the task the animals' body was slightly restrained in an acrylic tube, while the head remained freely movable to indicate the location of stimuli presented via speakers and/or on a LCD screen. Responses were detected using infrared light barriers on either side from center, coupled with a spout for rewarding correct answers. During the stimulus period, the maintenance of the central head-position was ensured through an additional, central light barrier. We evaluated the detection thresholds individually in both modalities, varying the stimulus-intensities between 10-60dB (auditory) and 0.01-0.38 Michelson contrast (visual) in a staircase manner. For the bimodal stimulation, intensity in one modality remained at threshold, while the intensity in the other modality was varied, again using the staircase procedure to obtain bimodal thresholds. We expected the animals to perform more accurate and faster in the bimodal cases, because congruent inputs from two modalities provide more reliable sensory evidence. As predicted, bimodal thresholds were reduced compared to both unimodal conditions. Additionally, all ferrets reacted faster and more precisely when the auditory stimulus was visually supported. In some subjects the multisensory response enhancement (MRE) was up to 35%. On the other hand, adding auditory information to the visual stimulation had no significant effect regarding the MRE, though all animals reached lower intensities in the visual-audio compare to the visual-only case, indicating a stronger behavioral relevance of vision compared to audition in ferrets. Taken together, the data demonstrate that principles of multisensory integration previously shown in other species also apply to crossmodal processing in the ferret brain.

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Poster

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Title: Behavioral evidence for opposing geotaxis by geomagnetic field and gravity in *Drosophila*

Authors: J.-E. BAE[1], S.-H. KWON[1], Y.-H. LEE[3], *K.-S. CHAE[2,1];
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[3]Korea Res. Inst. of Standards and Sci., Daejeon, Korea, Republic of

Abstracts: Geotaxis is the behavioral response of living organisms moving toward or away from the Earth and gravity is believed as the environmental cause to provoke geotaxis. However, the belief may be flawed because another physical factor surrounding Earth, the geomagnetic field (GMF), also varies in space like gravity. The GMF is regarded as a behavioral cue for the horizontal migration of magnetoreceptive animals such as birds and sea turtles and for body alignment of cattle, dear, and cockroach, etc. In contrast, it has never been considered a cue for vertical movement or altitudinal positioning of animal. Here using *Drosophila* model, we report hitherto unknown GMF-modulated positive geotaxis antagonizing negative geotaxis caused by gravity, and suggest the regulatory GMF parameters for the positive geotaxis. The various experimental GMF conditions including near-zero or strengthening, were generated using 3-axis Helmholtz coils. In several *Drosophila* strains, negative geotactic behavior under near-zero GMF condition was ascertained by several behavior assays such as tube-positioning, Y-maze, and free flight suggesting that GMF exerts positive geotaxis under ambient condition. The near-zero GMF-induced negative geotaxis was dependent on light intensity and necessitated 400-420 nm of light. Mutants deficient of geotaxis genes cry and Pdf, and gravity sensing gene pyx failed to show the geotaxis indicating that these genes are necessary for the geotaxis. In contrast, Z-axis strengthened GMF elicited significant positive geotaxis compared to control flies, confirming that ambient GMF opposes to gravity in modulating of geotaxis. In addition, a formula for relative strength of the GMF-modulated positive geotaxis is suggested based on the geotaxis data

from various GMF conditions, revealing that interplay of parameters such as vertical vector, total intensity, and declination is critical for the geotaxis. The results suggest crucial role of GMF-modulated magnetotaxis opposing to negative geotaxis by gravity to adjust vertical movement or altitudinal positioning of animal and a novel concept for GMF-based vertical coordinate for magnetotactic organisms.

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Poster

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Title: Decoding multisensory information from the parietal cortex: A comparison of maximum likelihood and artificial neural network decoders

Authors: H. MAO[1], Y. SHI[2], G. APKER[2], J. SI[1], *C. A. BUNEO[3];
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Abstracts: Multisensory integration is currently a topic of great interest due to its relevance for understanding perception, the control of action, and adaptation to neural prosthetic and sensory substitution devices. As a result we recently examined the integration of visual and proprioceptive signals in area 5 as monkeys reached to and actively maintained their arm position at multiple target locations in a frontal plane. On half of the trials both visual and non-visual feedback of the endpoint of the arm was available (V condition), while on the remaining trials visual feedback was withheld (NV condition). We previously reported that during the active maintenance of arm positions, vision of the arm decreased spiking activity and spiking variability in area 5. Using a maximum likelihood (ML) decoder, we also showed that such modulations were associated with improved decoding of arm position at the population level. Here we report additional decoding results using an ML decoder (Poisson spiking model) and

compare its performance with that of an artificial neural network (ANN), which makes no assumptions about spiking statistics. Performance of both decoders was validated with a leave-one-out procedure. When the ML decoder was applied to activity obtained from populations of position-modulated neurons (as determined by ANOVA), decoding accuracy increased with sample size, reaching a maximum decoding accuracy of ~85%. Decoding performance gain with vision reached a maximum of ~4% at a population size of ~10 neurons. When the decoder was applied to both tuned and untuned neurons, maximum decoding accuracy dropped to ~40% and peak performance gain with vision reached ~6% at a population size of ~150. When an ANN decoder was applied to position-modulated cells only, decoding accuracy also increased with sample size, reaching a maximum of ~80%, but peak performance gain with vision dropped to ~2% (at a population size of ~10). For the population of tuned and untuned neurons, maximum decoding performance reached ~35%. Peak performance gain with vision reached a maximum of ~1.25% at a population size of ~75 neurons, then decreased for larger populations. The results suggest that vision benefits decoding the most in smaller neural ensembles in area 5.

Disclosures: **H. Mao:** None. **C.A. Buneo:** None. **G. Apker:** None. **Y. Shi:** None. **J. Si:** None.

Poster

625. Multisensory and Motor Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 625.28/EE23

Topic: D.03. Multisensory

Support: NSFC Grant 31301882

Postdoctorfund of China C2013M542301

Title: Directionality and sharpness of neuronal responses to infrared stimuli in tectum of vipers

Authors: ***Q. CHEN**, F. GUANGZHAN, Y. TANG;
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Abstracts: Many animals can sense infrared radiation; however, only pit vipers were assumed to be capable of infrared imaging based on the camera-like pit structure and the infrared receptive topology in the optic tectum resembling the visual one. Reconstruction of infrared images in the central nerve system of vipers was proposed by using an idealized linear formalism. It is still unclear, however, whether the neuronal response can reflect the infrared imaging in the optic

tectum. Using a peltier as the warming source, a programmable robotic arm carrying the peltier moving along four directions (up-down, left-right, left up-right down and left down-right up) and reversal was constructed by our lab to test spatial preferences of infrared neurons. A device with a programmable shutter in front of the peltier was made for testing temporal features of infrared neurons. Our primary results show that the tectal neurons processing the infrared signal have the responsive preferences for moving directions of stimuli with the broader curves of the responsive pulses (50 ms duration) compared with the sharp curves of pulses in visual system (10 ms duration) on short-tailed pit vipers.

Disclosures: Q. Chen: None. **F. Guangzhan:** None. **Y. Tang:** None.

Poster

625. Multisensory and Motor Interactions

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Topic: D.03. Multisensory

Support: NIDCD grant RO1DC006914 to PMD

Title: Retronasal odorants modulate responses of taste cells in the nucleus of the solitary tract of the awake, behaving rat

Authors: *O. D. ESCANILLA, P. DI LORENZO;
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Abstracts: Gustatory and olfactory interaction is important in flavor perception. During food consumption, odors can be sampled orthonasally through the nose or retronasally through the mouth and the internal nares. In previous experiments, we found that olfactory and gustatory signals converge in the nucleus of the solitary tract (NTS) and that orthonasal odorants can modulate the responses of taste-responsive cells in this area. Here we studied whether odorants delivered retronasally will have the same effect on the initial stages of gustatory processing. Rats were implanted with an 8-micowire bundle electrode and allowed to recover for 7 days. Rats were then mildly water deprived and placed in an experimental chamber containing a lick spout for fluid delivery. Gustatory stimuli were 0.1M Sucrose, 0.1M NaCl, 0.01M Citric Acid, 0.0001M Quinine, and artificial saliva (AS). Odor and paired odor-taste stimuli were formulated by diluting each odorant, (0.01%) amyl acetate, (0.01%) acetic acid, (0.01%) phenylethyl alcohol and (0.01%) octanoic acid in either AS (for retronasal odor only presentations) or different

tastant solutions. Odor concentrations were below the detectable level for evoking gustatory or somatosensory responses in rats. Each odor, taste or taste-odor stimulus was presented for 5 consecutive licks separated by AS rinses that were on a variable ratio 5 schedule. A continuous stream of air flowed through an odor port located next to the lick spout to minimize odor contamination. Preliminary results show that retronasal odorants presented by themselves, can elicit a response from taste-responsive NTS cells while retronasal odorants paired with tastants can enhance or attenuate the taste responses in the NTS. These results suggest that retronasal and orthonasal odorants can affect taste responses in the NTS in similar ways. Further, data confirm that a subset of taste cells in NTS are multimodal implying that they may participate in the neural representation of a "food object," even at this early stage of processing.

Disclosures: O.D. Escanilla: None. **P. Di Lorenzo:** None.

Poster

625. Multisensory and Motor Interactions

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Topic: D.03. Multisensory

Support: German Research Foundation, Collaborative Research Centre, SFB 936/A3/B1.

TH is funded by the Emmy Noether program of the German Research Foundation.

Title: Oscillatory alpha activity reflects tactile spatial coordinates differently in sighted and blind individuals

Authors: *J. T. SCHUBERT[1], V. N. BUCHHOLZ[2], J. FÖCKER[3], A. K. ENGEL[2], B. RÖDER[1], T. HEED[1];
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Abstracts: Touch can be represented in skin-based and, when integrated with body posture, in space-based, external coordinates. Behavioral and ERP evidence suggests that sighted individuals automatically recode touch into external coordinates, whereas congenitally blind individuals rely on skin-based coordinates for touch representation, implying a crucial role of vision for external coding in touch. Oscillatory alpha (8-12 Hz) brain activity is thought to reflect

attention-related tactile processing. Therefore, we investigated how the reference frames involved in pre- and poststimulus attentional tactile processing are expressed in EEG alpha activity in sighted and blind humans. In each trial, an auditory cue instructed participants to direct attention to one hand, and a stimulus was presented 1000 ms later either to the attended or the other, unattended hand. A foot response was required to rare deviant stimuli at the attended location. To modulate the external coordinate of tactile stimuli, participants adopted an uncrossed or a crossed hand posture. Power modulations in the alpha band were analyzed in the 500 ms interval preceding stimulation, and 200-700 ms after stimulation onset. In the sighted, expectation of tactile stimulation following the auditory cue elicited a lateralization of alpha activity with uncrossed hands prior to stimulation, that was reduced in the crossed posture. Beamforming localized this posture-related effect in the intraparietal sulcus (IPS). In contrast, pre-stimulus alpha lateralization was unaffected by limb crossing for blind individuals.For sighted and blind groups, poststimulus alpha activity was reduced contralateral to tactile stimulation. In the sighted, hand crossing modulated alpha activity bilaterally, with a smaller alpha decrease for attended, but a larger decrease for unattended stimuli over centro-parietal regions. Blind participants, too, displayed these attention-related alpha band modulations, albeit to a lesser degree than the sighted. Susceptibility of alpha activity in the IPS of the sighted to hand posture suggests that the attentional processing reflected by this frequency band operates in external coordinates both prior to and following tactile stimulation. The stronger alpha decrease for unattended stimuli with crossed than with uncrossed hands may suggest that attention is directed on the basis of both skin-based and external coordinates in the sighted. In contrast, congenitally blind individuals seem to rely predominantly on skin-based coordinates for tactile attention.

Disclosures: **J.T. Schubert:** None. **V.N. Buchholz:** None. **J. Föcker:** None. **A.K. Engel:** None. **B. Röder:** None. **T. Heed:** None.

Poster

626. Eye Movement Behavior

Location: Halls A-C

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Program#/Poster: 626.01/EE26

Topic: D.06. Eye Movements

Support: 1R01 EY021286

Title: Foveal attention modulates saccade frequency during smooth pursuit

Authors: *S. J. HEINEN[1], E. POTAPCHUK[1], S. N. J. WATAMANIUK[2];
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Abstracts: Previously, we showed that foveal stimuli increased the number of saccades during pursuit, presumably because a salient foveal spot activated a position correction mechanism (Heinen et al., SfN 2013). As attention is normally linked to foveating saccades, it might be that foveal targets attract attention during pursuit, causing saccades to occur. To investigate this, we attempted to move attention in and out of the fovea. Observers pursued a stimulus of 4 peripheral dots arranged symmetrically about a central one. At a random time, one of the dots dimmed briefly, and observers had to identify it with a keypress. In a block of trials, either the central dot or the 4 peripheral dots were potential targets, drawing attention into or out of the fovea, respectively. Saccade frequency was highest when attention was drawn foveally, and reduced when it was drawn peripherally, supporting the idea that foveal attention during pursuit was modulating saccades. However, removing the central dot altogether further reduced saccades, indicating that the central spot still attracted attention even though observers knew it was not a potential target. To test whether this was because the attention "window" required to attend to the 4 peripheral dots encompassed the central one, we attempted to move attention completely away from the foveal dot by having observers perform the identification task on a peripheral, miniature version of the 5-dot stimulus (.5 deg diameter). Here the foveal pursuit target remained and the peripheral stimulus moved with it. Saccades were indeed reduced compared to when the attention window encompassed the center, but not as much as when the central dot was removed. The results suggest that attention modulates a position-correcting system used during pursuit, and are consistent with the idea that pursuit of foveal stimuli requires attention.

Disclosures: S.J. Heinen: None. **E. Potapchuk:** None. **S.N.J. Watamaniuk:** None.

Poster

626. Eye Movement Behavior

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Program#/Poster: 626.02/EE27

Topic: D.06. Eye Movements

Support: Action de Recherche Concertée

Fonds de la Recherche Scientifique

Université catholique de Louvain

Title: Physical causality guides saccades during visual pursuit initiation

Authors: K. WENDE[1], A. DROżDżEWSKA[3], B. FELTZ[3], *M. MISSAL[2];
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Abstracts: When a moving object collides with a stationary one that subsequently starts to move, humans 'perceive' a causal interaction. Causality cannot be sensed directly but must be inferred from the spatio-temporal characteristics of objects' trajectories. Albert Michotte (1946) hypothesized the existence of an automatic inference mechanism that could rapidly and implicitly recover the causal “Gestalt” of events. In the present study, we investigated how physical causality might influence catch-up saccade latency during visual pursuit initiation. We hypothesized that if causality is indeed processed implicitly in the visual system, then the initial catch-up saccade during pursuit initiation could have a shorter latency if the pursued target is moving in agreement with the laws of mechanics and a longer latency if the target is moving in a physically unlikely direction. Eye movements were recorded in 16 healthy humans while observing a launching display. The stimulus consisted of a large moving launcher entering the display from a masked periphery and moving toward the center of the screen where it collided with two smaller targets that subsequently started to move. One target moved in a direction that was in agreement with the laws of mechanics (oblique causal target, 'C') and the other target moved along a physically unlikely horizontal trajectory (non-causal target, 'NC'). Subjects were required to pursue the launcher and after the impact to continue to pursue one of the two smaller targets selected freely. Control conditions consisted in either pursuing a small cross instead of a large launcher or fixating a stationary cross prior to motion of the two smaller targets. In both control conditions, one target was moving obliquely and the other horizontally to exactly match the trajectories in the launching condition. We found that saccadic latencies were significantly shorter during launching when the causal target was selected (causal: 192±8ms, n=1758; non-causal: 208±6 ms, n=1629). In the control moving fixation cross condition there was also a significant difference between latencies of oblique and horizontal target motion (oblique: 184±5ms, n=1351; horizontal: 199±2ms, n=1400). However, no difference in latencies was found in the stationary fixation cross condition (189±4ms, n=1629 vs. 189±5ms, n=1572) suggesting that motion trajectory itself is not explaining differences of latencies measured. The effect of causality on catch-up saccade latency is consistent with the hypothesis that physical causality is an implicit prior that is automatically applied by the visual system to predict object motion.

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Poster

626. Eye Movement Behavior

Location: Halls A-C

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Program#/Poster: 626.03/EE28

Topic: D.06. Eye Movements

Support: ANR-13-APPR-0008 REM

Title: Saccadic reaction time distributions follow the matching law in a concurrent random interval reinforcement schedule

Authors: *L. MADELAIN[1,2];
[1]Psychology, Ureca - Univ. Lille 3, Villeneuve D'Ascq, France; [2]Inst. de neurosciences de la Timone, Marseille, France

Abstracts: Studies of decision-making process revealed that animals are sensitive to the sources and timing of rewards and use these variables to choose among alternatives. In particular experiments on foraging behavior indicate that the distribution of time among foraging sites is proportional to their relative value. These relations were expanded to a general principle of choice termed the matching law (Herrnstein, 1961) which states that the fraction of choices made to an option will match the fraction of total income earned from that option. Because saccadic reaction time distributions are known to be strongly affected by reinforcement schedules (Madelain et al, 2007) we now ask whether saccade latencies as well could match reinforcement proportions in a concurrent schedule. Our procedure was similar to the one used by Sugrue et al (2004) except that reinforcement depended on saccade latencies rather than on target choice. We had two subjects make saccades to a visual target stepping horizontally by 10 deg at a 0.6Hz rate. Fast and slow latencies (defined with respect to the first and last quartile of baseline RT distribution respectively) were reinforced in a concurrent Random Interval schedule with unsignaled changes of reinforcement ratios (9/1, 1/1 or 1/9) during extensive training. Using the generalized matching equation we found that saccade latencies were well controlled by the current schedule (sensitivity=0.704, bias=-0.004). A moving 30-trials temporal window revealed that local distributions of latencies were well correlated with the local ratios of obtained reward ($R2=0.79$, $P<0.05$). Interestingly, saccade peak velocities were higher in the “fast” than in the “slow” latencies. Simulations based on leaky integration of reward streams exhibit 80% agreement with human subjects demonstrating that a simple local choice rule may provide an adequate descriptive model of real matching behavior in this dynamic foraging task. These data indicate that saccade latency distributions follow the rules of other choice behavior and may depend on past behavior more than previously thought.

Disclosures: L. Madelain: None.

Poster

626. Eye Movement Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 626.04/FF1

Topic: D.06. Eye Movements

Title: Spatiotemporal distortions in visual scene reconstruction during and shortly after a saccade

Authors: *J. S. ATSMA[1], F. MAIJ[1], D. E. IRWIN[2], W. P. MEDENDORP[1];
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Abstracts: Although we frequently make saccades, we seem to perceive a stable, continuous visual scene. However, the post-saccadic reconstruction of the pre-saccadically viewed visual scene is not always perfect. Small changes in object location during a saccade are often not detected, an observation classically known as saccadic suppression of displacement (SSD). So far, little is known about the impact of SSD on visual targets other than the saccade target (ST). If the region around the ST is most important for anchoring visual stability, as the saccade target theory entails (McConkie & Currie 1996), one could expect that ST displacements are more precisely detected than other targets. Here, we compared SSD of the ST with SSD of the initial fixation target (FT) and a peripheral non-target (NT). The FT, ST, and NT formed an equilateral triangle with 15 deg sides; the saccade was always directed horizontally between FT and ST. During the saccade, two of the targets disappeared while one was displaced (by -5 to 5 degrees) parallel and orthogonal to the saccade direction. The displaced target was presented for three different durations (50 ms, 300 ms, and ~1000ms). Participants (n=8) indicated the remembered pre-saccadic position of this target by using a mouse cursor. We examined the precision (1/SD) and the bias (mean) of the position judgments. Our results show that displacements orthogonal to the saccade are detected more precisely than parallel displacements for both ST and FT, but not for NT. Geometrically, the mean position judgment for each of the targets showed compression towards a point within the triangle of targets. This compression was modulated by the duration of the post-saccadic stimulus, with more compression during the shorter displaced-target durations. These results put limits on the realm of the saccade target theory, providing a signature of the spatiotemporal transformations that are involved in visual scene reconstruction during a saccade.
Reference: McConkie, G.W. and Currie, C.B. (1996). Visual stability across saccades while viewing complex pictures. Journal of Experimental Psychology, 22, 563-581.

Disclosures: J.S. Atsma: None. **F. Maij:** None. **D.E. Irwin:** None. **W.P. Medendorp:** None.

Poster

626. Eye Movement Behavior

Location: Halls A-C

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Program#/Poster: 626.05/FF2

Topic: D.06. Eye Movements

Title: Change of gaze by retinal movements during locomotion by jumping spiders

Authors: D. B. ZUREK[1], *C. GILBERT[2];
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Abstracts: Visual properties of eyes are not uniformly distributed across the field of view, thus animals with moveable eyes change their direction of gaze to view objects of interest with the high acuity region of the retina. In jumping spiders, the principal eyes perform high acuity inspection and spectral analysis of objects. Their retinas can move to change the gaze direction through the lens, which is fixed in the exoskeleton. We have filmed these retinal movements in the horizontal plane as transparent spiders walked freely in a blank arena, tracked a horizontally moving target with retinal and body movements, and inspected a scaffolding to select an escape route. Retinal scanning movements occur independently or synchronously in the two eyes. Retinal movements may occur while the spider walks, but occur more frequently when stopped. The angular range of gaze movement is greater in the ipsilateral direction. Consequently, when a moving target approaches in the spider's peripheral visual field, the ipsilateral retina begins tracking the target first. As it passes into the contralateral field of view the other retina begins to follow the target more closely. When this retina reaches the end of its movement range, a body turn occurs.

Disclosures: D.B. Zurek: None. **C. Gilbert:** None.

Poster

626. Eye Movement Behavior

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Topic: D.06. Eye Movements

Support: R01 DC008585

Title: Time-frequency analysis of the precue effects on saccade latency in behaving monkeys

Authors: *J. HUANG[1,5], K. SONG[6], Y. XU[1], I. SIMPSON[1], K. KOSEK[2], Y. ZHOU[5], H. ZHU[1], W. ZHOU[1,3,4], H. LUO[6];
[1]Departments of Otolaryngology and Communicative Sci., [2]Departments of Ophthalmology, [3]Departments of Neurobio. and Anatom. Sci., [4]Departments of Neurol., Univ. of Mississippi Med. Ctr., Jackson, MS; [5]Dept. of Neurophysiol., Sch. of Life Sciences, Univ. of Sci. and Technol. of China, Hefei, China; [6]State Key Lab. of Brain and Cognitive Sci., Inst. of Biophysics, Chinese Acad. of Sci., Beijing, China

Abstracts: It has been well documented that a cue preceding a target modifies the saccade latency to the target. This cue effect on saccade latency has served as an effective model to study immediate plasticity in sensorimotor transformations. A recent study performed time-frequency analysis on precue effects in a visual discrimination task (Song et al., 2014). A surprising finding was that the precue induced dynamic oscillations at α-band frequencies (8 - 20Hz) in reaction time measurements. In the present study, we employed the time-frequency approach to examine the temporal characteristics of precue effects on saccade latency in a monkey. The monkey was trained to fixate a central target while a cue was presented for 75ms at left or right 10 degree. After a varying interval of cue-to-target, i.e., stimulus onset asynchrony (SOA) (20ms to 1020ms at a step of 20ms), a target was presented at the same (valid condition, 50%) or opposite (invalid condition, 50%) location with respect to the cue. The monkey was trained to make a saccade to fixate the target for juice rewards. Saccade latency was measured for each condition and plotted as a function of SOA. Compared to the control condition, there is a reduction in saccade latency in both valid and invalid conditions, indicting a general alert effect of the cue. Interestingly, we found that the reduction of saccade latency in the invalid condition was larger than that in the valid condition, indicating the monkey's interpretation of 50% cue validity was biased toward the invalid condition. The time-frequency analysis (the short-time Fourier transform) revealed that precue induced oscillations in saccade latency at α-band frequency at SOAs of 100ms, 300ms and 800ms. In other words, the α-band oscillations are modulated at θ band frequencies (3 - 5Hz). These preliminary results suggest that time-frequency analysis is a powerful tool to elucidate the temporal structures of the precue effects on saccade generation. Ongoing studies will further characterize these structures and investigate the underlying neural mechanisms.

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Poster

626. Eye Movement Behavior

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Program#/Poster: 626.07/FF4

Topic: D.06. Eye Movements

Support: Supported by NSERC.

Title: Visual and auditory targets elicit a unidirectional prosaccade switch-cost

Authors: *M. D. HEATH, F. E. STARRS, E. A. MACPHERSON, J. WEILER;
Univ. of Western Ontario, London, ON, Canada

Abstracts: An antisaccade is a non-standard task that requires decoupling standard (i.e., direct) stimulus-response relations and executing a response to a target's mirror-symmetrical location. As well, the completion of a directionally correct antisaccade is linked with changes in neural activity within high- and low-level oculomotor networks ¬- a modulation that is thought to reflect a presetting that prevents the evocation of a stimulus-driven prosaccade. Recent work by our group (e.g., Weiler and Heath 2014: Acta Psychologica) has shown that antisaccade presetting elicits a lingering inhibition of oculomotor planning mechanisms. In particular, the execution of an antisaccade to a (visual) target selectively delays the reaction time (RT) of a subsequent prosaccade; however, the converse switch does not influence RT (i.e., the unidirectional prosaccade switch-cost). The goal of the present investigation was to determine whether the aforementioned switch-cost is: (1) selectively tied to decoupling the standard response mapping of a visual stimulus, or (2) relates to a persistent and sensory-independent activation of a non-standard task-set. To accomplish our objective, participants randomly alternated between pro- and antisaccades in separate conditions wherein targets were defined via visual or auditory stimuli. As in previous work, results elicited a reliable unidirectional prosaccade switch-cost; that is, prosaccades preceded by antisaccades were associated with an increase in RT. Most notably, the magnitude of the switch-cost was equivalent between the visual and auditory conditions. As well, it is important to note that the switch-cost could not be attributed to a speed-accuracy trade-off in movement planning as the endpoint accuracy and variability of prosaccade switch trials was comparable to their non-switch trial counterparts. Taken together, results show that a sensory-independent task-set associated with antisaccades persists inertially and delays the planning of a subsequent prosaccade.

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Poster

626. Eye Movement Behavior

Location: Halls A-C

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Program#/Poster: 626.08/FF5

Topic: D.06. Eye Movements

Title: Blinking during torsional optokinetic eye movements partially resets eye torsion

Authors: ***M. F. KHAZALI**, A. SMILGIN, F. BUNJES, P. THIER;
Cognitive Neurol., Hertie Inst. For Clin. Brain Res., Tübingen, Germany

Abstracts: Torsional optokinetic nystagmus (tOKN) is a reflexive ocular motor response evoked by looking at a rotating stimulus. It consists of a slow phase in which the eye tries to follow the rotation of the visual stimulus in order to reduce the slip of its retinal image followed by a saccadic fast phase torsion (FP) in the opposite direction. The FP ensures that the eyes stay within the mechanicals limits of the plant. On the other hand, the FP inevitably impairs vision because it induces a "saccadic suppression" of the impact of the high velocity motion of the background image during the FP. Blinking, carried out at frequencies of 15 to 30/ min in order to keep the cornea moist, also causes a transient interruption of normal vision because of the co-occurrence of a blink related eye movement (EM) (Volkmann, Riggs et al. 1980). The raison d´etre of these EMs is not fully clear. They may help to hydrate the cornea. On the other hand, they may be a consequence of an important additional role of blinks, namely to protect the eyes against potentially harmful stimuli. Finally, blinking EMs may serve to exploit the EM related suppression of the visual distortions accompanying blinks. As EM related suppression decreases the time available for scrutinizing the visual world, we reasoned that coordinating blinks with FP might be a useful strategy. We tested this hypothesis in a group of 5 healthy human subjects in whom we evoked tOKN around the naso-occipital axis. We used video oculography to record blinks and eye position in 3D. We found that practically every blink was accompanied by a partial resetting of torsional eye position in the direction of the starting position, thereby partially compensating the deviation caused by the slow components of the tOKN. The amount of resetting depended linearly on the torsional eye deviation at blink onset. Surprisingly, the amount of resetting of eye position brought about by FP components independent of blinks was unrelated to eye deviation at FP onset. Blanking the optokinetic stimulus for a period corresponding to the duration of a blink did not cause a resetting of eye position. In accordance with this result, we also failed to find a correlation between blink duration and the amount of resetting. These

findings suggest that the oculomotor system shapes the eye movements required by blinks such as to at the same time serving the needs of the tOKN.

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Poster

626. Eye Movement Behavior

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Topic: D.06. Eye Movements

Support: Grant-in-Aid for Challenging Exploratory Research 24650105

Title: An advanced real-time monocular/binocular eye tracking system using a high frame-rate digital camera

Authors: ***K. MATSUDA**[1], A. TAKEMURA[1], K. MIURA[2], T. OGAWA[2], K. KAWANO[2]; [1]AIST, Tsukuba, Japan; [2]Kyoto Univ., Kyoto, Japan

Abstracts: We developed a new eye tracking system by adopting an IEEE-1394b or an USB3.0 digital camera that provides high sensitivity, high resolution, and high frame rate. The system is non-invasive and inexpensive and can be used for mice, monkeys, and humans. An infrared light illuminates one eye, and its reflected image on the cornea and the black image of the pupil are captured by the camera. The center of the pupil is calculated by fitting an ellipse and tracked over time. The reflected image on the cornea is used to compensate head-movements. The system has two-steps of calibration. Step-1, "passive calibration": when the subject spontaneously moves its eye, the system calculates the rotation center of the pupil, the rotation radius of the pupil, and the cornea curvature center in camera-coordinates. Step-2, "active calibration": when the subject fixates on small targets (from 3 to 9) on a computer display successively, the system provides a transition matrix for eye position from camera-coordinates to target-coordinates. Thus, after the passive and active calibration, we are able to measure the eye position in target-coordinates. However, since the active calibration is not realistic on mice, the eye movements of mice are recorded only in camera-coordinates. The eye position and pupil size data are read out on-line via computer network to be stored for off-line analysis and/or outputted as analog voltage through a digital-analog converter. The adoption of the WINDOWS 7, 8, 8.1 x 64 as the operation system makes this eye tracking system user-friendly. The program is available at https://staff.aist.go.jp/k.matsuda/eye/. Recent advances in inexpensive high-quality

digital cameras make the system applicable in measuring human binocular eye movements. We adopted a wide-field, hi-resolution, and hi-frame-rate camera of 2048x2048 pixels resolution and captured the images of both eyes simultaneously and calculated positions of the two eyes at each frame. Because of the high frame rate of the digital camera, the sampling rate of the system can be as high as 700Hz. By using this system, we succeeded in characterizing vergence eye movements of humans when ocular fixation shifts between two targets placed at different distances in the 3-D space.

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Poster

626. Eye Movement Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 626.10/FF7

Topic: D.06. Eye Movements

Support: NIH Grant 1R01 EY021286

Title: Interspersing fixation trials better reduces anticipatory pursuit than randomizing target direction

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Abstracts: Typical pursuit latency for humans is approximately 130 msec. However, it can be difficult to accurately identify pursuit onset because anticipatory pursuit prior to stimulus motion obscures the onset of stimulus-driven pursuit. Anticipatory pursuit can also impact measurement of open-loop pursuit parameters. To mitigate anticipatory pursuit, it is common to randomize pursuit direction. While randomization reduces anticipatory pursuit, it does not eliminate it (Kowler & Steinman, 1979; Heinen et al., 2005). Here we show that interleaving fixation trials among pursuit trials virtually eliminates anticipatory pursuit. Observers pursued a small spot target that moved at a constant speed. The target either started at the center of the display, or at a display edge, a condition that specifies target direction uniquely. As a baseline, we alternated or randomized pursuit direction (left & right). Then, pursuit direction was held constant, and within a block, either fixation trials or blank trials (no stimulus) were interleaved. Anticipatory velocity

was measured at target motion onset. We found that anticipatory velocity was highest when target motion was repeated in the same direction, or when target direction alternated left and right and target motion began at an edge. As expected, randomly alternating pursuit direction from a central position reduced anticipatory pursuit. However, it was reduced even more if pursuit trials alternated with fixation trials, and longer fixation trials resulted in a greater reduction. Fixation trials randomly interspersed among pursuit trials were the most effective at reducing anticipatory pursuit. Interleaving blank trials did not reduce anticipatory pursuit, nor did simply extending the random fixation period prior to stimulus motion onset, suggesting that knowing fixation trials were possible actively suppressed anticipation. The results demonstrate a heretofore undocumented interaction between fixation and pursuit circuitry.

Disclosures: S.N. Watamaniuk: None. **S.J. Heinen:** None.

Poster

626. Eye Movement Behavior

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Topic: D.06. Eye Movements

Support: Naturalia et Biologia (NEB)

Title: Sensitivity of catch-up saccades kinematics to repeated unperceived changes of target velocity

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Abstracts: Saccades and smooth pursuit are combined to ensure a clear vision while tracking a moving object. Because the pursuit gain (ratio between eye velocity and target velocity) is normally inferior to one, the eyes frequently lag behind the target (Meyer et al., 1985). Accurate foveation therefore requires catch-up saccades that are triggered by the central nervous system to cancel this growing error and bring the eye closer to the target. Interestingly, as initially pointed out by Dodge (1903), visual acuity is markedly decreased during saccades. McLaughlin (1967) took advantage of this phenomenon to design a new paradigm in which target location is systematically altered during the saccade by a constant displacement. A key point of his protocol is that subjects do not perceive the change of target position during the saccade. In the first trials, saccades land near the initial target position, thus requiring a corrective saccade towards the new

target position. However, after a series of similar trials, saccades land progressively closer to the modified target position. This well known behavior, called saccadic adaptation, has been extensively studied since. Here, we investigate using a new paradigm the effect of a change of target velocity during catch-up saccades on their kinematics. Subjects sat in dark room and looked at a screen (refresh rate: 144 Hz) located 60 cm ahead of them. They were required to look at a moving target. After a central fixation, the target jumped diagonally (randomly upward or downward: amplitude [5..15] deg. Horizontal step: -0.1*horizontal target velocity) and started to move horizontally (either leftward or rightward randomly) with a random velocity (amplitude: [20..30] deg/s). Subjects had to trigger a saccade towards the moving target to initiate the movement. During this saccade, the amplitude of the target velocity was systematically increased by 20 deg/sec. A test block was composed of 800 trials with a change of velocity. Before the test block, a block of 200 trials without a modification of target velocity was done to build the baseline behavior of the subjects. Subjects did not do more than one test block per day. Subjects did not perceived the modification of target velocity during the saccade. We analyzed the saccade trajectory and found an effect of the target velocity step on the horizontal component of the saccade. We also found a modification of the pursuit velocity at the end of the saccade correlated with the change of target velocity. Our results suggest that the unperceived change of target velocity during the saccade is taken into account by the central nervous system to alter both the saccade trajectory and the pursuit behavior.

Disclosures: **C. Kayal:** None. **B. Gaymard:** None. **P.M. Daye:** None.

Poster

626. Eye Movement Behavior

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Topic: D.06. Eye Movements

Support: BMBF Grant 01GQ1005C

GIF Grant 110879.1/2010

Title: Slow eye movements reflect human decision-making about visual motion direction

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Abstracts: The random-dot-motion (RDM) task, which tests motion signal perception in noise, has proved extremely useful in the study of sensory decision-making. Neuronal recordings in monkeys have revealed how motion-direction information is accumulated between stimulus onset and a monkey's decision. However, a non-invasive and easily-measured variable that reflects the dynamics of decision-making in humans at a fine temporal scale remains to be found. Here, we evaluate whether slow eye movements (SEMs) in humans viewing a RDM pattern are correlated with their ongoing decision-making process, inspired by the known parallels between motion perception and SEMs (like smooth pursuit). Human subjects viewed a low-coherence RDM pattern containing signal dots moving to the left or right and indicated their decision about motion direction using either a keyboard press (Experiment 1), a horizontal saccade (Experiment 2) or a vertical saccade (Experiment 3). Independent of response modality, their eyes slowly moved ("drifted") on average in the direction of the impending decision in all three experiments. Trials with SEMs were associated with better performance. The average SEMs on error trials indicated the direction of the subject's incorrect response, rather than the direction of the coherently moving dots. SEMs in the direction of the subjects' response were also found in trials using zero-coherence stimuli. SEMs appear to show a pattern similar to that of the neural activity of single neurons in monkey parietal cortex reported in the literature. SEMs may thus provide a non-invasive window into decision-making and allow the testing of motion-processing models with high temporal resolution.

Disclosures: S.B. Krishna: None. **E. Poland:** None. **S. Glim:** None. **B. Eichelberger:** None. **S. Treue:** None.

Poster

626. Eye Movement Behavior

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Program#/Poster: 626.13/FF10

Topic: D.05. Visual Sensory-motor Processing

Support: NWO-MaGW 404-10-142

Title: Neural mechanisms of visuomotor updating within an illusory context

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Abstracts: Performance of the double-step saccade task requires visuomotor updating: the spatial dimensions of the second saccade must be computed based on the initial retinal coordinates of the target and the metrics of the intervening first saccade. The posterior parietal cortex (PPC) has been implicated in this gaze-centered computation. What is the role of visuospatial contextual information in this updating process? We used 3T fMRI to examine how parietal representations during a double step saccade task are affected by a visual contextual illusion. While in the scanner, subjects briefly viewed a horizontal Brentano version of the Müller-Lyer illusion with a target at its middle vertex, while fixating at one of the two endpoints of the illusion. Next, the eyes' fixation point jumped to a position above or below the target, orthogonal to the orientation of the illusion. After a delay, subjects made a saccade to the remembered position of the target. Behavioral results showed that the visuomotor updating process is distorted by the Brentano illusion. The direction of the second saccade was not purely vertical, but had a small horizontal component in the direction of the illusion. Because the PPC has a gaze-centered organization, we expect a contralateral bias in BOLD response preceding the second saccade if the PPC contains an erroneous target representation due to the illusion. Our results suggest that target representations in the PPC are indeed affected by this contextual information.

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Poster

626. Eye Movement Behavior

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Program#/Poster: 626.14/FF11

Topic: D.06. Eye Movements

Title: Micro-meter magnitude components of eye movement separated from miniature head motion revealing pro- and anti-correlation vectors

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Abstracts: Study of miniature eye movements reveals that micro-saccades are important in visual perception and attention when we fixate our eyes to a specific spot. Details of miniature eye movements such as ocular drifts and tremors were yet to known due to various sources of noise and technical limitations. By developing a novel video occulography (VOG) using an ultra-high speed camera, micro-meter level images of eye and head were tracked by SURF method (Tanaka et al., 2014). As a result, we could extract pure components of miniature eye movements in tenths of micro-meter order, which is independent of head motion in a simple fixation task. Spatial, temporal, and frequency analyses reveal that two distinct types of miniature eye movements: one 10 to 50 micro-meter order, and the other smaller than this with higher temporal frequencies (60 to 100 Hz). Furthermore, we found two different types of miniature head motion, one which is comparatively large with the degree of 10 to 50 micro-meter order, and the other which is smaller with higher temporal frequencies. Correlation analysis between eye and head motion yielded in the both pro- and anti-correlation vector components of eye and head movements over seconds in both large and small scales, representing a miniature version of vestibular ocular reflex (VOR). These results illustrate a novel aspect of eye-head coordination of their micro meter magnitude.

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Poster

626. Eye Movement Behavior

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Title: Seeing space through time: Visual consequences of eye movement transients

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Abstracts: Humans acquire visual information by alternating fast relocations of gaze (saccades) with periods of "fixation", in which microscopic eye movements continually occur. Although it is well established that the visual system is highly sensitive to temporal transients, relatively little attention has been paid to how the modulations resulting from the continual alternation between macroscopic and microscopic eye movements affect spatial vision and its dynamics. To address this question, we report results from a combination of techniques, including spectral analysis of the spatiotemporal input signals to the retina, neural modeling of the responses of retinal ganglion cells, and psychophysical experiments with precise control of retinal stimulation. We first show that, during fixation on natural scenes, saccades and the smooth fixational motion of the eye (a movement commonly known as ocular drift) yield temporal modulations with highly different spatial distributions. Specifically, ocular drift equalizes temporal power over a broad range of spatial frequencies (Kuang et al., 2012), but saccadic transients leads to power at low spatial frequencies. In a model based on the responses of simulated retinal ganglion cells, the transition between these two spectral distributions yields specific predictions regarding the dynamics of contrast sensitivity: fixational eye movements enhance sensitivity to high spatial frequencies, while saccades mainly contribute to vision at low spatial frequencies. Finally, we validate these predictions by means of psychophysical tests of contrast sensitivity using high-resolution eye-tracking and a system for gaze-contingent display. We show that elimination of saccadic transients and fixational modulations selectively affects sensitivity at low and high spatial frequencies, respectively. These findings show that the interplay between saccadic and fixational eye movements results in a coarse-to-fine dynamics of visual perception in each intersaccadic interval.

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Poster

626. Eye Movement Behavior

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Program#/Poster: 626.16/FF13

Topic: D.06. Eye Movements

Title: Eye movements during a transition between different non-constant target velocity profiles

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Abstracts: Two types of eye movements are combined while tracking a moving object: smooth pursuit and saccade. Saccades are rapid redirections of the visual axis between two centers of interest. Smooth pursuit keeps the image of a moving target on the fovea. Because the pursuit gain is smaller than one (Meyer et al., 1985), the eye would increasingly lag behind the target without any correcting movements. Thus, "catch-up saccades" are triggered by the central nervous system (CNS) to cancel this growing position error between the eye and the target. Using a ramp-step-ramp paradigm, De Brouwer et al (2002) showed that a multiple regression using both position and velocity errors sampled 100 ms before saccade onset account for 96% of the variance of saccade amplitude. These authors suggested that this 100 ms delay represents the sensory delay of the visual system. Using a target with a non-constant velocity, Daye et al (2014) showed that catch-up saccades amplitude is better correlated with position and velocity errors sampled at saccade onset than with these parameters sampled 100 ms before saccade onset. Because of the inherent sensory delays, the authors suggested that the CNS uses an internal model of target movement to correct catch-up saccades amplitude. Daye et al. (2014) proposed that the same internal model could be used to control pursuit and to correct catch-up saccades amplitude. This study analyzes how the CNS controls eye movements (smooth pursuit and catch-up saccades) during the transition between two target trajectories with non-constant velocity profiles. After a 500 ms central fixation, subjects were required to track a horizontal moving target with a sinusoidal velocity (peak to peak displacement: 24°, frequency: 0.75 Hz). After either [2.375, 2.4375, 2.875, 2.9375] cycles of motion, the target jumped (amplitude randomly selected in [-1,0,1]°) and started to move with another non-constant velocity profile. The velocity could be either exponential, quadratic or sinusoidal (control condition without position step). Nine different target trajectories (4 exponentials, 4 quadratics and 1 control) were randomly intermixed during a block of 72 trials. Subjects did not do more than 4 blocks a day. After the pursuit initiation, eye velocity is correlated with target velocity during the first part of the protocol (sinusoidal velocity). Three hundred milliseconds after the change of target motion, eye velocity is better correlated with second target than with the sine if it has persisted. In between, our results suggest a smooth transition from one pursuit mode to the second. The analyses of catch-up saccades kinematics also show a transition.

Disclosures: E. Hainque: None. **E. Apartis-Bourdieu:** None. **P.M. Daye:** None.

Poster

626. Eye Movement Behavior

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Topic: D.06. Eye Movements

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Title: Prior experience biases the oculomotor response to a moving target

Authors: *N. DERAVET[1], J.-J. ORBAN DE XIVRY[1], G. BLOHM[2], P. LEFEVRE[1];
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Abstracts: The brain relies on prior experience to predict the trajectory of a moving target. This information is crucial when the target transiently disappears or when visual information is noisy. In a recent model, Orban de Xivry et al. (2013) suggested that the impact of visual and prior (predictive) information on smooth pursuit eye movements is weighted by their reliability. Here, we test this hypothesis. Thirteen subjects were asked to pursue a (visually noisy) moving target that consisted of a Gaussian spot with a standard deviation of 1.27 deg. In this experiment, a prior was built by presenting identical target motion (15 or 20 deg/s) for several trials in a row (training trials). After a series of training trials, the velocity of the target was either increased or decreased by 5 deg/s (catch trials). Catch trials (20 minus 5 deg/s or 15 plus 5 deg/s) were compared to training trials with (1) an identical number of preceding training trials and (2) matching target velocity (control trials). By selecting these trials, we made sure that visual information was identical between control and catch trials whereas prior information from training trials was different (20 vs. 15 deg/s or 15 vs. 20 deg/s). We compared the gain of the smooth pursuit response during steady-state pursuit and the features of catch-up saccades across catch and control trials. The eye velocity gain during catch trials was influenced by prior information. That is, when a catch trial with a 15 deg/s target followed a series of training trials with a 20 deg/s target, the observed velocity gain during the catch trials was higher than for 15 deg/s control trials (main effect of trial type: $p<0.001$). In other words, prior information about a 20 deg/s moving target influenced the visually-guided smooth pursuit response to a 15 deg/s target. The same pattern of response was observed when target velocity was reduced during the catch trial (from 20 deg/s to 15 deg/s). In this case, the steady-state eye velocity gain was higher during the catch trials than during control trials with identical target motion but different prior

(main effect of trial type: $p<0.001$). Furthermore, catch-up saccades were also influenced by prior information. Catch-up saccades made during catch trials with a 15 deg/s target (following training trials with 20 deg/s target) had higher amplitudes than saccades of corresponding 15 deg/s control trials ($p= 0.0175$). The opposite effect ($p<0.0001$) was found in catch trials (20 deg/s) following training trials with a slower target (15 deg/s). These observations suggest that prior information about target motion is readily incorporated in the motor commands that drive both smooth pursuit and catch-up saccades.

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Poster

626. Eye Movement Behavior

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Topic: D.05. Visual Sensory-motor Processing

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Title: Smooth pursuit eye movements in the common marmoset

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Abstracts: Smooth pursuit eye movements have been extensively studied in Old World primates (macaques) and humans providing critical insight into visual processing, choice, motor planning and plasticity. Pursuit eye movements allow the stabilization of slow moving objects on the retina by matching eye velocity with target velocity. This behavior is associated with specific cortical circuits that includes areas MT, MST and the FPA. To date, considerable variation has been reported across different New and Old World primate species for other specialized movements, such as fine grasping, along with corresponding anatomical variation in cortical-motor circuitry (Bortoff and Strick, 1993). Here we investigated if the common marmoset, a small New World primate, exhibits smooth pursuit eye movements. Two critical components are

required to generate smooth pursuit: 1) a moving stimulus and 2) the primate's selection a target to pursue in order to engage the tracking system. If the marmoset were to exhibit this behavior, it could provide an ideal platform to study sensory-motor transformations, as both parietal and frontal cortical areas are accessible given its lissencephalic brain. We measured smooth pursuit in two marmosets, previously trained to perform fixation tasks, using the Rashbass step-ramp paradigm. Initial pursuit eye movements were in the direction of the target motion indicating that pursuit is driven by target movement rather than target displacement. To determine how motion signals drive smooth pursuit we measured the relationship between target speed and eye acceleration during the open-loop interval. Initial eye acceleration strongly depended on the speed of horizontal target motion with faster targets evoking larger eye accelerations (4 deg/s: 32 deg/s2, 8 deg/s: 52 deg/s2), in agreement with previous measurements in macaques (Lisberger & Westbrook, 1985). We displayed faster targets (12 deg/s), but both animals had difficulty pursuing these targets, suggesting that smooth eye movements in marmosets are constrained to slower speeds than in macaques. Smooth eye movements were evoked by motion along both horizontal and vertical axes, but vertical pursuit had both smaller initial eye accelerations and reduced gain during maintenance. In sum, we have demonstrated that smooth pursuit eye movements naturally occur in marmosets to moving targets and these eye movements have similar characteristics to the eye movements observed in other non-human primates.

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Poster

626. Eye Movement Behavior

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Topic: D.05. Visual Sensory-motor Processing

Title: Characterization of the optokinetic and optomotor response in mice

Authors: *F. KRETSCHMER, T. C. BADEA;
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Abstracts: The mouse is the most common biological model organism due to it's fast reproduction cycle, relatively small size and the vast amount of genetic strategies that were developed to manipulate this species. To study the visual system of this animal various methodologies exist to examine the anatomy and physiology of the various tissues involved in the visual pathway. A limitation of these methods is that crucial questions e.g. whether or not a

specific drug can recover a vision deficit caused by a specific disease can only be addressed very indirectly and reveal little information about the properties of the actual visual performance of the animal as a whole. Since mice are unable to directly communicate their perception, objective behavioral criteria are needed to determine visual thresholds. Unfortunately mice are hard to train to perform behavioral tasks. In this study we present a novel setup that can be used to automatically evoke optokinetic and optomotor responses in mice. The resulting head and eye-movements can be recorded and quantified to determine various properties of the visual system in a robust, fast, and entirely objective way. Stimuli are presented on four computer screens surrounding the animal covering the whole field of view as textures on the surface of a virtual sphere. The sphere is positioned in such a way that the animal is always located in the center. Hence the distance to all areas of the stimulus is held constant. This is an important aspect to measure parameters like spatial acuity that are dependent on the perceived size and therefore on the distance between the head and the presented stimulus. Head and body movements are measured without the need to restrain the animal while the measurement of eye movements requires a fixation of the animal to keep the eye in the focus of a camera. The former are recorded by video tracking the snout and body axis of the animal in real time. This data is also used to objectively quantify optokinetic responses. Our self-developed video tracking algorithm continuously determines the location of the animal head that is used to readjust the position of the virtual sphere. The building plans and software of this setup are going to be released under an open source license in near future. We are currently using the setup to determine the spatial acuity, contrast thresholds, the temporal resolution, the visual field and the directional tuning in wild type mice. This data will help us to design experiments in future, establishing a baseline that enables us to analyze the visual deficits caused by mutations and diseases. For this purpose we already recorded various known mouse models for retinal degeneration at multiple time points.

Disclosures: **F. Kretschmer:** None. **T.C. Badea:** None.

Poster

626. Eye Movement Behavior

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Zurich Center for Integrative Human Physiology (ZIHP)

Betty and David Koetser Foundation for Brain Research

Title: Disconjugacy of eye movements in zebrafish larvae

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Abstracts: In zebrafish larvae, the two eyes mostly move in the same direction and their saccades occur at the same time. However, the eye movements appear to be disconjugate. First, the saccadic peak velocities and amplitudes during optokinetic stimulation and in the dark are significantly higher in the nasal-to-temporal (N-T) than the temporal-to-nasal (T-N) direction. Second, the time constant of centripetal eye drift in the dark is higher in the T-N direction than the N-T direction. Third, during optokinetic stimulation, the median eye velocity is lower in the N-T than in the T-N direction. Moreover, the beating field shifts more eccentrically when slow phases point in the N-T direction, although maximum slow-phase eye velocities are not significantly different between slow phases in the N-T and T-N directions. In this study, we investigated possible mechanisms of this horizontal disconjugacy using the top-down approach. A mathematic ocular motor model comprising an optokinetic system, an eye plant, burst neurons, and a velocity-to-position neural integrator (VPNI) was designed to simulate the larval eye movements. The model parameters were estimated based on the empirical data. A subsequent modeling-parameter analysis was performed to simulate how single subsystems affect the eye movements. The modeling results suggested that the ocular motor control signals in zebrafish larvae are separate for the two eyes and the two directions in the orbit (N-T vs. T-N), suggesting four distinct neural networks, i.e. two on both sides.

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Poster

626. Eye Movement Behavior

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Topic: D.05. Visual Sensory-motor Processing

Support: NSF IOS-0843354

Title: Measuring ocular drifts for the study of natural images

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Abstracts: As we scan natural images, the eye encounters fine as well as coarse detail. The information about this detail is acquired primarily during intersaccadic drift periods. Mounting evidence indicates that the drift motions of the eye are a feature that enhances fine detail, but physiological studies have not analyzed the effects of these microscopic drifts. Responses of visual neurons to natural images have generally been studied as if the eye is stationary during fixation. One reason for this situation is the challenge of measuring ocular drift with high accuracy and precision. We have been evaluating methodology for the purpose of studying neural responses to natural images with high precision during drift periods. To characterize the accuracy and precision of an eyetracker for measuring ocular drift requires utilization of a model eye or eyecoil that can mimic the signal from a real eye but be rendered absolutely stationary. This requirement has not been met for the most popular video eyetrackers, which have known artifacts related to decentration of the pupil. For two systems that are not affected by this artifact, an eyecoil (Remmel labs EM6) and a dual-Purkinje image eyetracker, (DPI v.6) we have made measurements of gain, noise and drift with model systems to assess the eyetrackers' ability to measure ocular drift. At maximum gain, the optimized eyecoil system with a bandpass of 0-320 Hz had an RMS noise level of 0.08 minarc and slow drifts over a duration of 10 min that were less than 0.03 minarc. The RMS noise level of a DPI eyetracker, measured with a model eye, was approximately 0.35 minarc for both the horizontal and the vertical axes. These values represent the best performance that could be obtained, but they will be degraded by potential artifacts that will be discussed. One such factor is unwanted micro head movements. The head is often only partially immobilized by means of a chin-rest during recordings with video eyetrackers, and residual head motion may occur even when the head is restrained with a bitebar. We have examined the amount of head motion under these different conditions and its impact on the quality of the recordings. We also compared standard calibration procedures with a novel procedure based on a gaze-contingent display and we find the new procedure can improve the accuracy of gaze localization by more than a factor of two on both axes. These results should facilitate development of standard procedures for optimizing study of the dynamics of microscopic ocular drift. Examples of drift measurements in the presence of natural images will be presented, perhaps with some neural responses as well.

Disclosures: **D. Snodderly:** None. **H. Ko:** None. **M. Poletti:** None. **M. Aytekin:** None.

Poster

626. Eye Movement Behavior

Location: Halls A-C

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Program#/Poster: 626.22/FF19

Topic: D.06. Eye Movements

Support: NIH EY022854

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Title: Color cannot be used as a contextual cue during rhesus monkey saccadic adaptation

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Abstracts: When the head does not move, rapid movements of the eyes called saccades are used to redirect the line of sight. Saccades are defined by a series of metrical and kinematic (evolution of a movement as a function of time) relationships. For example, the amplitude of a saccade made from one visual target to another is roughly 90% of the distance between the initial fixation point (T0) and the peripheral target (T1). However, this stereotypical relationship between saccade amplitude and initial retinal error (|T1-T0|) may be altered, either increased or decreased, by surreptitiously displacing a visual target during an ongoing saccade. This form of saccadic adaptation has been described in both humans and monkeys. We tested the hypothesis that a contextual cue (target color) could be used to evoke differential gain (actual saccade/initial retinal error) states in rhesus monkeys. We did not observe differential gain states correlated with target color during horizontal adaptation regardless of whether these targets were displaced simultaneously, sequentially, along the same vector as the primary saccade or perpendicular to it. These results are consistent with hypotheses that state that color cannot be used as a contextual cue and are interpreted in the context of previous studies of contextual saccadic adaptation in both humans and monkeys.

Disclosures: **A.L. Cecala:** None. **I. Smalianchuk:** None. **S.B. Khanna:** None. **M.A. Smith:** None. **N.J. Gandhi:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NHMRC grants 631000 & 1043933

BBSRC grant BB/J000620/1

Title: Phasic and tonic types of glycine-mediated inhibition dominate in parvalbumin positive dorsal horn interneurons

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Abstracts: The dorsal horn of the spinal cord is an important site for modality specific processing of sensory information related to nociception, touch, temperature and itch. Importantly, segregation of these modalities is essential for contextually relevant sensory experience. When modality segregation fails aberrant sensory experiences such as allodynia may emerge. We have recently described a population of inhibitory parvalbumin-positive interneurons (INs) with functional properties and connectivity that would enable them to segregate tactile and nociceptive information (Hughes et al, 2012 J Physiol 16:3927). These parvalbumin-positive INs receive weak excitatory synaptic input, which is surprising as the output of neural circuits is governed by the opposing action of excitatory and inhibitory inputs. Here we examine inhibitory drive to parvalbumin-positive INs using targeted patch-clamp recording in transgenic mice that express enhanced green fluorescent protein (eGFP) in parvalbumin-positive INs. Adult mice (2-3 months old, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated. Transverse spinal cord slices (300 um thick) were prepared from the lumbar cord. Recordings were made with a CsCl-based internal at 34^{o}C (holding potential -70 mV). Phasic and tonic forms of inhibition were detected in all recordings from parvalbumin-positive INs (n = 9). Miniature inhibitory postsynaptic currents (mIPSCs) had large mean amplitudes (124 ± 23 pA), fast rise (0.78 ± 0.09 ms) and decay times (6.12 ± 0.54 ms), and occurred at a relatively high frequency (1.31 ± 0.25 Hz). Bath application of bicuculline (10 uM) had minimal effect on mIPSC properties, whereas addition of strychnine (1 uM) completely abolished all mIPSCs. This suggests glycine-mediated synaptic inputs provide most of the phasic inhibitory input to parvalbumin-positive INs. The holding current in all parvalbumin-positive IN recordings decreased (mean amplitude = 104.69 ± 52.18 pA) during the application of strychnine, indicating that a tonic glycine-mediated inhibitory current also

regulated the activity of parvalbumin-positive INs. Together these data suggest both phasic and tonic glycine-mediated inhibition can modify the output of parvalbumin-positive INs and thus alter the capacity of spinal circuits to process and segregate tactile and nociceptive signals.

Disclosures: R.J. Callister: None. **K.M. Smith:** None. **D.I. Hughes:** None. **B.A. Graham:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: BFU 2012-37905, Spanish Ministry of Economy and Competitiveness

CCG2013/BIO-059, Universidad de Alcala

Title: Multiunit recordings of superficial dorsal horn nocicepitive neurons in a longitudinal slice of mice spinal cord

Authors: E. CISNEROS, I. MAZO, *C. ROZA, I. RIVERA-ARCONADA, J. A. LOPEZ-GARCIA;
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Abstracts: Central sensitization underlying chronic pain is conceived as an increased excitability of neural circuits processing pain signals in the spinal cord. Although most of the evidence supporting spinal sensitization arises from studies of individual neurones, adjustments in the temporal correlation of neuronal discharges might also occur and these need to be addressed with techniques allowing the simultaneous recording of a number of discrete elements. The spinal cord was extracted from > 5 weeks old mice via a dorsal laminectomy under anaesthesia (i.p. urethane 2 g/ Kg). We obtained a single longitudinal slice (~500 μm thick) sectioned in a vibratome containing the superficial layers of the cord and dorsal roots of lumbar segments 1 to 5. The slice was maintained *in vitro* by continuous superfusion of ACSF at 22±1 ºC. NeuroNexus multiple electrode arrangement (MEA) mounted on a motorised micromanipulator were carefully lowered down into the preparation to record multiple neurons. Extracellular recordings were performed in a total of 107 units clearly identified by spike sorting obtained from a total of 20 experiments. Most of the units with spontaneous activity (68/105) presented irregular

spontaneous discharges, however 37 showed specific patterns as revealed by their autocorrelograms (burst n = 9; duplets, n = 14; clock-like, n= 10 and short trains, n = 4). Most neurons showed synaptic responses to the activation of Aβ- (n = 20), Aδ- (n = 35) or C fibres (n = 15) and a small proportion showed wind-up to repetitive stimulation at C-fibre intensity (n = 14). NBQX (5 μM) produced a significant decrease in spontaneous firing (16/18) and wind-up (n = 3/3) and increased the input intensity required to evoke a synaptic response (n = 8/8). DAP-5 (50 μM) had no effect on spontaneous activity (n = 0/5) or in the input intensity (n = 3/3), but was able to decrease the wind-up (n = 3/3). Application of CP9999 (10 μM), NK1R-antagonist, inhibited spontaneous activity in most of the neurons (11/14), decreased wind-up responses (n = 4/4) but had no effect on the synaptic responses evoked by single stimuli. Apparently, synaptic activity in superficial dorsal horn neurons is largely mediated via AMPA and NK1 receptors, whilst wind-up seems to be mediated by both AMPA and NMDA receptors together with NK1. MEAs constitute a valuable tool to record simultaneously from small populations of neurons allowing long term stable recordings, favouring pharmacological approaches and reducing the number of animals used. Furthermore, differential effects of compounds on individual neurons recorded simultaneously might help understanding nociceptive circuits in superficial layers of the spinal cord.

Disclosures: E. Cisneros: None. **I. Mazo:** None. **C. Roza:** None. **I. Rivera-Arconada:** None. **J.A. Lopez-Garcia:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NIH Grant RO1 HL103773

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Title: Hyperexcitability of spinal neurons contributes to pain in a transgenic mouse model of sickle cell disease

Authors: *G. CATALDO[1], K. GUPTA[2], D. A. SIMONE[1];
[1]Diagnos. and Biol. Sci., [2]Med., Univ. of Minnesota, Minneapolis, MN

Abstracts: Pain is a major defining characteristic of sickle cell disease (SCD), is difficult to treat, and contributes significantly to a poor quality of life. Acute features of sickle pain and the development of chronic pain syndromes in many patients with SCD suggests that central neural mechanisms contribute to pain in this disease although the underlying mechanisms are poorly understood. Using a transgenic mouse model of SCD, electrophysiological approaches were used to determine whether nociceptive neurons in the spinal cord become sensitized during the disease process and contribute to hyperalgesia. Studies were carried out using Berkeley sickle mice (HbSS-BERK) homozygous for the expression of human sickle hemoglobin, which show severe hematologic phenotype, organ damage and characteristic features of pain observed in human sickle cell disease. Littermate controls (HbAA-BERK) expressing normal human hemoglobin on the same mixed genetic background we also used. This unique transgenic mouse provides an opportunity to investigate neural mechanisms that mediate pain in SCD. Mice were tested for mechanical hyperalgesia then anesthetized with isoflurane before a laminectomy exposed the lumbar spinal cord. Extracellular recordings were made from single dorsal horn neurons with receptive fields (RFs) located on the plantar surface of the hind paw. RFs were identified by stroking the skin and applying mild pressure and mapped using von Frey filaments. Neurons were classified functionally as WDR or HT using mechanical stimuli of graded intensities. The discharge rate of spontaneous activity was recorded for 3 minutes. Mechanical response thresholds and responses evoked by suprathreshold mechanical stimuli were also determined. Comparisons of discharge rates were made between control and sickle mice. Nociceptive dorsal horn neurons in sickle mice were sensitized as evidenced by enlarged RFs (HbAA 16.5mm^2 ± 2.2, HbSS 39.6mm^2 ± 3.1), increased rate of spontaneous activity (HbAA 0.23 ± 0.19, HbSS 8.7 ± 4.3), lower mechanical thresholds (HbAA 26.8mN ± 3.0, HbSS 15.6mN ± 3.2), enhanced responses to mechanical stimuli, and prolonged after-discharges following mechanical stimulation as compared to control mice (data combined for WDR and HT neurons). Direct recordings of enhanced action potential activity in dorsal horn neurons from sickle mice suggest that central sensitization may be a key factor contributing to chronic pain in patients with SCD. These findings are essential to elucidating the mechanisms that underlie central sensitization in SCD in order to identify novel targets for treating the debilitating pain associated with this disease.

Disclosures: G. Cataldo: None. **K. Gupta:** None. **D.A. Simone:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

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NIH 09196

Title: Intradermic capsaicin increases autogenic and heterogenic PAD in Aδ articular afferents as part of a control mechanism that regulates information flow in nociceptive afferents

Authors: *A. O. RAMIREZ[1], E. F. HERNÁNDEZ[2], P. RUDOMIN[2,3];
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Abstracts: Previous studies in the anesthetized cat have indicated that, in contrast with tendon organ afferents, autogenic primary afferent depolarization (PAD) of single afferents in the posterior articular nerve (PAN) is relatively small, but can be increased by capsaicin-induced skin inflammation. Since at present there is limited information on the type of articular afferents that express the capsaicin-induced autogenic PAD, we have now extended these studies and examined PAD of single slow conducting PAN afferents. PAD was inferred from changes in the current required for single afferent fiber antidromic activation by stimuli applied within the region where the PAN evoked field potentials were largest (L6-L7 segments 0.6 to 2.2 mm depth). The examined fibers had conduction velocities and peripheral thresholds in the Aδ range (34.2 ± 1.2 m/s and 2.8 ± 0.3 xT, mean ± SE). Two hours after the intradermic injection of capsaicin, PAN nerve stimulation (3 pulses 1.2- 5xT, 700 Hz, applied 35 ms before the test pulse) reduced the intraspinal threshold of 8 PAN fibers from 94.6 ± 1.3%, relative to control threshold, to 87.2 ± 1.5%. That is, it produced PAD. In 7 fibers, before capsaicin, PAN stimulation increased the intraspinal threshold to 113.3 ± 2.5% (PAH). However, after capsaicin, these same stimuli produced PAD since they reduced the intraspinal threshold to 90.9 ± 4.4%. In 6 cases, after capsaicin, PAN stimuli below the peripheral threshold of the examined fiber produced PAD (from 105.8 ± 2.9% to 88.2 ± 3.6%). In 9 fibers, in addition to the facilitation of the autogenic PAD, the intraspinal threshold produced by stimulation of the cutaneous SP and SU nerves changed from 91.1 ± 2.3% to 84.6 ± 2.4% and from 93.6 ± 2.1% to 82.4 ± 9.9% respectively. These observations provide evidence that nociceptive stimulation induced by intradermic capsaicin increases transmission along the pathways producing autogenic and heterogenic PAD of Aδ, presumably nociceptive, PAN afferents. An increased autogenic PAD of these afferents would limit the activation induced by nociceptive stimulation of the skin and could function as part of a self-regulating control of nociceptive information in this pathway. The increased autogenic and heterogenic PAD could result from intrinsic changes in spinal neuronal connectivity as well as from changes in descending control.

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Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Support: NIH R01 Grant DE022880

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Title: Stress induces pain transition by potentiation of AMPA receptor phosphorylation

Authors: S. LIU[1,2], Y. YANG[1], C. LI[1,2], H. FANG[1], O. FURMANSKI[1], J. SKINNER[1], R. JOHNS[1], R. HUGANIR[1], *F. TAO[1,3];
[1]John Hopkins Univ. Sch. Med., BALTIMORE, MD; [2]Zhengzhou Univ. Basic Med. Col., Zhengzhou, China; [3]Texas A&M Univ. Baylor Col. of Dent., Dallas, TX

Abstracts: Table of Contents **Aim of Investigation:** Chronic pain after surgery is a serious issue in clinical practice that has received increased interest in recent years. However, it is unclear how the transition from acute to chronic postsurgical pain occurs. Previous studies have shown that psychosocial and socio-environmental factors are involved in the development of chronic postsurgical pain. In the present study, we investigated the molecular mechanism underlying pain transition after surgery. **Methods:** Wild-type and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor phosphorylation mutant mice were used in this study. Social defeat stress and plantar incision were carried out as described previously. Western blotting was conducted to analyze AMPA receptor phosphorylation. Surface biotinylation, cross-linking, and pHluorin assays were conducted to detect AMPA receptor trafficking. Electrophysiological recording was performed for long-term potentiation (LTP) induction. **Results:** We found that social defeat stress markedly enhanced plantar incision-induced AMPA receptor phosphorylation in the spinal cord and greatly prolonged plantar incision-induced pain, but stress alone did not produce pain behaviors. We also found that targeted mutation of AMPA receptor GluA1 phosphorylation site Ser831 significantly inhibited stress-induced prolongation of incisional pain. In addition, we observed that stress significantly increased GluA1 membrane surface expression and GluA2 internalization in the spinal dorsal horn neurons, and that stress hormone corticosterone induced GluA1 membrane insertion and GluA2 internalization in

cultured spinal cord neurons. Our electrophysiological experiments showed that subthreshold 10-Hz stimulation induced LTP in AMPA receptor phosphomimetic mutant mice, suggesting that AMPA receptor phosphorylation can lower the threshold for LTP induction and increase the probability of synaptic plasticity. **Conclusion:** AMPA receptor phosphorylation may play an important role in stress-induced pain transition and the development of chronic postsurgical pain. *(Supported by the Blaustein Pain Research Fund and NIH R01 Grant DE022880)*

Disclosures: **S. Liu:** None. **Y. Yang:** None. **C. Li:** None. **H. Fang:** None. **R. Huganir:** None. **O. Furmanski:** None. **F. Tao:** None. **J. Skinner:** None. **R. Johns:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NIH Grant NS46606

National Cancer Institute grant CA124787

Title: Morphological and physiological characteristics of a subgroup of GABAergic spinal lamina II neurons in CCI mice

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[1]Anesthesiol., The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [2]Dept. of Integrative &Pharmacology, The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstracts: The processing of sensory, including nociceptive, information in spinal cord dorsal horn is critically modulated by spinal GABAergic neurons. GABAergic neurons in spinal dorsal horn have been classified morphologically and physiologically, but the morphological and physiological properties under the neuropathic pain condition had not been investigated. We used a transgenic mouse strain co-expressing enhanced green fluorescent protein (EGFP) and the GABA-synthesizing enzyme GAD67 to compare the morphological and physiological properties change of GABAergic neurons in sham control and CCI neuropathic pain mice. Whole-cell patch-clamp technique was used for intracellular labelling and physiologically characterize EGFP-expressing lamina II neurons in spinal cord slices. The EGFP labelled neurons in both sham and CCI mice morphologically exhibited islet, central and vertical cells but no radial cell was observed. The length of cell dendrities is significantly shorter in CCI mice (155.96 ± 18.29 μm) compared with sham control mice (334.93 ± 29.48 μm). The resting membrane potential of

the EGFP-expressing neurons in sham and CCI mice is no significant difference (-60.5 ± 3.2 verse -61.8 ± 3.5mV). When stimulated by rectangular current injection, the firing pattern of these EGFP-expressing neurons showed tonic, delayed, initial and irregular firing pattern, which also have been observed in CCI mice. These results suggest that GABAergic neurons are morphologically shrink with the excitability no change in neuropathic pain condition. The morphological changes may contribute to the neuropathic pain of CCI mice.

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Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Program#/Poster: 627.07/FF26

Topic: D.08. Pain

Title: Properties of recombinant isolectin B4 (IB4): Binding and immunostaining

Authors: M. D. KOHLS, *D. A. LAPPI, L. R. ANCHETA;
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Abstracts: Isolectin B4 (IB4) is a protein found in the seeds of *Griffonia simplicifolia*, a woody climbing shrub native to western and central Africa. Although initially used as an identifier and agglutination agent for B-type red blood cells, it has since become widely used in the neurosciences as a neuronal tracer, for labeling specific populations in the spinal cord, and as a targeting moiety for delivering toxins to specific cells. Recent developments in response to competition from the nutritional supplement industry have reduced the available supply of seeds from which the native protein is purified. In order to create a consistent supply of pure and active IB4 we have determined the full nucleotide sequence of the IB4 gene, cloned it from *Griffonia* genomic DNA, and expressed recombinant IB4 in *E. coli*. The recombinant IB4 (rIB4) was purified and tested in several activity assays against the native protein. A fusion protein of rIB4 and GFP was created to demonstrate the use of this protein in immunostaining. *Griffonia* also contains isolectin A that agglutinates A-type red blood cells - the A and B lectins form tetramers with varying subunit combinations. These tetramers are potential sources of contamination in preparations of the native protein. rIB4 is completely free of any A lectin contamination. The rIB4 is highly pure, and has identical activity to the native protein.

Disclosures: **M.D. Kohls:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems, Inc. **D.A. Lappi:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems, Inc. **L.R. Ancheta:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems, Inc..

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Title: Modulation of nociceptive processing at the spinal cord level by PAR2 receptors

Authors: *P. MRÓZKOVÁ[1,3], J. PALECEK[2];
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Abstracts: Modulation of synaptic transmission in the spinal cord dorsal horn plays a key role in the development of pathological pain states. Protease-activated receptors (PARs) are a family of four G-protein-coupled receptors (PAR1-4) activated by proteases. The role of PAR2 receptors in pain perception is established in the peripheral tissues. However, the role of PAR2 receptors on the central branches of DRG neurons in the spinal cord is not fully understood. The present study aimed to study the role of PAR2 in nociceptive processing and modulation of synaptic transmission in the superficial dorsal horn (DH) neurons. Whole-cell patch clamp recordings of miniature - mEPSCs, spontaneous - sEPSCs and evoked - eEPSC currents (by dorsal root

stimulation) were made from superficial DH neurons in acute spinal cord slices prepared from Wistar rats 21 days old, with the presence of strychnine (5uM) and bicuculline (10uM), at -70mV holding potential. TTX (0.5uM) application was used for mEPSC detection. Application of PAR2 agonist SLIGKV-NH2 (PAR2 AP, 100μM) induced inhibition of mEPSC frequency. In a second set of experiments PAR2 AP application induced sEPSC frequency increase and also increased the amplitude of eEPSC recorded in the superficial DH neurons. Application of the inactive peptide VKGILS-NH2 (100uM) did not evoke any change in the mEPSCs, sEPSC frequency or eEPSC amplitude. The changes in the mEPSC, sEPSC and eEPSC induced by the PAR2 AP application were dependent on activation of protein kinase C. Application of PKC inhibitor (staurosporin 250nM) prevented all changes evoked by the SLIGKV-NH2 alone. Additionally, effect of PAR2 AP was also prevented by application of TRPV1 antagonist SB366791 (10uM). Our results suggest that presynaptic PAR2 receptors may play an important role in modulation of nociceptive synaptic transmission in the spinal cord dorsal horn. Further experiments are needed to fully evaluate the role of spinal cord PAR2 receptors in pain modulation under control and pathological conditions.

Disclosures: P. Mrózková: None. **J. Palecek:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Support: FCOMP-01-0124-FEDER-029623 PTDC/NEU-SCC/0347/2012

FCOMP-01-0124-FEDER-029623 PTDC/NEU-NMC/0494/2012

Title: Monosynaptic convergence of somatic and visceral C-fiber afferents onto spinal lamina I neurons: A mechanism of referred pain

Authors: E. FERNANDES[1], L. L. LUZ[1], E. KOKAI[2], M. SIVADO[2], P. SZUCS[2], *B. V. SAFRONOV[1];
[1]IBMC, Porto, Portugal; [2]Univ. of Debrecen, Dept. of Physiol., Debrecen, Hungary

Abstracts: Referred pain is a phenomenon of feeling pain at a site other than the site of its origin. Examples include myocardial ischemia during a heart attack where pain is often felt in the

neck, shoulders and back rather than in the chest, the site of the injury. One of the most widely accepted theories of referred pain suggests that inputs from somatic and visceral afferents can converge within the spinal cord. However, it is not known whether these afferents converge directly (monosynaptically) onto a specialized neuron, or, alternatively, a polysynaptic processing is required. Furthermore, little is known about the anatomical classes of neurons involved in somatovisceral integration. Here we used the isolated spinal cord preparation with preserved greater splanchnic and intercostal Th9 peripheral nerves, to show that nociceptive C-fibers of somatic and visceral origin converge monosynaptically onto a subgroup of lamina I neurons in the thoracic (Th9-Th8) spinal cord. Neurons receiving converging inputs were both anterolateral-tract projection neurons and local-circuit neurons. Two other subgroups of lamina I neurons were specialized in receiving monosynaptic either somatic or visceral inputs. Thus, our data show that different classes of lamina I neurons process somatic-specific, visceral-specific and converging somatovisceral inputs. The monosynaptic somatovisceral convergence of C-fiber afferents on lamina I neurons provides physiological mechanism of referred pain at the spinal cord level.

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Poster

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Topic: D.08. Pain

Support: Stryker Corporation

Title: Temporal dynamics of dorsal horn projection neuron responses to spinal cord stimulation depend on stimulation frequency and GABAergic inhibition

Authors: ***T. ZHANG**[1], J. J. JANIK[2], W. M. GRILL[1];
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Abstracts: The putative mechanism underlying pain relief by spinal cord stimulation (SCS) is suppression of the activity of sensory dorsal horn projection neurons by segmental inhibition activated by SCS. However, SCS may also excite dorsal horn neurons, and in some cases, SCS excites and inhibits the same neuron. The interaction between the excitatory and inhibitory

influences of SCS may partially explain why the efficacy of clinical SCS depends on stimulation frequency, but the temporal dynamics of this relationship have not been characterized. We recorded the responses of antidromically identified low-threshold (LT), wide dynamic range (WDR), and nociceptive-specific (NS) projection neurons in the lumbar dorsal horn to different frequencies of SCS in acute experiments in healthy, urethane anesthesized (1.6 g/kg) male adult Sprague-Dawley rats. In subsets of experiments, we administered either bicuculline (3 μg i.t.), a GABAa antagonist (12/51), or CGP 35348 (100 μg i.t.), a GABAb antagonist (8/51), to assess the effects of modulating GABAergic inhibition on neuronal responses to SCS. 32/51 antidromically identified projection neurons exhibited significant responses to SCS versus baseline. Both the responder and non-responder groups included LT, WDR, and NS neurons, but non-responders were more likely to be NS neurons and responders were more likely to be LT neurons ($p<0.05$, Pearson's Chi-Squared). Individual projection neurons exhibited a variety of responses to SCS, including transient inhibition, transient excitation, and combinations of inhibition and excitation, and both the magnitude and type of the post-stimulus response varied with stimulation frequency, often within the same neuron. Bicuculline enhanced transient excitatory responses in 3/12 neurons and unmasked excitatory responses in 3/12 neurons; CGP 35348 did not significantly affect responses to SCS. The temporal dynamics of neuronal responses depend on the SCS frequency even for the same neuron, and these responses are modulated by GABAergic inhibition, suggesting that managing the interaction between excitatory and inhibitory influences from SCS may be a way to inform more optimal SCS parameter selection.

Disclosures: **T. Zhang:** None. **W.M. Grill:** None. **J.J. Janik:** A. Employment/Salary (full or part-time):; Stryker Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stryker Corporation.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 627.11/FF30

Topic: D.08. Pain

Support: HIN NS069909-01

Dana Foundation

Title: Distributed spinal fmri responses to noxious cold and heat stimuli in anesthetized monkeys

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Abstracts: In this study we compared noxious cold and heat stimuli evoked functional MRI (fMRI) responses at the cervical spinal cord in anesthetized squirrel monkeys. Three monkeys were scanned in a 9.4 T Varian magnet using a saddle-shaped surface transmit-receive coil positioned over the neck of the animal. Axial high-resolution Magnetization Transfer contrast (MTC) images (0.25x0.25x3 mm3) were acquired to enhance the visualization of grey and white matter. FMRI images were obtained with a gradient-echo multi-slice sequence (TE=6.5 ms; TR=24 ms; flip angle = ~12°, volume acquisition time = 1.54 s, slice = 5, 0.5x0.5x3 mm3 resolution). Two distal finger pads were stimulated with blocks (in 21 s on and 30 s off circles) of single or multiple temperatures, ranging from innocuous cool and warm (15 and 40 °C) to noxious cold and heat (4 and 47.5 °C) via a Medoc ATS probe. After physiological noise, slice timing and motion corrections, functional MRI data were analyzed with AFNI software. We found that noxious cold and heat stimulation (4 and 47.5 °C) produced reproducible fMRI responses in bilateral dorsal and ventral horns in two (out of five) consecutive spinal segments (3 mm in thickness), indicating localized spinal stimulus-evoked fMRI responses. FMRI signal time courses obtained from the ipsilateral dorsal horn differed significantly from those observed in the white matter. The nociceptive cold and heat responses were reproducible across imaging runs and sessions within each animal and across animals (n=3). Expanding on previous observations of fMRI responses to innocuous thermal stimulation in human subjects(Stroman et al., 2012), here we report distributed nociceptive cold and heat responses in both the dorsal and ventral horns of cervical spinal cord in anesthetized monkeys. Our data indicate that high-resolution MRI with novel MT contrast permits improved visualization and segmentation of spinal grey matter, thereby allowing better localization of nociceptive responses in the dorsal and ventral horns.

Disclosures: **P. Yang:** None. **F. Wang:** None. **L. Chen:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

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Title: Spinal cord astrocytes release endocannabinoids due to the activation of their CB1 receptors

Authors: *M. ANTAL[1], Z. HEGYI[1], T. OLÁH[1], A. KISS[1], Á. KőSZEGHY[1], K. HOLLÓ[1], T. PATONAY[1], L. CSERNOCH[1], A. MINCIC[2];
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Abstracts: Bidirectional communication between neurons and glial cells mediated by endocannabinoid signaling has recently been demonstrated. Endocannabinoids released by neurons can activate CB1-Rs on astrocytes, resulting in glutamate release from astrocytes and a consecutive glutamate-mediated modulation of neural functions. Endocannabinoid mediated neuron-glia-neuron communication can play a major role in the regulation of many brain functions including pain processing in the superficial spinal dorsal horn. Concerning neuron-glia-neuron regulatory mechanisms neural-glial ensembles of the superficial spinal dorsal horn underlying pain processing can be an interesting functional unit of the central nervous system, since here in addition to CB1-Rs, astrocytes also express DGLα and NAPE-PLD, synthesizing enzymes of the two major endocannabinoids, 2-AG and anandamide, respectively. Thus, in the present experiment we explored the effect of astrocytic CB1-R activation on changes in intracellular Ca2+ concentration, possible Ca2+-mediated activation of DGLα and/or NAPE-PLD and consecutive release of 2-AG and/or anandamide from astrocytes. We demonstrated that application of CB1-R agonists evokes transient increase in intracellular Ca2+ concentration in astrocytes within the superficial spinal dorsal horn. To investigate whether this Ca2+ transient can activate DGLα and/or NAPE PLD, we established a primary astrocyte culture from astrocytes isolated from the spinal dorsal horn. We showed that, similar to the *in vivo* findings, cultured astrocytes express CB1-Rs as well as DGLα and NAPE-PLD. We showed that application of CB1-R agonists evokes elevation of intracellular Ca2+ concentration also in the cultured astrocytes. Measuring the 2-AG and anandamide content of the culture medium, we showed that the elevation of intracellular Ca2+ concentration of astrocytes is accompanied with a robust elevation of both 2-AG and anandamide concentration in the culture medium. The results indicate that spinal cord astrocytes can release endocannabinoids due to the activation of their CB1-Rs. We hypothesize that similarly to the cell culture environment astrocytes can release endocannabinoids also in the superficial spinal dorsal horn. The released 2-AG and anandamide then can act on neural CB1-Rs, influencing both excitatory and inhibitory neurotransmission. Thus, the astrocytic endocannabinoid signaling apparatus may influence ongoing spontaneous

network activity and integrated activity of neuronal ensembles in the pain processing neural networks of the superficial spinal dorsal horn.

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Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Support: CIHR Grant MOP 12942 (YDK)

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CIHR 106033 (LEL)

Title: α5GABA-A receptors in the superficial dorsal horn regulate central sensitization

Authors: *J. PÉREZ-SÁNCHEZ[1], R. P. BONIN[1], L.-E. LORENZO[1], C. LABRAKAKIS[2], E. M. BRIDGWATER[3], B. A. ORSER[3], Y. DE KONINCK[1];
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Abstracts: The tonic activation of extrasynaptic α5 subunit-containing GABA-A (α5GABA-A) receptors is known to regulate neuronal excitability in the CNS. In the spinal cord, tonic inhibition may be important for the control of nociception and has been suggested to contribute to hyperalgesia. However, the role of α5GABA-A receptors in nociceptive information processing remains elusive. We therefore explored the distribution of α5GABA-A receptors in the dorsal horn of the spinal cord and their functional contribution to pain processing. Immunohistochemical analysis showed that α5GABA-A receptors are localized in superficial

layers in the dorsal horn of the spinal cord, consistent with a role for these receptors in pain processing. In behavioural tests, mice lacking the α5GABA-A receptors (Gabra5−/−) exhibited increased responses in phase 2 of the formalin test, which has been associated with central sensitization in the dorsal horn. In addition, mechanical hyperalgesia produced by intraplantar administration of capsaicin was larger in Gabra5−/− as compared to wild type (WT) mice. However, baseline acute nociception was similar between Gabra5−/− and WT mice. Taken together, our data suggest that α5GABA-A receptors contribute to central sensitization in the dorsal horn of the spinal cord. Whole-cell recordings from dorsal horn neurons from Gabra5−/− and WT mice will show the contribution of α5GABA-A receptors in tonic inhibition in the spinal cord.

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Poster

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Topic: D.08. Pain

Support: NIH Grant NS70814

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Title: Electrical stimulation of low-threshold afferent fibers depresses synaptic transmission of high-threshold afferent inputs in lamina II dorsal horn neurons

Authors: Q. XU[1,2], A. D. SDRULLA[3], V. TIWARI[3], S.-Q. HE[3], F. YANG[3], S. N. RAJA[3], X. DONG[1], *Y. GUAN[3];
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Abstracts: The pain “gate control” theory postulates that activity of low-threshold/large myelinated afferent fibers (Aβ-fibers) drives a feed-forward inhibition of spinal nociceptive transmission. However, it is unclear how electrical stimulation of Aβ-fibers (Aβ-ES) modulates neuronal excitability and synaptic transmission in substantia gelatinosa (SG, lamina II) neurons,

which are important for relaying, integrating, and modulating converging nociceptive inputs in superficial dorsal horn. Therefore, we examined synaptic mechanisms in SG neurons that may contribute to pain inhibition from electro-analgesia therapies that use Aβ-ES. By conducting *in vitro* patch-clamp recording in spinal cord slices from mice (4-5 weeks old), we examined whether Aβ-ES at the dorsal root inhibits the evoked excitatory postsynaptic current (eEPSC) to high-threshold afferent inputs (i.e., C-fiber) in SG neurons. We calibrated the stimulus strength for Aβ-ES (10 μA, 0.1 ms) to activate only low-threshold afferent fibers by recording compound action potentials at the proximal dorsal root in response to increasing intensities of stimulation applied at the distal site (0-1.0 mA, 0.1 ms). The paired-pulse test stimulation (500 μA, 0.1 ms, 400 ms apart) was applied at an intensity that activates C-fibers. Aβ-ES (50 Hz, 5 min) induced a gradual and prolonged decrease in C-fiber eEPSC amplitude in SG neurons from both naïve mice and mice at 1-2 weeks after an L5 spinal nerve ligation. The paired-pulse ratio (amplitude 2nd eEPSC / 1st eEPSC) increased after Aβ-ES in these cells, suggesting that the inhibition of eEPSC by Aβ-ES may partly involve presynaptic mechanisms. Further, Aβ-ES inhibited both monosynaptic and polysynaptic C-fiber eEPSC. This study suggests that Aβ-ES at 50 Hz may depress the synaptic transmission of high-threshold afferent inputs in SG neurons. Future studies may reveal whether this novel form of synaptic depression, which is induced by Aβ-fiber activity and mostly mediates non-noxious inputs, contributes to pain inhibition from Aβ-ES therapy.

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Poster

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Topic: D.08. Pain

Support: NIH grant AR063772

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Title: Optogenetic dissection of pain and itch circuitry in the spinal dorsal horn

Authors: *J. HACHISUKA, K. M. BAUMBAUER, L. M. SNYDER, H. R. KOERBER, S. E. ROSS;
Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstracts: Skin sensations, such as pain, touch, hot, cold and itch are processed in the spinal dorsal horn and relayed to the brain. There are multiple types of excitatory and inhibitory interneurons, which form circuits that modulate skin sensation. Recently, many molecules that relate to pain and itch have been localized in specific subsets of these interneurons. However, little is known about the role of these neurons in the spinal circuitry. In order to address these questions, we used specific mouse cre lines (e.g. neurotensin cre) crossed with cre-responsive channelrhodopsin (Ai32). The roles of these interneurons were investigated by recording from retrogradely-labeled spinoparabrachial tract cells in an *ex vivo* preparation consisting of whole lumbosacral spinal cord/DRG/nerve and skin. We performed whole cell patch clamp recordings from labeled cells visualized first by fluorescent microscopy and then oblique infrared LED illumination for establishing whole cell recordings. We first recorded from the channelrhodopsin-expressing neurons to confirm that blue light stimulation evoked inward current in voltage clamp mode and action potentials in current clamp mode. We next recorded from the projection neurons that are identified by retrograde labeling and examined whether they receive input from the different populations of interneurons. Finally, we identified the cell's receptive field by applying mechanical and thermal stimulation to the skin, and used the combination of blue light stimulation of the spinal cord with natural stimulation of the skin. The results of these experiments are providing novel insights of the circuitry of pain and itch sensation in the spinal cord.

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Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: Grant-in-Aid for Young Scientists (B) (No. 23792575)

Title: Low-threshold mechanoreceptive Aδ- and C-afferent units contribute to the inhibition of nociceptive transmission via the spinal μ-opioid system

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[1]Tokyo Metropolitan Inst. of Gerontology, Tokyo, Japan; [2]Dept. of Chiropractic, Univ. du Québec à Trois-Rivières, Trois-Rivières, QC, Canada

Abstracts: Background and aim Pain may subside by touching the skin near the painful site. As an underlying mechanism of touch-induced analgesia, the most common belief is that afferent inputs conveyed by large-diameter afferent fibers modulate spinal activity induced by small-diameter afferent fibers. However, the details of this theory have not been confirmed. Recently, we found that gentle mechanical cutaneous stimulation (touch) with a soft disc covered with microcones (with texture is similar to that of a finger), but not with a flat disc, inhibits nociceptive somatocardiac reflexes in anesthetized rats and conscious humans. The aim of the present study is to identify the types of cutaneous afferent fibers and spinal opioid receptors that contribute to the anti-nociceptive effects of microcone touch. Methods & Results The present study consists of two experiments performed on deeply-anesthetized rats. Touch was applied to the inner thigh for 10 minutes. In the first experiment, unitary activity of skin afferents was recorded from the saphenous nerve using bipolar wire electrode, and responses to touch using a microcone disc and flat disc (control) were compared. In total, 38 low-threshold mechanoreceptive afferents with slowly adapting property were obtained. Based on conduction velocity, recorded units were classified into Aβ (n = 13), Aδ (n = 12) and C (n = 13) fibers. During microcone touch, greater increments of discharge rate were observed in the majority of low-threshold mechanoreceptive Aδ and C afferent units, whereas most Aβ afferents similarly responded to the two types of touch. In the second experiment, we examined the effect of an intrathecal injection of opioid receptor antagonists on the inhibitory effects of microcone touch on heart rate responses to noxious heat stimulation. Heat stimulation was applied to the lower back of rats using a Peltier thermode and altered heart rate by 10.9 ± 1.2 bpm. The magnitude of the heart rate response was suppressed by microcone touch in saline-injected rats by approximately 36 % and the suppression continued even 10-15 minutes after touch terminated. Such an inhibition was blocked in naloxone- (non-selective opioid receptor antagonist) or CTOP- (μ-opioid receptor antagonist) injected rats. Naltrindole (δ-opioid receptor antagonist) did not influence the effect of microcone touch. Conclusion The present study suggests that low-threshold mechanoreceptive Aδ and C afferents excited by touch activate the μ-opioid system in the spinal cord, leading to the inhibition of nociceptive transmission that contributes to somatocardiac reflexes.

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Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NIH grant R21 NS074146

Title: Impact of spinal interneuron disinhibition on somatosensory processing in neuropathic pain

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Abstracts: Neuropathic pain is a debilitating condition whose symptoms arise from the abnormal processing of somatosensory input. Reduction of synaptic inhibition in the spinal dorsal horn can account for many features of neuropathic pain including mechanical allodynia, a phenomenon in which innocuous tactile stimulation to be mistakenly perceived as painful. Disinhibition derives at least in part from chloride dysregulation caused by down¬regulation of the potassium-chloride co-transporter KCC2. But it remains unclear how different types of spinal neurons are affected and, in turn, how this impacts network-level processing of sensory input. Recording *in vivo* from anesthetized rats, we found that pharmacological blockade of either KCC2 or GABAA receptors caused spinal interneurons to become hyperresponsive to light tactile stimulation, but whereas blockade of carbonic anhydrase by acetazolamide significantly reversed the effects of KCC2 blockade, it had no effect after GABAA receptor blockade. Given that acetazolamide has antiallodynic effects in behavioural studies, our data confirm the importance of KCC2 downregulation as a disinhibitory mechanism contributing to neuropathic pain. We also found that cutaneous receptive fields (RFs) were dramatically expanded after KCC2 blockade, consistent with normally subliminal excitatory input being unmasked by the reduction of inhibition. Indeed, whereas stimulation around the RF normally reduced the response to concurrent stimulation inside the RF, such stimulation enhanced responses after KCC2 blockade. This effect - the switch from inhibition to excitation by stimulation outside the normal RF - was significantly greater in excitatory interneurons than in inhibitory interneurons. Cell types were distinguished *in vivo* on the basis of spike waveform, spiking pattern, spontaneous spike rate, and modulation by KCC2 blockade; their identities as excitatory and inhibitory neurons were established by comparison with *in vitro* recordings from identified cell types. Our data demonstrate that disinhibition arising from distinct molecular mechanisms is differentially susceptible to different therapeutic interventions. Furthermore, although excitatory

and inhibitory interneurons both experience disinhibition, we show that excitatory interneurons are disproportionately affected because of their synaptic connectivity. The resultant changes in network-level processing are important for how disinhibition manifests the clinical features of neuropathic pain.

Disclosures: K. Lee: None. **S.A. Prescott:** None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: Louise and Alan Edwards grants in Pain Research

Rita Allen Foundation-American Pain Society

Title: Three-dimensional reconstruction of synaptic relationship between interneurons of dorsal horn of the spinal cord

Authors: *H. PETITJEAN[1], S. PAWLOWSKI[2], A. DAVIDOVA[1], A. RIBEIRO-DA-SILVA[2], R. SHARIF NAEINI[1];
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Abstracts: The dorsal horn of the spinal cord is the first central region where somatosensory information is processed. A complex network of excitatory and inhibitory interneurons integrates peripheral inputs before transferring them to supraspinal sites. How the different elements of this network are interconnected is not well established. In this study, we propose a quantification method to analyze the synaptic appositions from identified inhibitory neurons to identified excitatory neurons. This method uses transgenic reporter mice, immunostainning, confocal imaging and image processing using the IMARIS software. Our method allows us to generate a 3-D reconstruction of neuronal processes and detect close synaptic appositions inferior to 100nm. This approach enabled us to establish the connectivity pattern of parvalbumin interneurons onto their excitatory interneurons target. Furthermore, our results indicate that a single inhibitory interneuron can perform appositions onto multiple target excitatory interneurons. By combining this approach with behavioral experiments, we will examine

whether interfering with this pattern of connectivity impacts the processing of sensory information in the dorsal horn, especially in the context of chronic neuropathic pain.

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Poster

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Program#/Poster: 627.19/GG6

Topic: D.08. Pain

Title: Dopamine modulation of synaptic transmission in lamina I neurons of the dorsal horn spinal cord

Authors: *M. PUOPOLO;
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Abstracts: The dorsal horn spinal cord is the first relay station where sensory information from the periphery and deeper tissue are received, integrated, and relayed to higher brain structures. Descending fibers from the Central Nervous System play a critical role in the integration of the sensory information in the dorsal horn spinal cord. While the contribution of descending noradrenergic, serotonergic, and GABAergic fibers is well established, the contribution of descending dopaminergic fibers from area A11 of the hypothalamus is poorly understood. The aim of the present project was to investigate whether dopamine can modulate the synaptic transmission between primary afferent fibers and projecting neurons located in lamina I of the dorsal horn spinal cord. We used the patch clamp technique in an intact spinal cord preparation *in vitro* isolated from rats (P14-21). Excitatory post synaptic currents (EPSCs) were recorded in whole-cell voltage clamp configuration (Vhold= -70 mV) from lamina I neurons. The external solution was (in mM): 125 NaCl, 2.5 KCl, 1.25 $NaH_2PO_4.H_2O$, 26 $NaHCO_3$, 1 MgCl2, 2 CaCl2, 20 Glucose, 0.01 bicuculline, 0.005 strychnine, and continuously oxygenated with 95/5% O_2/CO_2. The internal solution was (in mM): 125 $CsMeSO_3$, 10 NaCl, 2 MgCl2, 10 HEPES, 10 EGTA, 4 MgATP, 0.3 NaGTP, 14 Phosphocreatine, 5 QX-314, pH adjusted to 7.2 with CsOH. The dorsal root was stimulated with a suction electrode (0.1 ms duration, 350-500 μA constant current output) at 0.01 Hz. Lamina I neurons included in the study had large size (cross-sectional soma area of 339.28 ± 123.67 μm^2), and cell capacitance of 70.57 ± 21.18 pF, n=22, consistent with projecting neurons. Experiments were carried out at 35 ± 1 ºC. 20 μM dopamine reduced

the EPSC (measured as the charge transfer) to 46.1 ± 29.2 % (n=14), with respect to control. The inhibitory effect of dopamine on the EPSC could be mimicked by both D1/D5 and D2 dopamine receptor agonists. 10 μM SKF 81297 reduced the EPSC to 59.1 ± 25.5 % (n=5), with respect to control. When 20 μM quinpirole were added on top of 10 μM SKF 81297, the EPSC was further reduced to 53.9 ± 36.2 % (n=5), with respect to control. When the afferent fibers were stimulated with paired stimuli (2s apart), the ratio between the second and the first EPSC was increased from 0.43 ± 0.19 in control to 0.76 ± 0.22 (n=7) in the presence of 20 μM dopamine, consistent with a presynaptic effect of dopamine. The data presented here suggest that dopamine can modulate the synaptic inputs from primary afferent fibers onto projecting lamina I neurons. The effect of dopamine is mediated by activation of both D1/D5 and D2 dopamine receptors. Increase in the PPD ratio suggests a presynaptic effect of dopamine.

Disclosures: M. Puopolo: None.

Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

Support: The Reynolds Family Spine Laboratory

Title: Reduced pain sensitivity in plasma membrane calcium atpase 2 (pmca2) heterozygous mice

Authors: *V. KHARIV[1,2], W. DONG[2], R. F. HEARY[2], S. ELKABES[2];
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Abstracts: Plasma membrane calcium ATPase 2 (PMCA2) is a calcium extrusion pump expressed in excitable cells. In the spinal cord, it is primarily localized to neurons in both the dorsal and ventral horns. Our earlier investigations indicated that PMCA2 is essential for the survival of spinal cord neurons, as silencing of PMCA2 expression causes neuronal death *in vitro*. Moreover, we reported that PMCA2 deficiency is associated with motor abnormalities and a reduction in motor neuron number, *in vivo*. It is not yet known whether PMCA2 plays a role in sensory function. To address this issue we assessed thermal and mechanical sensitivity in the $PMCA2^{+/-}$ mice and wild type littermates.The hotplate paw withdrawal and Von Frey filament

tests indicated that naïve, female PMCA2$^{+/-}$ mice are significantly less sensitive to thermal or mechanical stimuli than wild-type controls. This difference was not observed in male mice, demonstrating sex specificity. Following spinal cord injury, a condition that can elicit neuropathic pain, female PMCA2$^{+/-}$ mice continued to exhibit significantly less thermal sensitivity. To investigate the mechanisms underlying the sensory alterations in the PMCA2$^{+/-}$ mice, we initiated studies on the dorsal root ganglia (DRG) and the dorsal horn (DH). As we did not find expression of PMCA2 in the DRG, we focused on molecular changes in the DH. There was a significant, 40% reduction in the levels of NeuN, a general neuronal marker, in the DH of PMCA2$^{+/-}$ mice compared to the wild type. Since GABAergic interneurons, NMDA receptor-expressing neurons and metabotropic glutamate receptor (mGluR)-expressing neuronal and non-neuronal cells contribute to central pain mechanisms, we quantified NMDAR2B, mGluR1, mGluR5, and glutamic acid decarboxylase (GAD; the rate limiting enzyme in the synthesis of GABA) protein levels in the DH. mGluR5 levels were significantly decreased by 62% in the DH of the PMCA2$^{+/-}$ mice compared to the wild type. In contrast, mGluR1 levels were significantly increased by 2-fold. No significant differences were observed in NMDAR2B or GAD levels in the DH of PMCA2$^{+/-}$ and WT mice.These findings suggest that PMCA2 plays a role in mechanisms implicated in nociceptive processing. It remains to be determined how a reduction in PMCA2 affects mGluR1 and mGluR5-expressing cells in the DH and whether these alterations contribute to PMCA2-dependent changes in sensory function.

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Poster

627. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: Brain Canada

Quebec Pain Research Network

CIHR

Weston Foundation

Title: A role for the transcription factor Lmx1b in pain modality discrimination

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Abstracts: One important aspect of nociception is the ability to discriminate between modalities such as noxious heat versus mechanical stimulation. Electrophysiological studies suggest that neuronal circuits devoted to specific pain modalities are organised into “labeled lines”, such that pain sensation is processed by neurons primarily devoted to the sensation of a particular pain modality. While molecular correlates of such sorting in vertebrates are evident at the level of primary sensory neurons, few molecular markers of modality discrimination have been found in the dorsal horn of the spinal cord. Using a candidate gene approach we focussed on the transcription factor Lmx1b, which is expressed in dorsal horn neurons and essential for their normal development, but its role in nociception has not been characterised in postnatal animals. To assess the behavioral consequences of deleting Lmx1b in the spinal cord, we generated a conditional Lmx1b knockout mouse line using a spinal cord-specific Cre recombinase driver. Such mice show robustly lowered sensitivity to mechanical noxious stimulation but have normal thermal nociception. We are currently exploring the possibility that Lmx1b defines a population of dorsal horn neurons devoted to mechanical pain sensation, in line with the observation that human LMX1B mutations result in modality-specific nociception defects.

Disclosures: A. Kania: None. **N. Szabo:** None. **R.V. Da Silva:** None. **S. Sotocinal:** None. **J. Mogil:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.01/GG9

Topic: D.08. Pain

Support: KAKENHI #25893280

Title: Role of hydrogen peroxide and TRPA1 in visceral hyperalgesia in trinitrobenzene sulphate-induced colitis

Authors: *Y. KOGURE[1,2], S. WANG[1,3], K.-I. TANAKA[1], S. YAMAMOTO[1], N. NISHIYAMA[1], K. NOGUCHI[2,3], Y. DAI[1,2,3];
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Med., Nishinomiya, Japan; [3]Traditional Med. Res. Ctr., Chinese Med. Confucius Inst. at Hyogo Col. of Med., Kobe, Japan

Abstracts: [Background and aims]Inflammatory bowel disease (IBD) is associated with chronic abdominal pain. Recent studies indicate that reactive oxygen species such as hydrogen peroxide (H_2O_2) may activate transient receptor potential ankyrin 1 (TRPA1). In this study, we investigated the role of H_2O_2 and TRPA1 in visceral hyperalgesia in rats following trinitrobenzene sulphate (TNBS)-induced colitis. [Methods]Adult male Sprague-Dawley rats (200-250 g) were used. Colonic inflammation was induced by intra-colonic administration of TNBS. Visceromotor response (VMR) against colorectal distention (CRD) was recorded to evaluate the painful response at 1, 3, 7 and 14 days after TNBS treatment. An antioxidant, N-acetyl-L-cysteine (NAC) or a TRPA1 antagonist, HC-030031 was intravenously administrated to the TNBS-(or vehicle-) treated rats. A group of rats were killed one day after TNBS treatment, 0.5-1 cm length of colonic tissue from the inflamed region was removed and then processed for assessment of its H_2O_2 contents. In a parallel experiment, intra-colonic H_2O_2-induced painful response in naïve rats and the effect of intravenous HC-030031 were measured by the VMR. [Results]TNBS treatment resulted in a significant increase in the VMR that maintained to at least 14 days. The H_2O_2 contents in the inflamed region of the colon in TNBS-treated rats were significantly higher than that in vehicle-treated rats. Intravenous administration of NAC or HC-030031 significantly suppressed the enhanced VMR in TNBS-treated rats without any effect on vehicle-treated rats. Moreover, we found that blockade of TRPA1 by HC-030031 significantly reversed the H_2O_2-induced visceral hyperalgesia in naïve rats. [Conclusion]These results suggest that H_2O_2 contents of the colonic tissue increase in the early stage of TNBS-induced colitis. The increased H_2O_2 may contribute to the visceral hyperalgesia by activating TRPA1. *This work was supported by the KAKENHI #25893280 (S.W.).

Disclosures: **Y. Kogure:** None. **S. Wang:** None. **K. Tanaka:** None. **S. Yamamoto:** None. **N. Nishiyama:** None. **K. Noguchi:** None. **Y. Dai:** None.

Poster

628. Visceral Pain

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Program#/Poster: 628.02/GG10

Topic: D.08. Pain

Support: KAKENHI #25893280

Title: TRPA1, but not TRPV1 contributes to the colonic motility-dependent visceral pain in rats

Authors: Y. HAO[1,2], S. WANG[1,4], Y. KOGURE[1,3], S. YAMAMOTO[1], H. MIWA[2], K. NOGUCHI[3,4], *Y. DAI[1,3,4];
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Abstracts: Aims: The transient receptor potential vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1, respectively) channels as a pain sensor are well recognized. Visceral pain is a major sign or symptom of many gastrointestinal diseases. Colonic dilatation, ischemia and inflammation are believed to cause visceral pain. The present study was conducted to investigate the role of these two TRP channels on colonic motility-dependent visceral pain. Methods: Adult male Sprague-Dawley rats (200-250 g) were used. Experimental colitis was induced by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS), or oral administration of 4% (w/v) dextran sodium sulfate (DSS) added in drinking water. The colonic motility was evaluated by the intracolonic pressure, which was assessed by a pressure sensor connected with an end-closed, water-filled balloon inserting into rats' colon. Visceral pain was measured by electromyography what presented by visceromotor response (VMR) of the external oblique. Both pressure and VMR were recorded by the PowerLab data acquisition device. Capsaicin, allyl-isothiocyanate (AITC), serotonin and other chemical reagents were administrated intrarectally or intraperitoneally. Results: Substantial increases of both colonic motility and VMR were induced by intra-peritoneal administration of serotonin or bethanechol to naïve rats. The serotonin- or bethanechol-induced VMR was inhibited by their receptor's antagonist ondansetron or 4-diphenylacetoxy-N-methyl-piperidine methiodide (4-DAMP), respectively. It is of interest that intrarectal injection of AITC, but not capsaicin significant potentiated both colonic motility and VMR, which were completely blocked by 4-DAMP. Moreover, a clearly increase of VMR with an enhanced basal colonic motility was observed in rats with DSS- or TNBS-induced colitis when compared to naïve rats. The DSS- or TNBS-induced increase of VMR was blocked by 4-DAMP. Conclusion: Intensive colonic motility may induce visceral pain in naïve rats. This colonic motility-dependent visceral pain is related to the activity of TRPA1, but not TRPV1. Moreover, the colitis-induced visceral pain is accompanied with the increase of colonic motility.
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Poster

628. Visceral Pain

Location: Halls A-C

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Program#/Poster: 628.03/GG11

Topic: D.08. Pain

Support: R03 DK088011

P20 GM104936

P30 HD002528

P20 RR016475

Madison and Lila Self Graduate Fellowship

Title: Exercise ameliorates urinary bladder hypersensitivity and dysfunction in maternally-separated female mice

Authors: *A. N. PIERCE, R. WANG, J. M. RYALS, J. A. CHRISTIANSON;
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Abstracts: Exposure to early life stress serves as a risk factor for developing chronic pelvic pain disorders and comorbid mood disturbances. Urogenital pain patients often suffer from depression and/or anxiety, which has been attributed to altered functioning of specific brain regions that express corticotropin-releasing factor (CRF) including the hypothalamic-pituitary-adrenal (HPA) axis that regulates stress response and influences the perception of pain. Voluntary exercise has been shown to attenuate molecular and behavioral changes associated with a dysfunctional HPA axis; however, the therapeutic potential of exercise to ameliorate visceral hypersensitivity arising from early life stress has not been determined. To address this issue, we examined the effect of voluntary wheel running on bladder sensitivity and function in female mice that underwent neonatal maternal separation (NMS), a well-established model of early life stress. C57Bl/6 mice were born in-house and either were separated as litters from their dams for 3 hours/day from postnatal day 1-21 (NMS) or remained undisturbed (naïve). At 4 weeks of age, naïve and NMS mice were pair-housed with free access to running wheels (Exercised group [Ex]) or remained in standard caging without wheels (Sedentary group [Sed]). At 8 weeks of age, mice were assayed for bladder sensitivity, micturition analysis, and evidence of altered HPA axis output. NMS-Sed mice displayed significantly increased visceromotor response (VMR) during urinary bladder distension (UBD) compared to naïve-Sed mice. NMS-Ex mice had significantly diminished VMR compared to NMS-Sed mice and were not different from either naïve-Sed or naïve-Ex. Exercise also prevented significant increases in both micturition frequency and total urine output resulting from NMS. Both serum corticosterone and urinary bladder mast cell degranulation were significantly increased in NMS-Sed mice, compared to naïve-Sed mice, indicating

increased HPA axis activation. Voluntary exercise significantly reduced mast cell degranulation in urinary bladder from NMS mice, compared to sedentary counterparts. Taken together, these findings provide novel insight on the efficacy of exercise, an easily translatable clinical intervention, as a potential treatment strategy for chronic visceral hypersensitivity and neuroimmune dysfunction associated with early life stress.

Disclosures: **A.N. Pierce:** None. **R. Wang:** None. **J.M. Ryals:** None. **J.A. Christianson:** None.

Poster

628. Visceral Pain

Location: Halls A-C

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Program#/Poster: 628.04/GG12

Topic: D.08. Pain

Support: R03 DK088011

P20GM104936

P30HD002528

P20GM103418

Title: The impact of early life stress and voluntary exercise intervention on comorbid mood and urogenital pain disorders in male mice

Authors: *I. FUENTES, A. N. PIERCE, R. WANG, J. A. CHRISTIANSON;
Univ. of Kansas Med. Ctr., Kansas City, KS

Abstracts: Exposure to early life stress, such as childhood neglect or abuse, low socioeconomic status, or witnessing violence or parental discord in the home, has been shown to permanently alter the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the response to stress and influences pain perception. Disruption within the HPA axis has been shown to contribute to both mood and functional pelvic pain disorders, including interstitial cystitis, irritable bowel syndrome and prostatitis. We have investigated the impact of neonatal maternal separation (NMS), a commonly used rodent model of early life stress, on behavioral indicators of depression, pelvic organ sensitivity, and micturition in male mice, as well as the therapeutic potential of a voluntary exercise intervention. C57Bl/6 mice were born in house and

either separated as litters for 3 hours per day from postnatal day 1-21 (NMS) or received no handling outside of normal husbandry care (naïve). At 4 weeks, mice were pair housed in cages equipped with free running wheels (exercised groups [Ex]) or remained in standard caging (sedentary groups [Sed]). Adult NMS-Sed mice displayed depressive-like behavior measured as significantly lower sucrose intake during a two-choice preference assay; however, sucrose intake was not significantly altered in NMS-Ex mice, compared to naïve-Sed or naïve-Ex. NMS-Sed mice exhibited significantly lower withdrawal thresholds to von Frey monofilament application to the perigenital region than naïve-Sed mice. Voluntary exercise prevented this phenotype as the perigenital mechanical sensitivity of NMS-Ex mice was not significantly different from either naïve-Sed or naïve-Ex mice. Furthermore, NMS-Sed mice displayed increased micturition frequency and total urine output over a 1h testing period, compared to naïve-Sed mice; however, no significant differences were observed between NMS and naïve groups that were exercised. Toluidine blue staining revealed a significant increase in mast cell degranulation in both the bladder and prostate in NMS-Sed mice compared to naïve-Sed; and preliminary protein and mRNA expression studies suggest an improper negative regulation of the HPA axis, both within the hypothalamus and from higher limbic structures. Taken together, these results suggest that alterations within, and downstream of, the HPA axis are likely contributing toward our observed behavioral changes resulting from early life stress. We have also provided evidence that voluntary exercise may be a potent therapeutic option for patients suffering from comorbid mood and pelvic pain disorders.

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Poster

628. Visceral Pain

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Topic: D.08. Pain

Support: NINDS NS0050758

NIDDK DK094593

NS081707

Title: Optogenetic dissection of bladder nociception and function in mice

Authors: *V. K. SAMINENI[1], J. J. DEBERRY[3], M. PULLEN[2], B. A. COPITS[2], B. M. DAVIS[3], R. W. GEREAU[2];
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Abstracts: Although bladder pain afflicts 3-6% of women in the United States, its pathophysiology is poorly understood. Moreover, these pain conditions are resistant to many analgesics that alleviate somatic pain, suggesting that there are distinct mechanisms of bladder pain. To advance our understanding about peripheral nerve fibers that innervate the bladder and their role in development of bladder pain, we characterized three transgenic mouse lines expressing the light-activated channel, channelrhodopsin (ChR2), fused to EYFP in Advillin, TRPV1 and Nav1.8-Cre expressingdorsal root ganglion (DRG) neurons. Prior reports suggest the possibility that TRPV1 is expressed in the urothelium, in TRPV1-ChR2 mice no fluorescence could be detected in the urothelium, indicating that the TRPV1 promoter drives no (or very little) expression in these cells. In Nav1.8-ChR2 mice, the parasympathetic postganglionic neurons found at the base of the bladder in the pelvic ganglion also expressed ChR2-EYFP. TRPV1-ChR2 expression was seen in dense projections to lamina I and II of the superficial dorsal horn, consistent with expression of TRPV1 in all unmyelinated sensory fibers during development. In the Nav1.8-ChR2 mice, projections are also seen in lamina III, a layer thought to receive both myelinated and unmyelinated input. Illumination of DRG neurons expressing ChR2 under control of the TRPV1 promoter led to robust action potential generation, even at very low light levels. In order to assess bladder nociception, we determined the visceromotor response (VMR) during graded bladder distension (20-60 mmHg), both prior to and during 473nm blue laser stimulation of the bladder. We observed robust sensitization bladder responses after activation of ChR2 expressing TRPV1 and Nav1.8 neurons in the bladder. We are currently exploring the physiological consequences of ChR2 activation in a range of fiber types directly innervating the bladder, to elucidate the cellular basis of bladder sensitivity via the use of optogenetics in mice.

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Poster

628. Visceral Pain

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Topic: D.08. Pain

Support: NIH R01 DK093525

NIH R01 NS35790

APS Future Leaders in Pain Research

Title: Prostatitis induces bladder hypersensitivity via neural cross-talk

Authors: *E. S. SCHWARTZ, E. E. YOUNG, B. FENG, G. F. GEBHART;
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Abstracts: Background: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a condition that involves the prostate, bladder, and pelvic floor, and causes symptoms that include frequent and urgent need to urinate, pain or burning when urinating, and pain radiating to the back, abdomen, and/or colorectum. The purpose of the study is to provide evidence of existing pathways in the development of organ cross-sensitization by demonstrating that bladder hypersensitivity develops following the induction of chronic prostatitis. Furthermore, we demonstrate sensory (afferent) co-innervation of the mouse prostate and bladder, changes in putative mediators in both prostate and bladder, and bladder afferent sensitization. **Methods**: Retrograde tracers were injected into the bladder (DiI) and/or prostate (fast blue, FB). Prostatitis was produced by injecting 0.1 mg zymosan in 1% FB into the dorsal lobe of the mouse prostate; sham mice received 1% FB. After zymosan/sham injection, DRG were collected and examined for innervation density, somata size, and content/expression (via single cell PCR) of putative nociceptive markers. Prostate and bladder histology, immunohistochemistry, and RNA were examined weekly. Cystometry, urinary bladder distension and bladder afferents were also assessed at these times. **Results:** We found that > 50 % of DRG afferent somata are shared by both the urinary bladder and prostate. Evaluation of mRNA content following prostate inflammation showed a significant upregulation in pro- and anti- inflammatory cytokines (i.e., TNF-α and IL-10) and growth factor NGF as well as changes in mRNA content in FB-labeled DRG somata which showed TRPV1, TRPA1, and ASIC-3 to be relatively abundant; expression was significantly increased during prostatitis. Significantly, bladder function, responses to distension, and bladder afferents were sensitized during prostatitis. **Conclusions:** These experiments revealed that prostate and bladder share afferent innervation via convergent DRGs. Experimental prostatitis significantly increased urinary bladder voiding frequency and decreased volume per void compared to controls and induced hypersensitivity to bladder distension. These observations provide mechanistic support for CPPS and prostate to bladder cross-organ sensitization.

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Poster

628. Visceral Pain

Location: Halls A-C

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Topic: D.08. Pain

Support: NIH Grant NS37424

Title: Estrogen receptor alpha and beta differentially mediate MAPK signaling pathway activation in the female rat spinal cord

Authors: *Y. JI, J. KARPOWICZ, S. PANDYA, D.-Y. CAO, R. TRAUB;
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Abstracts: We previously reported that the visceromotor response to colorectal distention fluctuates across the estrous cycle, sensitivity peaking as the plasma estrogen level reaches its apex during proestrus. Ovariectomy (OVx) decreases visceral sensitivity which is restored by subcutaneous (s.c.) or spinal (intrathecal) estradiol replacement, suggesting estrogen facilitation of visceral nociceptive processing occurs at the level of the spinal cord. Furthermore, s.c. and intrathecal administration of the estrogen receptor alpha (ERα) agonist PPT reversed the inhibitory effect of OVx similar to E2, suggesting ERα in the spinal cord may mediate cyclic changes in visceral sensitivity. Consistent with an increase in visceral sensitivity, s.c. E2 or PPT increases pERK1/2 expression in the spinal cord. In contrast, s.c. administration of the ERβ agonist, DPN, was antinociceptive, decreasing the visceromotor response. In the present study, we used an *in vitro* spinal cord slice preparation to further investigate the mechanisms underlying estrogen receptor mediated spinal signal transduction. OVx rats were injected with Oil, E2 (50μg), PPT (1mg/kg) or DPN (10mg/kg) and spinal cord slices were obtained 4h later. Incubation of spinal slices from E2 and PPT rats with L-glutamic acid (100 μM) for 5 to 30 min induced an increase in pERK expression compared with adjacent vehicle treated slices. In contrast, slices from Oil or DPN treated rats did not show any change of ERK phosphorylation after 5 min incubation. Longer term incubation (15 or 30 min) with L-glutamic acid decreased pERK expression. In a separate study, ER agonists were bath-applied to spinal cord slices from OVx rats to further explore the mechanisms underlying ER agonist mediated changes in response to glutamate. Bath application of E2 (1nM) and DPN (10nM) did not change the pERK/ERK level at 5 min, but increased ERK phosphorylation at 15min-1h. Bath application of PPT (10nM), on the other hand, had no effect on ERK phosphorylation at 5-15min, but caused a small increase in pERK2/ERK2 at 1h. Taken together, these data suggest activation of ERα and ERβ

differentially modulate MAPK signaling pathways at the level of the spinal cord which may contribute to differing effects on behavioral responses to visceral stimuli.

Disclosures: **Y. Ji:** None. **J. Karpowicz:** None. **S. Pandya:** None. **D. Cao:** None. **R. Traub:** None.

Poster

628. Visceral Pain

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Program#/Poster: 628.08/GG16

Topic: D.08. Pain

Support: NSFC81070884

NSFC81230024

NSFC31300909

Title: Adrenergic signaling mediates pancreatic hyperalgesia through activation of purinergic receptors in primary sensory neurons in rats with chronic pancreatitis

Authors: S. F. HU[1], H.-Y. ZHU[1], Y. ZHOU[1], Y. ZHOU[1], *G.-Y. XU[2];
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Abstracts: Objective: The mechanism of pain in chronic pancreatitis (CP) is poorly understood and its treatment remains difficult, in large part due to our lack of knowledge about the neurobiological mechanisms in nociception. The aim of this study was designed to investigate roles of norepinephrine (NE) and P2X receptor signaling pathway in the pathogenesis of pancreatic pain in a rat model of CP. Methods: CP was induced in male adult rats by intraductal injection of trinitrobenzene sulfonic acid (TNBS). Pancreatic hyperalgesia was assessed by referred somatic behaviors to mechanical stimulation of rat abdomen. DiI dye injected into pancreas was used to label pancreas innervating dorsal root ganglion (DRG) neurons. P2X receptor (P2XR)-mediated responses in pancreas DRG neurons were measured using calcium-imaging and whole cell patch clamp recording techniques. Western blot analysis was performed to examine protein expression. Results: Administration of purinergic receptor antagonist suramine or A317491 attenuated pancreatic pain in CP rats. Expression of P2X3Rs and ATP-induced current density of pancreas DRG neurons were markedly enhanced after TNBS

injection. The sensitization of P2X3Rs was reversed by β adrenergic receptor antagonist propranolol but not by α adrenergic receptor antagonist phentolamine. CP was associated with an upregulation of expression of β2 adrenergic receptors in DRGs. The NE concentration in DRGs and pancreas was significantly enhanced in TNBS-treated rats. TNBS injection also produced a 2-fold increase in ATP-induced calcium signals and amplified the NE-induced potentiation of ATP responses when compared with controls. Using triple labeling techniques, we showed that β2 adrenergic receptors were colocalized with P2X3Rs in pancreatic DRG neurons. Furthermore, incubation of DRG neurons with NE significantly enhanced ATP-induced intracellular calcium signals, which was abolished by propranolol but not by phentolamine, and partially blocked by protein kinase A inhibitor H-89. Forskolin, an adenylyl cyclase activator, markedly enhanced ATP-induced responses of DRG neurons. Importantly, the pancreatic referred somatic hyperalgesia in CP rats was markedly attenuated by systemic administration of propranolol but not by phentolamine. Conclusions: Sensitization of P2X3Rs, which was likely mediated by adrenergic signaling in primary sensory neurons, contributes to pancreatic pain, thus identifying a potential target for treating pancreatic pain caused by inflammation.

Disclosures: **S.F. Hu:** None. **H. Zhu:** None. **Y. Zhou:** None. **Y. Zhou:** None. **G. Xu:** None.

Poster

628. Visceral Pain

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Topic: D.08. Pain

Support: grants4targets from Bayer Pharmaceutical

Title: RvD1 and Chemerin alleviated inflammatory signs associated with endometriosis in rats

Authors: ***N. DMITRIEVA**[1], C. M. GARCIA PASCUAL[2], G. SUESS[2], R. SHIRLEY[2];
[2]Psychology, [1]Florida State Univ., TALLAHASSEE, FL

Abstracts: Endometriosis (ENDO) is a chronic estrogen-dependent painful condition that is characterized by the presence of endometrial growths outside the uterus. Inflammatory processes associated with ENDO such as increased local production of inflammatory mediators, increased permeability of the vascular bed within the growths to blood proteins and infiltrating leukocytes, suggest that chronic pain associated with ENDO has inflammatory origin. RvD1, 17(R)-RvD1 and RvE1 are pro-resolving lipid mediators (Resolvins) that are locally biosynthesized during the

resolution phase of acute inflammation. Chemerin is an endogenous peptide ligand for Chem23R, a receptor that can also become activated by RvE1. Recent findings indicate that these Resolvins can alleviate inflammatory signs including pain in some chronic conditions. We studied the effects of RvD1, 17(R)-RvD1 and Chemerin on increased vascular permeability of ectopic endometrial growths assessed by Evans Blue extravasation in rats with surgically-induced ENDO. In another group of ENDO rats, we studied the effect of i.p. injections these molecules on vaginal hyperalgesia, which was monitored telemetrically. Both Resolvins and Chemerin, but not vehicle, significantly decreased vascular permeability in ectopic endometrial tissue. 17(R)-RvD1 and Chemerin also significantly alleviated severity of vaginal hyperalgesia. Our results suggest that Resolvins and Chemerin can be effective in reducing inflammatory signs including visceral pain in women with ENDO.

Disclosures: N. Dmitrieva: None. **C.M. Garcia Pascual:** None. **G. Suess:** None. **R. Shirley:** None.

Poster

628. Visceral Pain

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Program#/Poster: 628.10/GG18

Topic: D.08. Pain

Support: NIG Grant DK093525

Title: Colonic inflammatory molecules do not underlie inflammatory, post-, and non-inflammatory colorectal hypersensitivity in the mouse

Authors: *J.-H. LA, G. F. GEBHART;
Dept. Anesthesiol., Ctr. For Pain Research, Univ. Pittsburgh, PITTSBURGH, PA

Abstracts: BACKGROUND & AIMS: Colorectal hypersensitivity develops during, after and even in the absence of colorectal inflammation. In the absence of colonic inflammation, a low-level inflammatory milieu has been hypothesized to underlie the hypersensitivity. We tested this hypothesis by examining [1] the effect of dexamethasone in three mouse models of colorectal hypersensitivity and [2] colonic inflammatory molecules/mediators quantified by qRT-PCR. METHODS: Visceromotor responses to colorectal distension were quantified as a measure of sensitivity. On day 1, mice received intracolonic saline (control), TNBS (inflammatory on day 3; post-inflammatory on day 15), or acidified hypertonic saline (non-inflammatory).

Dexamethasone was administered once daily for two (TNBS day 3) or four days (days 11-14 in other models). RESULTS: Dexamethasone did not attenuate colorectal hypersensitivity in any model, but did inhibit gene expression of inflammatory molecules. Principal component analysis revealed six correlated groups of molecules: 1 (COX-2, IL-1β, Follistatin, IL-6, and Inhibin βA); 2 (Activin R2B, COX-1, substance P, and CGRPα and β; 3(TGF-β1, TGF-β2, POMC, and BAMBI); 4 (MCPT-1, IL-10RA, and TNF-α); 5 (IL-10 and TGF-βR1); and 6 (CGRPα and PGE2 synthase). No group was correlated with colorectal hypersensitivity. Gene expression of molecules in Groups 1 and 2 were up- and down-regulated, respectively, only in the inflammatory model. Group 3 distinguished non- from post- and inflammatory models, and Group 5, post- from the inflammatory model. CONCLUSIONS: Accordingly, a low-level colonic inflammatory milieu of candidate inflammatory molecules is neither sufficient nor necessary for development of colorectal hypersensitivity.

Disclosures: J. La: None. **G.F. Gebhart:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.11/GG19

Topic: D.08. Pain

Support: Helsinn Research Support

Title: Attenuation of visceral and somatic nociception by peripheral administration of ghrelin agonists

Authors: E. MOHAMMADI[1], *C. PIETRA[2], K. TYLER[1], R. NORTHRUP[3], B. GREENWOOD[1];
[1]Dept. of Physiol., VA Med. Ctr., University of Oklahoma Health Sciences Center, OK; [2]Helsinn Healthcare SA, Lugano- Pazzallo, Switzerland; [3]Helsinn Therapeut. Inc, Bridgewater, NJ

Abstracts: Background: Previous studies suggest that ghrelin inhibits nerve injury associated neuropathic pain though an inhibition of proinflammatory cytokines. Whether ghrelin-receptor mediated mechanism attenuate visceral or somatic pain in the absence of inflammation remains to be determined. This study investigated the effects of two potent and selective ghrelin agonists, one peripherally restricted, ipamorelin (IPA) and one peripheral and centrally acting , namely HM01. The efficacy of both compounds was investigated in experimental models of non-

inflammatory visceral hypersensitivity and somatic mechanical allodynia. **Methods**: Acute visceral hypersensitivity was induced experimentally in rats by infusing acetic acid into the colon at a concentration (0.6%) known to sensitize visceral afferents in the absence of mucosal inflammation. Colonic sensitivity was assessed via a visceromotor behavioral response (VMR) quantified as the number of abdominal contractions in response to graded isobaric pressures (0-60 mmHg) of colorectal distension (CRD). Somatic mechanical allodynia was quantified by the number of ipsilateral paw withdrawals in response to a calibrated von Frey filament. IPA was administered intravenously at 0.01, 0.1, and 1 mg/kg whereas HM01 was administered orally at 1, 3, 10, or 30 mg/kg. **Results**: Compared to vehicle controls, both IPA and HM01 caused a significant attenuation of visceral hypersensitivity and somatic allodynia. While HM01 was effective at all doses tested only the highest dose of IPA (1 mg/kg i.v.) showed efficacy. A comparison between the minimal effective doses is shown in the table below:

Groups	**Visceral Hypersensitivity(60mmHg) # of contractions**	**Somatic Allodynia Force (g)**
Vehicle Control	32± 1.6	51± 2.4
IPA (1 mg/kg i.v.)	18± 1.0**	117± 2.9****
HM01 (1 mg/kg p.o.)	24± 0.7**	98± 2.8****

** $p<0.01$, **** $p<0.0001$ compared to VEH **Conclusion**: We have shown for the first time that ghrelin-mediated mechanisms are involved in visceral and somatic nociception in the absence of an overt inflammatory nerve injury. Furthermore, a ghrelin agonist with both central and peripheral activity showed superior efficacy over a peripherally restricted agonist.

Disclosures: **E. Mohammadi:** 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.; Helsinn SA. **C. Pietra:** A. Employment/Salary (full or part-time):; C. Pietra, Helsinn SA. **K. Tyler:** 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.; Helsinn SA. **B. Greenwood:** 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.; Helsinn SA. **R. Northrup:** A. Employment/Salary (full or part-time):; Helsinn Therapeutics Inc..

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.12/GG20

Topic: D.08. Pain

Support: NIH Grant AR047410

Title: Increased glutaminase and aspartate aminotransferase in rat sacral dorsal root ganglion neurons during acute colonic inflammation

Authors: *K. E. MILLER[1], R. JOHN[1], M. B. ANDERSON[1], C. KIM[1], K. TYLER[2], B. GREENWOOD-VAN MEERVELD[2];
[1]Dept Anat. & Cell Biol, Oklahoma State Univ. Ctr. Hlth. Sci., TULSA, OK; [2]Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstracts: At the onset of inflammation, nociceptive sensory neurons respond by altering their profile of protein expression. In somatic inflammatory pain models, the amounts of glutaminase (GLS) and aspartate aminotransferase (AST), enzymes for glutamate production, are increased in dorsal root ganglion (DRG) neurons within 24 hrs following the onset of inflammation. Our previous findings using a trinitrobenzene sulphonic acid (TNBS) model of inflammatory bowel syndrome (IBS) showed that sacral DRG neurons have elevated GLS and AST in sacral DRG neurons at 30 days post-inflammation. The current study was to determine if GLS and AST are elevated early in the DRG after the onset of visceral inflammation. **Methods**: In Sprague Dawley rats, an acute colonic inflammation was induced by intracolonic infusion of TNBS (50 mg/kg, 25% ethanol, 0.5 ml enema). Saline-enema and naïve rats were used as controls. A disease activity index (DAI) assessed the level of colitis. On days 1 & 2, sacral 1 DRG were isolated and processed for glutaminase (GLS) and aspartate aminotransferase (AST) immunohistochemistry and image analysis. **Results**: Compared to saline-enema treated or naive controls (n = 4/group), TNBS-induced rats had an increase in both AST and GLS-immunoreactivity (ir) in DRG neurons. AST-ir was elevated 23% over controls at day 1 and 37% at day 2 of inflammation. No change was detected in GLS-ir at day 1, but GLS-ir was elevated 55% at day 2. **Summary**: An inflammatory insult to the colon significantly increases GLS- and AST-ir within sacral DRG neuronal cell bodies within 24-48 hr. **Conclusion**: Our results suggest that elevated glutamate metabolism in primary sensory neurons may lead to increased glutamate production in and release from spinal or colonic primary afferent terminals. This may lead to visceral hypersensitivity by glutamate-mediated central and/or peripheral sensitization.

Disclosures: K.E. Miller: None. **R. John:** None. **M.B. Anderson:** None. **C. Kim:** None. **K. Tyler:** None. **B. Greenwood-Van Meerveld:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.13/GG21

Topic: D.08. Pain

Support: This work was supported by a department of Veterans Affairs Merit Grant to Dr. Beverley Greenwood-Van Meerveld.

Title: Altered stress-induced visceral nociception in adult female rats previously exposed to unpredictable early life adversity

Authors: *S. KENNEDY[1], B. GREENWOOD-VAN MEERVELD[2];
[2]Oklahoma Ctr. for Neuroscience, Dept. of Physiology, VA Med. Ctr., [1]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstracts: Early life adversity (ELA), such as neglect or abuse, has been indicated as a risk factor for the development of stress axis dysfunction in adulthood, specifically in females. Our laboratory has previously shown that unpredictable, but not predictable, ELA induces visceral hypersensitivity in adult female rats, and this effect is estrogen-dependent. However, it remains to be determined whether ELA has long lasting effects on visceral nociceptive responses to stress axis activation in adulthood. It is our hypothesis that following ELA, adult stress exposure induces allodynia and hyperalgesia in a sexually dimorphic manner. Neonatal rats underwent classical conditioning using unpredictable or predictable odor-shock pairing or an odor only control. In adulthood, visceral sensitivity was assessed via visceromotor behavioral response (VMR) quantified as the number of abdominal contraction in response to graded pressures (0-60 mmHg) of isobaric colorectal distension (CRD). Adult stress was induced using *acute* (1-day) or *chronic* (7-day) exposure to a 1-hr water avoidance stressor (WAS). In adulthood *acute* WAS increased the VMR to CRD in all female rats, however rats that experienced unpredictable ELA exhibited significantly ($P<0.001$) more pain behaviors (60 mmHg: 43.5±1.2 abdominal contractions in 10 min) compared to predictable ELA (33.1±2.5) or odor only controls (31.7±0.8). In adult female rats, *chronic* WAS increased the VMR to CRD, however the number of abdominal contractions in response to 60 mmHg CRD was significantly ($P<0.001$) greater in rats exposed to either unpredictable ELA (44.0±0.6 abdominal contractions in 10 min) or predictable ELA (43.0±1.7) compared to controls rats (32.3±0.5). In male rats, exposure to WAS increased colonic sensitivity to luminal distension, however prior exposure to neonatal ELA did

not correlate with increased magnitude of the WAS-induced VMR to CRD in adult life. In summary, this study suggests a novel consequence of ELA on visceral nociception in adulthood that is sex-specific. We demonstrated that ELA unmasks an exaggerated nociceptive response to an adult stressor in female rats. However, ELA does not serve as a risk factor for enhanced visceral nociception following adulthood stress in male rats. Taken together, these data indicate that ELA may interact with the female sex hormone estrogen to disrupt negative feedback mechanisms underlying stress axis homeostasis and responsivity to adulthood stressors. This dysregulation may ultimately predispose females to stress-induced visceral pain in adult life.

Disclosures: S. Kennedy: None. **B. Greenwood-Van Meerveld:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.14/GG22

Topic: D.08. Pain

Support: NCCAM -5R01AT007137

Title: Neurological differences between female irritable bowel syndrome patients and healthy controls during expectation of safety from abdominal threat

Authors: Z. GILL[1], J. LABUS[1], J. HONG[1], C. LIU[1], B. NALIBOFF[1], *E. A. MAYER[2], K. TILLISCH[1];
[1]Ctr. for Neurobio. of Stress UCLA, Los Angeles, CA; [2]Med., Oppenheimer Family Ctr. For Neurobio. of Stress, LOS ANGELES, CA

Abstracts: Background: IBS is a common gastrointestinal disorder characterized by chronic abdominal pain, alteration in bowel habits, and symptom-related anxiety. Previous studies have shown that IBS patients have abnormal brain responses to the expectation of visceral pain in regions associated with emotional arousal and visceral sensation. It is unknown whether individuals with IBS exhibit normal responses to 1) the expectation of a non-visceral threating stimulus or 2) the presence of a safety condition within the context of a threat paradigm. **Aims**: To identify differences in functional neural activity between IBS patients and healthy controls during the expectation of a threatening abdominal shock and during a designated safe period. **Methods**: 23 female IBS patients and 10 female healthy controls (ages 18-50) were recruited by advertisement. We recorded the blood oxygen level-dependent (BOLD) response to an

abdominal threat paradigm using a 3 Tesla Siemens Trio scanner. The paradigm was a jittered block design which included the following conditions: safe (no shock will be delivered), threat (shock may be delivered), and a neutral cross hair condition during which patients were told that no shock would occur. A two sample t-test was performed within SPM8 and regions of interest in the emotional arousal and sensory circuitry were evaluated. **Results**: We identified significant BOLD differences between the IBS group (31.4 years, SD=9.53) and the healthy control group (26.1 years, SD=5.38). Region of interest analysis using small volume correction in SPM8 showed significance in bilateral dorsomedial nuclei of the thalamus (left:-2, -16, 6 (XYZ), Z=4.59, p=0.002, family-wise error (FWE) corrected, and the right: 2, -14, 8, Z=4.03, p=0.015, FWE corrected). Brain activity in these regions were correlated with patient self-report of abdominal pain on a 10 point numeric rating scale and revealed significant positive correlations in both the left [r(23)=23, p=0.007] and right [r(23)=23, p=.012] thalamus. No differences were identified between groups in the threat period of the paradigm. **Conclusions**: The thalamus is associated with expectation-related neural activity consistent with increased afferent sensitivity. IBS patients' exhibiting a significantly greater level of activity in this region during expectation of safety suggests that those with IBS may have abnormalities in the deactivation of the afferent processing network. It is possible that this central alterations plays a role in the increased sensitivity of IBS patients to visceral sensations, and cardinal IBS symptoms such as bloating and abdominal pain.

Disclosures: Z. Gill: None. **E.A. Mayer:** None. **J. Labus:** None. **B. Naliboff:** None. **K. Tillisch:** None. **J. Hong:** None. **C. Liu:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.15/GG23

Topic: D.08. Pain

Support: NorthShore Research Career Development Award

Title: Behavioral and physiological characterization of the molecules involved in menstrual pain

Authors: *K. M. HELLMAN[1,2], F. F. TU[2];
[1]OB/GYN, NorthShore Univ. HealthSystem, Chicago, IL; [2]Obstetrics & Gynecology, Univ. of Chicago, Chicago, IL

Abstracts: Contemporary research has focused on the central nervous system's contribution to menstrual pain, yet the impact of peripheral factors remain inadequately characterized. This has hamstrung efforts to develop effective targeted therapeutic strategies for dysmenorrhea. Although it is reputed that cytokines elicit pain by increasing uterine contractility resulting in ischemia, studies investigating their effects on uterine function *in vivo* are limited. Studies in humans reported elevated levels of prostaglandin (PGF2α), oxytocin, platelet-activating factor (PAF) and transient receptor potential vanilloid (TRPV) in dysmenorrhea. To establish whether these candidate molecules are sufficient to elicit abdominal pain and what their effects are on uterine physiology, we characterized pain behavior following their administration in a mouse model. While oxytocin elicited torso stretching resembling labor in mice, there was no evidence of pain or hyperalgesia. In contrast, PGF2α and the PAF receptor agonist, Carbamyl PAF (CPAF) produced behavior indicative of abdominal pain and hyperalgesia, but not torso stretching. Uterine pressure was increased by oxytocin and CPAF, but not by PGF2α. In control experiments, no effects were observed in vehicle treated mice or when CPAF was administered to PAF-R KO mice. In wild-type mice, CPAF elicited hypoxemia mediated by the elevated intrauterine pressure. Following CPAF exposure, the TRPV agonist capsaicin improved perfusion and oxygenation. Thus, PAF may contribute to uterine pain by increasing uterine contractility, leading to hypoxemia, while TRPV may serve a protective role by increasing perfusion. Future work extending these findings using our *in vivo* model can be used to evaluate therapeutic approaches for menstrual pain.

Disclosures: K.M. Hellman: None. **F.F. Tu:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.16/GG24

Topic: D.08. Pain

Support: SUPPORTED BY RO1 NS 11892

Title: Changes in sensory and sympathetic innervation of pelvic organs and ectopic uterine growths during the development and stabilization of endometriosis (ENDO)-induced vaginal hyperalgesia in the rat

Authors: *S. L. MCALLISTER[1], B. K. GIOURGAS[2], K. J. BERKLEY[2];
[1]Program in Neuroscience/Psychology, [2]Florida State Univ., Tallahassee, FL

Abstracts: Endometriosis is a painful disorder defined by extrauteral endometrial growths (ectopic growths). How the growths contribute to pain symptoms, such as dyspareunia (vaginal hyperalgesia) is poorly understood. A rat model (ENDO) is created by autotransplanting on abdominal arteries pieces of uterus. These ectopic growths form cysts that accrue a sprouted sensory and sympathetic innervation that appears gradually over a six-week period and then later stabilizes (McAllister et al. 12; 13), giving rise to three distinguishable phases: initial, transitional (TRANS), and established (ESTAB): 1-2 wks; 4-6 wks; and 8-10 wks post-ENDO surgery, respectively. The hyperalgesia develops after the cysts are initially innervated and the innervation has become functional. The hyperalgesia then becomes variable during TRANS, and is stabilized by ESTAB (McAllister et al. 12). These and other findings strongly support the hypothesis that innervation of the cysts' contributes significantly to the vaginal hyperalgesia during the entire course of its development and stabilization (Stratton & Berkley 11). Additional studies from our lab during TRANS suggest that the sympathetic innervation of the vaginal canal also contributes to vaginal hyperalgesia, with no contribution from innervation of the eutopic uterus (McAllister et al 12, 13). Here we tested how innervation of the vaginal canal and eutopic uterus might contribute during the ESTAB phase. METHODS: The density of sensory and sympathetic innervation of both tissues was assessed with quantitative immunohistochemistry using antibodies to calcitonin gene related peptide (CGRP; identifies sensory C-fibers) and either tyrosine hydroxylase or vesicular monoamine transporter (TH; VMAT2: identifies sympathetic fibers). Three groups were studied (n=4/group): (i) ENDO, (ii) shamENDO (control surgery), (iii) and Naïve (no surgery). Vaginal canal and eutopic uterine samples were harvested when the rat was in proestrus during the ESTAB phase. RESULTS/CONCLUSIONS: There were no significant group differences in innervation of the vaginal canal or eutopic uterus during the ESTAB phase. These findings support the hypothesis that different peripheral neural mechanisms underlie ENDO-induced vaginal hyperalgesia during the course of its initial-to-transitional development and then during stabilization.

Disclosures: S.L. McAllister: None. **B.K. Giourgas:** None. **K.J. Berkley:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.17/GG25

Topic: D.08. Pain

Support: NIH NS37424

Title: Epigenetic modulation of stress-induced visceral hypersensitivity in female rats

Authors: D.-Y. CAO, G. BAI, J. M. KARPOWICZ, Y. JI, *R. J. TRAUB;
Neural and Pain Sci., Univ. of Maryland Sch. of Dent., Baltimore, MD

Abstracts: Visceral hypersensitivity induced by chronic stress is a laboratory model for irritable bowel syndrome (IBS) and can be used to explore the molecular mechanism underlying IBS. Stress may alter gene expression which in turn produces or modulates visceral sensitivity. Epigenetic mechanisms are heavily involved in stress and potentially in visceral pain. In this study, we examined the effect of histone acetylation as an epigenetic mechanism at the spinal level on stress-induced visceral hypersensitivity. Subchronic stress was produced by forced swim (FS) for three consecutive days (10, 20, 20 min) in female Sprague-Dawley rats. Histone hyperacetylation at the spinal level was induced by inhibition of histone deacetylase via intrathecal injection of suberoylanilide hydroxamic acid (SAHA). Visceral sensitivity was tested by the visceromotor response (VMR) to colorectal distension and expression of metabotropic glutamate receptor 2 and 3 (mGluR2, 3) in the lumbosacral (LS, i.e. L6-S1) dorsal spinal cord was examined. Our results show that stress significantly increased visceral sensitivity over baseline 1 and 5 days after the last FS. The immobility time during the first 5 minutes of FS increased on consecutive days suggesting the rats developed a depressed state. Spinal administration of SAHA (40 μg/day) both prevented (pre-treatment with drug 30 min before each FS) and reversed (post-treatment with drug for 3 days after the last FS) visceral hypersensitivity induced by FS. Western blot data showed SAHA pre-treatment significantly increased acetylation of lysine 9 on histone 3 (H3K9ac) in the LS dorsal spinal cord compared with vehicle treated rats. SAHA pre-treatment also increased expression of mGluR3, but not mGluR2, in the LS dorsal spinal cord 1 day post FS. Chromatin immunoprecipitation showed that SAHA pretreatment significantly increased binding of H3K9ac, but not H3K18ac, to the mGluR3 promoter. Intrathecal injection of the mGluR2/3 antagonist LY341495 reversed the SAHA-evoked attenuation of the stress-induced visceral hypersensitivity, but did not alter FS-induced visceral hypersensitivity in vehicle treated rats. These results reveal that increasing histone acetylation in the spinal cord attenuates psychophysical stress-induced visceral hypersensitivity by increasing mGluR3 activity and indicate a novel approach to relieve stress-induced visceral pain, especially for IBS patients.

Disclosures: D. Cao: None. **R.J. Traub:** None. **G. Bai:** None. **J.M. Karpowicz:** None. **Y. Ji:** None.

Poster

628. Visceral Pain

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Program#/Poster: 628.18/GG26

Topic: D.08. Pain

Support: NIH R37 DK54824

Title: Effect of protamine sulfate and potassium chloride on the inhibitory rectovesical reflex in an isovolumetric bladder model

Authors: S. PERSYN[1], *L. A. BIRDER[2], J.-J. WYNDAELE[1];
[1]Dept. of Urology, Univ. of Antwerp, Fac. of medicine, Antwerp, Belgium; [2]Univ. Pittsburgh Sch. Med., PITTSBURGH, PA

Abstracts: ABSTRACT PURPOSE: In pelvic physiology a coordinated activity of the lower urinary tract and colorectum is necessary for normal function. A dysfunction in this neural cross-organ communication often leads to co-morbidity of bladder and colorectum. Previous studies have shown an inhibitory rectovesical reflex (IRVR), elicited by a noxious colorectal distension (CRD) (60mmHg). Increasing colorectal afferent activity, mechanically and pharmacologically, significantly enhanced the IRVR. Our goal was in part to explore the influence of a compromised urothelial barrier on bidirectional colon-bladder interactions. MATERIALS AND METHODS: We combined protamine sulfate (PS) (10 mg/ml for 30 min; transurethral injection), which has been demonstrated to compromise urothelial barrier function, and a high concentration of potassium chloride (KCL) (300 mM). Two different groups of female Sprague-Dawley rats (200-250 g) were compared. The first group underwent two different protocols: control saline and KCL filling. The second group underwent three different protocols: control saline filling, saline filling after PS treatment and KCL filling after PS treatment. In all animals isovolumetric contractions with ligated urethra were studied. The protocol was approved by the Animal Ethics Committee of the University of Antwerp (EC 2013-06). RESULTS: The inhibitory effect of a noxious CRD on isovolumetric bladder contractions was observed in a control experiment before starting the different parts of the study. KCL inhibited isovolumetric bladder activity, which is reflected in a decreased contraction frequency (BCF) (18.67%) and an increased intercontraction interval (28.59%) and bladder capacity (14.51%). The effect of noxious CRD was increased 3-fold during KCL compared with saline. In rats with a compromised barrier (PS treatment), the noxious CRD led to a 3-fold increased inhibition with saline alone and up to 5-fold with KCL. In addition, the KCL-induced bladder contractility in PS treated rats was inhibited (BCF reduction: 25.71%). CONCLUSIONS: This study shows an inhibitory effect of intravesical KCL filling on the isovolumetric bladder contractility which was greater in rats with a compromised urothelial barrier. In addition, KCL filling results into an increased IRVR elicited by a noxious CRD, which was stronger after PS exposure. Our results suggest a stronger IRVR in rats with an increased bladder afferent stimulation, which is more prominent with a compromised urothelial barrier.

Disclosures: **S. Persyn:** None. **L.A. Birder:** None. **J. Wyndaele:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.19/GG27

Topic: D.08. Pain

Support: Department of Veterans Affairs Merit Grant awarded to BGVM

Title: Chronic mechanical allodynia and visceral hyperalgesia induced by elevated amygdala corticosteroids: Importance of histone acetylation

Authors: *C. LIGON[1], L. TRAN[3], B. GREENWOOD-VAN MEERVELD[2];
[1]Oklahoma Ctr. for Neurosciences, [2]Physiol., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [3]Mayo Clin., Scottsdale, AZ

Abstracts: Objective: We previously demonstrated that acute exposure of the central amygdala (CeA) to elevated corticosterone (CORT) induced persistent somatic allodynia, visceral hyperalgesia, decreased glucocorticoid receptor (GR) and increased corticotrophin-releasing factor (CRF) expression in the CeA. Using the same model, we hypothesize epigenetic mechanisms such as histone deacetylation contribute to the maintenance of chronic somatic allodynia and visceral hyperalgesia induced by elevated CORT in the CeA. Methods: Animals were stereotaxically implanted with CORT or CHOL micropellets and bilateral CeA cannula (n=7-8/group). Post-surgery (days 21-28), animals were infused daily with either 0.5µl of vehicle (VEH; 0.1% DMSO/Saline) or the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA; 100-500 ng/µl) bilaterally into the CeA. Somatic sensitivity was measured using an electronic von Frey test and visceral sensitivity was assessed via a visceromotor behavioral response (VMR) quantified as the number of abdominal contractions in response to colorectal distension (CRD). Results: Following 28 days, CORT implants onto the CeA significantly ($P<0.001$) lowered mechanical somatic threshold from 69.3 ± 4.45g to 44.7 ± 2.54g. SAHA treatment restored mechanical thresholds to 74.5 ± 2.11g ($P<0.001$). Results of the VMR following CRD showed that in CORT implanted rats there was a significant increase ($P<0.001$) in the number of contractions at the highest distension pressure of 60 mmHg pressure in CORT treated animals (33.9 ± 1.9) compared to CHOL (19.5 ± 0.8), indicative of visceral hyperalgesia. The increase in the number of abdominal contractions in response to CRD was attenuated by SAHA treatment ($P<0.001$; 20.75 ± 0.99). Conclusion: Protection of histone acetylation prevents

the sustained nociceptive behaviors induced by elevated CORT in the CeA, indicating that epigenetic programming, in particular histone modifications, may be critical in the maintenance of visceral and somatic pain induced by amygdala activation.

Disclosures: C. Ligon: None. **L. Tran:** None. **B. Greenwood-Van Meerveld:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.20/GG28

Topic: D.08. Pain

Support: SFI Grant 07/CE/B1368

SFI Grant 12/RC/2273

Title: Negative allosteric modulation of the mglu7 receptor reduces visceral hypersensitivity in a stress-sensitive rat strain

Authors: *R. D. MOLONEY[1], R. M. O'CONNOR[2], M. KALINICHEV[4], T. G. DINAN[3], J. F. CRYAN[2];
[1]Alimentary Pharmabiotic Centre/ Neurogastroenterology, [2]Anat. and Neurosci., [3]Psychiatry, Univ. Col. Cork, Cork, Ireland; [4]Addex Therapeut., Geneva, Switzerland

Abstracts: Introduction: Glutamate, the main excitatory neurotransmitter in the central nervous system exerts its effect through both ionotropic and metabotropic receptors. mGlu7 receptors, the most highly conserved isoform, are abundantly distributed in the brain, especially in those regions, such as the amygdala, known to be crucial for the emotional processing of painful stimuli. Understanding the role of mGlu7 receptors has been hampered by the lack of selective pharmacological tools. Visceral hypersensitivity, a hallmark of irritable bowel syndrome (IBS), is a poorly understood phenomenon manifesting as an increased sensitivity to visceral stimuli. The pathophysiology of visceral hypersensitivity is not well understood, however glutamate has long been associated with somatic pain processing leading us to postulate that crossover may exist between these two modalities. Moreover, stress has been shown to exacebate visceral pain. ADX71743 is a novel, recently described, centrally penetrant negative allosteric modulator of mGlu7 receptors. Thus we can use this tool to explore the possible involvement of this receptor in the mediation of visceral pain in a stress-sensitive model of visceral hypersensitivity, namely

the Wistar Kyoto rat. Methods: Colorectal distension (CRD) was performed to assess visceral sensitivity in adult male Wistar Kyoto (WKY) rats (250-300g;Harlan, UK). Postures defined as visceral pain behaviours were abdominal retractions and/or abdominal withdrawal reflex. ADX71743 (50, 100, 150mg/kg s.c.) was administered 30 minutes prior to commencement of CRD. All behavioural analysis was performed by a trained observer blind to treatments. Results: ADX71743 dose-dependently reduced visceral hypersensitivity in the Wistar Kyoto rat as exhibited by increased visceral sensitivity threshold ($F(3,30) = 3.092$, $p<0.05$) with concomitant reductions in total number of pain behaviours ($F(3,30) = 6.582$, $p<0.01$). Conclusion: These findings show for what is to our knowledge the first time that mGlu7 receptor signalling plays a role in visceral pain processing. Thus, negative modulation of the mGlu7 receptor may be a plausible target for the amelioration of visceral pain in disorders such as IBS where there is an immense medical need.

Disclosures: R.D. Moloney: None. **R.M. O'Connor:** None. **M. Kalinichev:** A. Employment/Salary (full or part-time):; Addex Therapeutics. **T.G. Dinan:** None. **J.F. Cryan:** None.

Poster

628. Visceral Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 628.21/GG29

Topic: D.08. Pain

Support: NIH Grant RO1-NS035790

IASP John J. Bonica Trainee Fellowship

Title: Bladder hypersensitivity following cystitis is accompanied by altered bladder cytokine expression

Authors: *A. D. SHAFFER, J.-H. LA, G. F. GEBHART;
Anesthesiol., Univ. of Pittsburgh Ctr. For Pain Res., Pittsburgh, PA

Abstracts: Background: Painful bladder syndrome/interstitial cystitis (PBS/IC) is a chronic, debilitating disorder characterized by increased urinary frequency, urgency, and pain in association with small bladder volume. These symptoms are modeled in animals by administration of the antineoplastic drug cyclophosphamide (CYP) that induces dose-dependent

cystitis and bladder hypersensitivity. It has been theorized that constant, low-grade inflammation may contribute to PBS/IC. As a first step to test this hypothesis, we measured pro- and anti-inflammatory cytokines in the bladders of mice treated with CYP, and analyzed the correlation between the cytokines and bladder hypersensitivity. Methods: A chronic CYP treatment regimen (100 mg/kg i.p., 3 doses over 5 days) was used to induce bladder hypersensitivity in the absence of histologic damage to the bladder wall. The visceromotor response (VMR) to bladder distention was measured as an index of bladder nociception on the day following the last CYP treatment. Subsequently, gene expression of pro- and anti-inflammatory cytokines in the bladder was quantified using qRT-PCR. Results: CYP produced bladder hypersensitivity as indicated by an increase in VMR magnitude. This hypersensitivity was accompanied by an upregulation in the gene expression of Inhibin βA, the subunit of Activin A, in the bladder. Importantly, Inhibin βA expression was significantly correlated with VMR magnitude, suggesting a role for this cytokine in bladder hypersensitivity. In contrast, the gene expression of IL-1β, TNF-α, IL-6, TGF-β1, and IL-10 were not altered by chronic CYP. Conclusions: CYP-induced cystitis can change Inhibin βA gene expression in the bladder, which is correlated with the degree of bladder hypersensitivity. This supports the notion that low-grade inflammation involving altered cytokine expression may contribute to bladder hypersensitivity in PBS/IC. Manipulations targeting Activin A may reveal potential treatments to reduce symptoms in PBS/IC.

Disclosures: A.D. Shaffer: None. **J. La:** None. **G.F. Gebhart:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.01/GG30

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH/NINDS NS077446

Title: A new, automated kinematic locomotor assessment system for mice with SCI

Authors: *D. BASSO[1,2,3], L. C. FISHER[1,2], S. WHITE[1], A. L. SHEETS[4];
[1]Hlth. and Rehabil. Sci., Ohio State Univ., COLUMBUS, OH; [2]Ctr. for Brain and Spinal Cord Repair, [3]Neurosci. Grad. Studies Program, The Ohio State Univ., Columbus, OH; [4]Ohio State Univ. Consultant, Columbus, OH

Abstracts: Debilitating neurological conditions are often accompanied by motor or behavioral impairments which are replicated in rodent experimental models. Scientists must test new treatments by measuring functional recovery in fast moving, small animals like mice. We aimed to develop and test a quantitative behavioral and locomotor assessment system for mice with spinal cord injury (SCI). Using markerless motion tracking during open field locomotion, we collected 3 dimensional kinematics with 10 cameras (100Hz) for 2 minutes. Twenty-two mice were analyzed - naïve (n=8); mild (n=5), moderate (n=5) and severe SCI (n=4). The animal's 3D shape was reconstructed in each video frame using background subtraction and shape-from silhouette techniques. The total animal volume was divided into front and rear halves, and the two centers of volume (CoV) provided a measure above and below the mid-thoracic SCI. The rear CoV height and speed served to classify mouse behavior and motor impairments. Behaviors included directed locomotion, exploratory locomotion, meandering, standing, and rearing. SCI decreased behaviors, speed and rear CoV height which were significantly correlated with greater lesion size ($r=.65-.7$; $p<.01$). Further system-generated metrics are being explored to better differentiate mild from moderate SCI. This automated system appears to generate behavioral and motor outcomes that are sensitive to SCI severity, which will reduce errors and personnel resources.

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Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.02/GG31

Topic: D.12. Kinematics and EMG

Support: NIH R01-NS069214

Title: Only moderate intensity of gamma fusimotor drive can stabilize a single joint in neuromorphic emulation

Authors: *C. M. NIU, W. SOHN, T. D. SANGER;
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Abstracts: An important role of the gamma system is to allow the spindle to maintain its high sensitivity over a wide range of muscle lengths during reflex and voluntary contractions. Due to the difficulty of intervening gamma systems in-vivo, the quantitative linkage between gamma activity and movement behavior has not been established. To this purpose, we developed the technique of emulating a sensorimotor system using spiking neurons, realistic muscles and spindles on neuromorphic VLSI chips. The system allows us to create biologically realistic reflex accommodating 1 joint, 2 muscles, 256 spindles and 2,048 neurons. Using the emulated system, we sweep different combinations of gamma dynamic and static values and analyze their effects in stretch reflex. We show that the perturbed joint will retain its original position at a speed that is significantly affected by gamma fusimotor drive, which provides both the position-dependent component via gamma static and a dynamic damping via gamma dynamic in the closed-loop control. We also show that in certain combinations where gamma static exceeds 200Hz, the joint fails to stabilize by either oscillating or swinging out of physiological range; in contrast. Our results suggest that only moderate intensity of gamma drive is allowed to stabilize a joint. In voluntary movements, the contraction of the agonist muscle tends to slack the spindle fiber that undermines its sensitivity for proprioceptive feedback. In order to maintain spindle fibers taut, it is suggested that gamma fusimotor drive would coactivate with inputs to the alpha-motoneuron pool. In future work we will vary the intensity of alpha-gamma coactivation to test its effect on voluntary movements. The hypothesis is that alpha-gamma coactivation effectively suppresses the stretch reflex in the antagonist muscle, and therefore establishes a new equilibrium position in the limb with minimal co-contraction.

Disclosures: C.M. Niu: None. **W. Sohn:** None. **T.D. Sanger:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.03/GG32

Topic: D.10. Spinal Cord Injury and Plasticity

Support: CNPq 483404/2013-6

CNPq: 506024/2013-0

FAPESPA: 003/2014

FUNADESP: 8100234/2013

Title: Grasping ability impairment after unilateral corticospinal tract lesion

Authors: *W. A. CARVALHO[1], J. C. TEIXEIRA[2], S. J. V. CRUZ[2], W. G. LEAL[3], P. BAHIA[2], A. PEREIRA[4];
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Abstracts: Rodents can use their forepaw in dextrous ways to manipulate and eat food pieces of various shapes and textures. We investigated the effect of unilateral corticospinal tract lesion (UCTL) on skilled-reaching forepaw movements. Nine adult (both sex) Wistar rats (CEPAE/UFPA protocol BIO0079-12) weighing 250-300g were divided in sham (n=3) and lesioned (n=6) groups. Animals were anesthetized with 0.1ml xylazine (9mg/kg) and 0.7ml ketamine (72mg/kg). A partial laminectomy was performed at C4 level to expose the dorsal columns. Using a micropipette we injected 20pMol of the vasoconstrictor endothelin-1 close to the medial dorsal artery at a depth of 1 mm from the pial surface. Five minutes later, the micropipette was gently withdrawn, the animal was sutured and then returned to its home cage. The staircase test was used to evaluate forepaw (PP: preferred and NPP: non-preferred) movements before and 3, 7 and 14 days post-lesion (DPL). The sham group showed no difference in the average number of pellets retrieved compared to the baseline (PP: 9.00±1.00, NPP: 7.33±0.58) 3 DPL (PP: 7.67±1.53, NPP: 7.00±2.00) 7 DPL (PP: 8.67±2.52, NPP: 8.00±1.00) 14 DPL (PP: 9.33±0.58, NPP: 8.33±1.53). The lesioned group, however, displayed a significant impairment 3 and 7 DPL with spontaneous recovery in 14 DPL: baseline (PP: 9.67±1.37, NPP: 5.17±1.17) 3 DPL (PP: 2.33±1.03, NPP: 4.67±1.37) 7 DPL (PP: 2.67±0.52, NPP: 5.83±1.17) and 14 DPL (PP: 5.67±1.75, NPP: 6.33±1.03). Our results show that UCTL impairs skilled forepaw movements in rats. The staircase test was efficient to provide quantitative measures of skilled reaching for both the preferred and non-preferred paw.

Disclosures: W.A. Carvalho: None. **J.C. Teixeira:** None. **S.J.V. Cruz:** None. **W.G. Leal:** None. **P. Bahia:** None. **A. Pereira:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.04/GG33

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH 1 R01 NS074882-01A1

NIH 1 F31 NS080512-01

NIH HHSN271200800036C

Foundation for Physical Therapy Florence P. Kendall Post-Professional Doctoral Scholarship

Title: Morphological changes and neuronal hyperactivity in lumbar locomotor networks remote to mid-thoracic spinal cord injury

Authors: *T. D. FAW[1,2,3], C. N. HANSEN[1,3], L. C. FISHER[1,3,4], S. D. KERR[1,3,4], J. A. BUFORD[1,2,3,4], D. M. BASSO[1,2,3,4];
[2]Neurosci. Grad. Studies Program, [3]Ctr. for Brain and Spinal Cord Repair, [4]Sch. of Hlth. and Rehabil. Sci., [1]The Ohio State Univ., Columbus, OH

Abstracts: Spasticity occurs in ~75% of people with spinal cord injury (SCI), with most reporting a negative impact on quality of life. While spasticity is clinically defined as a velocity dependent increase in tonic stretch reflexes, its pathophysiology is largely unknown. One possible mechanism involves dysfunction of spinal interneurons. It is unclear how spasticity and/or interneuronal dysfunction relate to learning and plasticity after SCI. Previously, we showed learning and adaptive plasticity in the lumbar cord at chronic but not acute time points after mid-thoracic SCI only in the presence of sparing. To identify the role of interneurons in this effect, we used Golgi staining and neuron tracing software to quantify morphological changes of interneurons in Lamina VI/VII of L3-L6 from naïve and spinal cord injured, female Sprague-Dawley rats. We quantified 5 dendrites / animal to model reactive plasticity and examine the effects of sparing in naïves (n=3) and at acute (7d) and chronic (42d) time points after T8 spinal cord transection (TX; Acute TX n=5; Chronic TX n=4) or severe SCI contusion (SCI; Acute SCI n=4; Chronic SCI n=5). Dendritic spines increased up to 90μm from the soma after chronic injury regardless of sparing (Naïve = 70.5 +/- 10.2, Chronic TX = 113.4 +/- 9.4, Chronic SCI = 112.2 +/- 3.5; $p<.05$). After TX, the greatest spine density occurred close to the soma (0-30μm) and increased over time (Naïve = 18.4 +/- 3.6, Acute TX = 30.7 +/- 3.2; Chronic TX = 38.6 +/- 4; $p<.05$). Interneuron volume decreased over time with TX (Acute TX = 17871.8μm3 +/- 1207; Chronic TX = 8535.1μm3+/- 1147; Naïve = 22759μm3 +/- 460; $p<.05$) and was partially attenuated by sparing (Chronic SCI = 16789.1μm3 +/- 528). In sister groups, neuronal excitability was quantified by stimulus intensity to produce an ankle flexion force of 0.4-0.5N in the tibialis anterior muscle. Stimulus intensity decreased over time after TX (Acute n=6, 1.53mA +/- 0.23; Chronic n=5, 0.64mA +/- 0.19; Naïve n=8; 2.3mA +/- 0.14; $p<.05$) and was partially attenuated by sparing at 42d (Chronic SCI n=17; 1.53mA +/- 0.14). We are now differentiating excitatory vs. inhibitory dendritic spines with VGLUT1,2 and GAD67 in Thy1-YFP mice to better understand the spine density changes observed after SCI and TX. Together, we show maladaptive interneuronal changes following SCI that worsen over time with complete SCI and are mitigated in the presence of sparing. This aligns with the clinical manifestation of

hyperreflexia and spasticity. Evaluating spine density, soma size, and neuronal excitability provide novel outcome measures for interventions designed to reduce spasticity and promote functional recovery following SCI.

Disclosures: **T.D. Faw:** None. **C.N. Hansen:** None. **L.C. Fisher:** None. **S.D. Kerr:** None. **J.A. Buford:** None. **D.M. Basso:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.05/GG34

Topic: D.12. Kinematics and EMG

Support: NIH Grant AR052345

NIH Grant K12HD073945

NIH Grant AR050520

Title: Prolonged immobilization and unloading leads to profound and long-lasting changes in spinal excitability

Authors: ***E. L. LAWRENCE**[1], A. NAGAMORI[1], F. J. VALERO-CUEVAS[1,2], J. M. FINLEY[2];
[1]Dept. of Biomed. Engin., [2]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstracts: Both strength losses due to atrophy and increases in spinal excitability as measured by Hoffman's reflex (H-reflex) have been associated with prolonged unloading of the lower extremity (Clark et al., 2006; Leukel et al., 2014). However, these studies only consider the effects of unloading or immobilization in healthy subjects, and may not reflect the time course of recovery following unloading due to injury. Therefore, the purpose of this study was to evaluate the time course of recovery of spinal excitability and muscle contractile efficacy after prolonged immobilization and unloading after injury. Our study consisted of a sample of convenience in which the experimental participant (34F) was prescribed a regimen of lower limb unloading following a surgical procedure (open reduction and internal fixation) to repair a fibula fracture and ankle dislocation in the lower left extremity. The participant underwent 37 days of non-weightbearing ankle immobilization post-surgery and an additional 15 days of ankle immobilization during ambulation. On the first two days after cast removal and at one-week

intervals up to 5 weeks, we assessed spinal excitability and muscle contractile properties by measuring H-reflexes and maximal M-waves in the soleus bilaterally. During each experimental session, we first recorded recruitment curves for the M-wave and H-reflex. Then, for each leg, we recorded 20 H-reflexes at a stimulation intensity that produced a controlled M-wave equal to 10% of the respective limb's M-max. We found reduced Mmax values in the unloaded limb compared to the loaded limb ($p=0.005$) over all data collections, which is suggestive of atrophy and is consistent with previous studies (Clark et al., 2006; Leukel et al., 2014). We further report a significantly higher ratio of Hmax/Mmax for the first two weeks after cast removal ($p=0.05$) and nearly significant differences the third week post-cast removal ($p=0.1$). While not significant, differences continued to exist for 2 more weeks. These results suggest that spinal excitability is heightened following immobilization, consistent with previous reports. Surprisingly, we found that the changes in spinal excitability and muscle contractile required five weeks to return to baseline despite an immediate increase in weight-bearing activity after the cast was removed. These results add to our understanding of the time course of central changes in lower-limb neuromuscular function following prolonged unloading by demonstrating that changes in spinal excitability and muscle contractile properties may persist for much longer than a single week as demonstrated previously.

Disclosures: E.L. Lawrence: None. **A. Nagamori:** None. **F.J. Valero-Cuevas:** None. **J.M. Finley:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.06/GG35

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Alberta Innovates - Health Solutions

CIHR

SCITCS

Vanier Canada

Rick Hansen Institute

Project SMART

Title: The modulation of corticospinal input to the legs during arm and leg cycling

Authors: *R. ZHOU[1], S. KIM[2], S. CHONG[2], V. MUSHAHWAR[2];
[1]Ctr. for Neurosci., [2]Univ. of Alberta, Edmonton, AB, Canada

Abstracts: Locomotion is a complex task that relies on the integration of cortical input, sensory feedback, and activity in spinal networks. Arm activity can significantly modulate the performance of the legs in various types of locomotion including walking, cycling and swimming. While some studies have demonstrated the coupling between the cervical and lumbar regions of the spinal cord, there are scarce findings about how cortical input to the legs is modulated by active arm involvement during locomotion. The goal of this study was to investigate the modulation of the corticospinal input to the lower limb muscle, vastus lateralis (VL), while actively engaging arms in coordination with the legs during rhythmic cycling. VL was chosen due to its dominant contribution during leg cycling. Sixteen adults with intact nervous system were recruited. Each participant underwent four experimental conditions: leg cycling only with the arms stationary (ASLC), arm cycling only with the legs stationary (ACLS), arm and leg cycling (ALC) and stationary arms and legs (ASLS). For each participant, the maximum electromyographic (EMG) activity of VL was maintained at the level of ~10% of the maximum isometric voluntary contraction (MIVC) across all conditions. During each condition, transcranial magnetic stimulation (TMS) was delivered, in a randomized order, at four different time points of VL activation within a cycling revolution. The time points included the time of onset, mid-rising, peak and mid-descending EMG activity in VL. The intensity of TMS stimuli was selected to evoke half of the maximal motor evoked potential (MEP) with ~10% MIVC of VL muscle. The MEPs in VL were collected and compared across all conditions and time points using a two-way ANOVA. The results showed significant differences in the amplitudes of MEPs across conditions and time points ($p \leq 0.05$). In particular, Fisher's LSD post-hoc test indicated that ALC ($p=0.02$) and ASLC ($p=0.018$) are significantly different from ASLS. However, ALC and ASLC were not significantly different from ACLS. This finding suggested that dynamic arm and leg activation strongly modulate the corticospinal pathway to the legs. Furthermore, arm cycling alone may modulate the descending drive to the legs to the same extent that simultaneous arm and leg cycling does. While further investigations are needed, the findings indicate the importance, and perhaps equivalent impact, of active arm movement in modulating the strength of the corticospinal drive to the legs. The work provides support for the need to engage the arms actively in lower limb rehabilitation for people with stroke or spinal cord injury for a more effective restoration of leg function.

Disclosures: R. Zhou: None. **S. Kim:** None. **S. Chong:** None. **V. Mushahwar:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.07/GG36

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Swiss Continence Foundation

Swiss Academy of Medical Sciences

Title: A urodynamic model to study the lower urinary tract function in awake spinal cord injured rats

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Abstracts: Current urodynamic rat models are not directly translational to humans since most measurements are made in anesthetized animals and since the external urethral sphincter function is not considered. Narcotics highly influence lower urinary tract function. The assessment of sphincter activity is essential to understand detrusor sphincter dyssynergia, a common consequence of spinal cord injuries. We developed a urodynamic model with electromyography (EMG) of the external urethral sphincter for quantification of lower urinary tract function in awake female rats. Urodynamic catheter and EMG electrodes were implanted into female Sprague Dawley rats. Starting one day postoperatively, urodynamic measurements including urethral sphincter EMG were performed in awake, slightly restrained animals. Controls had catheter implantation only. Rats were also assessed under urethane anesthesia for comparison to standard models. There was no difference between catheter only and the EMG electrode implanted animals. Voiding activities (volumes, bladder compliance, voiding pressure) in awake animals were very different from animals under urethane anesthesia. Implanted rats could be followed with repeated measures over up to 6 weeks without complications. The model is currently applied to rats with large lesions of the thoracic spinal cord.

Disclosures: **M.P. Schneider:** None. **F.M. Hughes:** None. **A.K. Engmann:** None. **M. Gullo:** None. **T.J. Purves:** None. **H. Kasper:** None. **M. Tedaldi:** None. **M.E. Schwab:** None. **T.M. Kessler:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.08/HH1

Topic: D.12. Kinematics and EMG

Support: BRI-U Laval Res. Chair in CP

Title: Effects of attention and acute physical exercise on cutaneous reflexes in children with cerebral palsy

Authors: *J. I. VOISIN[1,2], C. GANE[2], S.-K. DUFOUR[2], D. WYSS[3], K. ZABJEK[3,4], J. ANDRYSEK[3,5], D. B. MALTAIS[1,2], L. J. BOUYER[1,2];
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Abstracts: Background: Individuals with cerebral palsy (CP) commonly experience physical fatigue related to physical activities such as walking. This may negatively impact on functional mobility skills (Maltais et al, 2013, Gane et al., 2013) and on academic performance (Berrin et al., 2007,). However the mechanisms by which these negative effects arise remain poorly understood. To begin to address this issue, we examined the effects of acute fatiguing physical exercise on attention and on cortical control, the later as measured by cutaneous reflexes. Methods: Eight children with spastic CP (6-13 years old, 5 males, GMFSC level I) were evaluated pre and post fatiguing physical exercise. The exercise was a maximal graded shuttle run test (SRT) specific for children with CP (Verschuren et al., 2006) where the children ran back and forth on a 10 m track at increasingly faster speeds until they could no longer maintain the pace. Cutaneous reflexes were tested in a seated position, while the stimulated foot was dorsiflexing against resistance. The participants were lightly touched over the dorsal part of the right or left foot (stimulation unseen but side known). They were instructed to pay attention to this stimulation and report it. They were to ignore the (stronger) mechanical cutaneous stimulation used to evoke the cutaneous reflexes (which were on either the same or opposite foot). Independent variables were focus of attention (directed to the same foot as the stimulated one or to the opposite foot) and pre/post exercise. The dependent variable was the amplitude of the short latency reflexes (peak2peak amplitude from 20 to 50 ms post stimulation) recorded from the right and left anterior tibialis following the cutaneous stimulation of the corresponding foot (cutaneous branch of the sural nerve). Results and Discussion: Increased cutaneous reflexes were found after SRT (from 0.13 to 0.16 mV) and when attention was oriented to the stimulated

foot (from 0.12 to 0.17 mV) (paired t-test, $p<0.05$). These results suggested that both divided attention and acute physical fatigue may negatively impact performing motor-based activities of daily life such as walking in cognitively demanding environments.

Disclosures: J.I. Voisin: None. **C. Gane:** None. **S. Dufour:** None. **D. Wyss:** None. **K. Zabjek:** None. **J. Andrysek:** None. **D.B. Maltais:** None. **L.J. Bouyer:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.09/HH2

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Exercise-dependent increase in mTOR activity regulates KCC2 expression and reflex recovery after SCI

Authors: S. F. CHOYKE, *M.-P. COTE;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstracts: Plasticity in spinal pathways is a key target for therapeutic strategies aiming at restoring both sensory and motor function after spinal cord injury (SCI). The serine-threonine kinase mammalian target of rapamycin (mTOR) has recently emerged as a promising pharmacological target for the treatment of SCI. mTOR expression is up-regulated in an exercise-dependent manner after SCI and can be regulated by tyrosine kinase receptor activation. We recently showed that BDNF expression is correlated with exercise-dependent reflex recovery and with the expression of the potassium chloride cotransporter 2 (KCC2), and that both required TrkB activation. Not only is mTOR involved in the regulation of neuronal cell growth, survival and differentiation, but also in dendritic development, synaptogenesis and synaptic plasticity. Interestingly, although KCC2 function primarily influences the efficacy of GABAergic signaling, its abundant presence in dendritic spines has attracted growing interest. Suppressing KCC2 in mature neurons influences synaptic efficacy independently of GABAergic function whereas an increase in KCC2 levels promote dendritic spine development. Motoneurons display marked loss of dendritic membrane and branching after SCI which is not observed in exercised animals. We hypothesized that mTOR contributes to KCC2 upregulation in SCI animals that are exercised. Adult female Sprague-Dawley rats underwent a complete spinal cord transection at T12. Animals were assigned to one of these 3 groups: vehicle, rapamycin or rapamycin + Ex. Rapamycin treatment (i.p.) was given every other day starting 1 day after surgery for the

duration of the study. In a terminal experiment, H-reflexes were recorded from interosseus muscles following stimulation of the tibial nerve at 0.3Hz, 5Hz and 10Hz. Our data show that preventing mTOR action during exercise hindered spinal reflex recovery, with KCC2, BDNF and TrkB upregulation in the lumbar spinal cord. Our results suggest that the exercise-dependent increase in KCC2 expression and return of spinal reflexes requires mTOR activity. The role of KCC2 in restoring endogenous inhibition and remodeling of the dendritic tree of motoneurons with exercise will be discussed.

Disclosures: S.F. Choyke: None. **M. Cote:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.10/HH3

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Wings for Life

SMRRT (CIHR team grant)

Spinal Cord Chair

Title: Interlimb cutaneous reflex transmission after thoracic hemisection during walking on a flat treadmill or on a ladder treadmill

Authors: *A. KUNDU, M. ESCALONA, H. DELIVET-MONGRAIN, J.-P. GOSSARD, S. ROSSIGNOL;
Neurosci., Univ. De Montreal, Montreal, QC, Canada

Abstracts: Previous work has shown that adult cats can recover quadrupedal stepping within about three weeks following a unilateral hemisection even if some deficits such as left-right asymmetry persist. Treadmill training can further improve the locomotor performance. Thus neuroplastic changes occur in the remnant supraspinal structures and also at the level of the spinal cord. The present work aims at assessing the potential role that could be played by cutaneous spinal reflexes in such recovery. Adult cats were selected based on their ability to walk regularly for several minutes on the flat treadmill (FTM) and on a ladder-treadmill (LTM) consisting of rungs attached to the treadmill at regular intervals of 8 cm which requires more supraspinal control. The cats were chronically implanted with EMG electrodes in seven muscles (four hindlimb, one back and two forelimb muscles) and a cuff electrode on the right and left Superficial Peroneal Nerve (SPN). A left hemisection was performed at the T10 level. Cutaneous reflexes were elicited systematically in various phases of the step cycle by stimulating the SPN while the cats were walking on the FTM or on the LTM, before and after the hemisection. Preliminary results show that the cutaneous reflexes measured in Semitendinosus (knee flexor-hip extensor) on the FTM and LTM are phase-dependent, being increased during the swing phase and mostly absent during stance. One week after hemisection, the peak reflex amplitude is about half of that seen in the control. When walking on the FTM with an intact spinal cord, reflexes were generally smaller than on the LTM. Post hemisection, their amplitude first

decreased but then gradually increased. Cutaneous reflex in the contralateral and forelimb extensor muscles were also observed. During a terminal acute experiment (decerebration and curarization), SPN on both sides were stimulated during fictive locomotion. Plots of reflex amplitude vs St burst amplitude showed an increased gain of transmission on the left as compared to the right. This work suggests that crossed and interlimb reflexes participate in the adaptation of walking on the LTM and also after hemisection, two conditions requiring more supraspinal controls. Work supported by Wings for Life, SMRRT (CIHR team grant) and Spinal Cord Chair.

Disclosures: A. Kundu: None. **M. Escalona:** None. **H. Delivet-Mongrain:** None. **J. Gossard:** None. **S. Rossignol:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.11/HH4

Topic: D.10. Spinal Cord Injury and Plasticity

Support: CIHR

Title: Mitigation of pressure ulcers using intermittent electrical stimulation

Authors: L. R. SOLIS[1], P. SERES[2], *V. K. MUSHAHWAR[3];
[2]Biomed. Engin., [1]Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. Alberta, Edmonton, AB, Canada

Abstracts: Pressure ulcers are a major medical complication that affects people with reduced mobility and impaired sensation. Pressure ulcers can develop at the skin and progress inwards, or at the bone-muscle interface and progress outwards. The latter is classified as deep tissue injury (DTI) and is caused by prolonged loading of soft tissue between a bony prominence and a surface. The excessive mechanical deformation of the muscle triggers the onset of damage, which is then exacerbated by ischemia and reperfusion. We suggested the use of intermittent electrical stimulation (IES), delivered through a system called “Smart-e-Pants” to prevent the formation of DTI in populations at risk, particularly those with spinal cord injury (SCI). The use of IES can counteract both the mechanical and vascular pathways leading to DTI formation (Solis et al 2007, 2011). More importantly, IES can effectively prevent the formation of DTI (Solis et al 2007, 2013. Curtis et al 2011). IES accomplishes this by mimicking the subconscious

postural adjustments (fidgeting) that people with full mobility perform as a result of discomfort. This intermittent activation of loaded muscles consists of bouts of stimulation lasting for 10 sec, followed by 10 min of stimulation cessation. In this study we explored the effectiveness of IES to mitigate tissue damage that has already occurred. The study was conducted in rats with a SCI in which a DTI was caused by externally loading the triceps surae muscle. The applied load was proportional to the load that people with SCI experience while sitting in a wheelchair. IES was applied for 4 hours initiated at different time points (1, 3, and 6 days post onset of DTI). Tissue damage was assessed through the use of MRI and histological methods. The effectiveness of IES to mitigate damage was compared against the natural progression of DTI at the same time points in control groups of rats. In rats that received IES, DTI in the muscle was mitigated by ~50% compared to the groups that did not receive IES. This result is significant because it provides evidence that IES while effective at preventing DTI, can also mitigate damage that had already started. This is of particular importance for its clinical implementation, as in practice, DTI develops unbeknownst to caregivers due to the current lack in effective DTI detection methods. For this reason, a system that can be used both to prevent the onset of DTI as well as mitigate damage that had already taken place could help to reduce the incidence of pressure ulcers, particularly those of deep origin. This not only improves quality of life but also reduce the large costs associated with their treatment, which in the USA are at least $11 Billion/year.

Disclosures: L.R. Solis: None. **V.K. Mushahwar:** None. **P. Seres:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.12/HH5

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Wings For Life

CIHR Team Grant (SMRRT)

CRC chair

Title: Comparing locomotion on a flat and on a ladder treadmill in cats before and after spinal hemisection

Authors: *M. ESCALONA, A. KUNDU, H. DELIVET-MONGRAIN, J.-P. GOSSARD, S. ROSSIGNOL;
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Abstracts: Locomotor evaluation after spinal lesions is usually done on a flat treadmill which requires little voluntary adaptation. To evaluate the effect of spinal hemisection affecting ascending and descending pathways we modified our conventional treadmill to evaluate cats walking at various speeds on a flat treadmill (FTM) or on a ladder treadmill (LTM) by fixing quadrangular rungs (35cm w, 5cm h) spaced at every 8cm on the FTM. This set-up allows us to record and average EMG activity and kinematics for several consecutive cycles in various conditions (on FTM or LTM, with an intact cord and after hemisection). Four cats were implanted with EMG electrodes in the main flexor and extensor muscles of the forelimbs and hindlimbs on both sides and recorded together with synchronized video of the left side. After a control period of 3wks to collect baseline values, a left hemisection was performed at T10. For the next 6 weeks 2 cats were trained on the LTM and the 2 others on the FTM. Once a week, for 6 weeks, the stepping (EMG, Kinematics) were evaluated at speeds from 0,4 to 0,8 m/s on the FTM. All protocols were approved by the Université de Montreal´s Ethics Committee. The hindlimb movements of the 2 cats walking on the LTM with an intact cord were very similar to those recorded during walking on the FTM: there was a small increase in knee and ankle flexion and increased velocity of knee and ankle extension in the later part of stance before contact with the rungs. This normalization of kinematics was however achieved through some significant EMG changes. In one cat the hip extensor-knee flexor Semitendinosus (St) discharge occurring at the end of stance was increased in amplitude; more remarkably, a 2nd burst occurring just before foot contact was as large as the first, suggesting a voluntary control to place the foot accurately on the rungs. After hemisection, the second St burst disappeared on the left but was maintained on the right which continued to walk on the rungs as did the forelimbs. The knee extensor was markedly increased in the second part of stance. This cat walked in between the rungs with the left hindlimb while the second cat was able to step on the rungs for several but irregular step cycles. Kinematic changes on the FTM might have reflected not only the effect of the hemisection but also the effect of training on the LTM since the 2 other cats not trained on the LTM did not show these changes on the FTM. This paradigm thus allows to investigate more accurately the contribution of spinal pathways to voluntary locomotion and help understand the mechanisms of locomotor recovery after partial spinal cord lesions. Supported by Wings For Life, CIHR Team Grant (SMRRT) and a CRC chair.

Disclosures: M. Escalona: None. **A. Kundu:** None. **H. Delivet-Mongrain:** None. **J. Gossard:** None. **S. Rossignol:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.13/HH6

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Evaluation of the antioxidant effect of dapsone in a model of traumatic spinal cord injury in rat based on the amount of reduced glutathione

Authors: *A. S. ROJAS[1], C. RIOS[2], A. DIAZ-RUIZ[2], D. NICOLAS[2], G. BALDERAS[2];
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Abstracts: Introduction: Traumatic spinal cord injury(TSCI) is a condition caused by irreversible damage on neurological function. One mechanism observed after TSCI is apoptosis, inducing the loss event of adjacent healthy tissue.Therefore, the search for treatments aimed at reducing the process is paramount.Dapsone(DDS) is a drug capable of inhibiting oxidative damage, inflammatory response after a process of ischemia/reperfusion in rats.Previously in the laboratory showed that the DDS protects tissue damage and promotes functional recovery in rats. **Objective:**To evaluate the antioxidant effect of dapsone based on the reduced amount present in the acute phase after trauma in a rat model glutathione. **Method:**Female Wistar rats(200-250g) were used, being assigned randomly into 11 groups: sham 4 groups(n=16), 7 groups of rats with TSCI(n=56). The animals were anesthetized with pentobarbital and underwent a laminectomy or TSCI level T-9 using the New York University team Spinal Cord Impactory.Subsequently, three different doses of Dapsone depending the group they belonged to(12.5,25 and 37.5mg/kg) were administered.The animals were sacrificed 24 hours after surgery. **Results:**The values obtained from the analysis of the amount of GSH in the injured groups are similar no significant when compared with the control group(TSCI+Vehicle) differences. **Conclusions:**The findings of this study showed that the antioxidant effect of dapsone is unrelated to the regulation of GSH, because there is no reduction in the neurodegenerative effects when using dapsone treatment in the acute phase of TSCI(first 3-5 hours), so that the evaluation of other markers of oxidative damage is suggested.

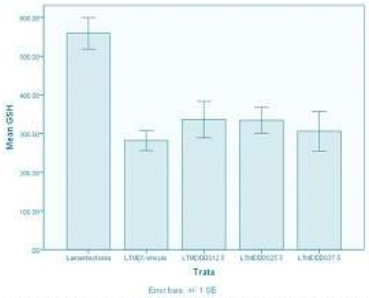

Figure 1. Chart in which the mean values ± standard error of the determination of GSH in animals with laminectomy and TSCI evaluated 24 h after the surgical procedure are shown, values are expressed in mg of GSH / mg protein. Laminectomy: A surgical procedure without injury. TSCI: Traumatic injury to the spinal cord. TSCI / vehicle: spinal cord injury + saline. TSCI /DDS12.5: spinal cord 12.5mg/kg + dapsone. TSCI /DDS25: spinal cord 25mg/kg + dapsone. TSCI /DDS37.5: spinal cord 37.5mg/kg + dapsone. GSH: reduced glutathione. One way ANOVA followed by Tukey test.

Disclosures: **A.S. Rojas:** None. **C. Rios:** None. **A. Diaz-Ruiz:** None. **D. Nicolas:** None. **G. Balderas:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.14/HH7

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Grant DA 031197

NIDA Drug Supply Program

Mission Connect, a project of the TIRR Fourdation

Title: Evaluating the necessity of the KOR in the morphine-induced attenuation of function after SCI

Authors: ***M. ACEVES**, M. A. HOOK;
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Abstracts: Opioids are one of few effective analgesics for the treatment of pain following spinal cord injury (SCI). Unfortunately, we have shown that morphine administered in the acute phase of SCI, irrespective of the route of administration, compromises recovery of locomotor function, increases mortality and pain reactivity, and suppresses weight gain in a rodent contusion model

(Hook et al., 2007, 2009, 2011). These adverse effects appear to depend on activation of the kappa opioid receptor (KOR). Selective activation of the KOR, using GR89696, undermined locomotor recovery and decreased weight gain. In the current study, we tested whether activation of the KOR is necessary to produce the adverse effects of morphine using norBNI, a selective KOR antagonist. Subjects received a moderate spinal contusion (T12), and an intrathecal cannula was implanted. Baseline locomotor function (BBB) and pain reactivity (tail-flick) were assessed 24 hours following injury. Subjects were then pretreated with norBNI (0, .08, or .32 μmol), followed by morphine (0 or .32 μmol). Pain reactivity was re-assessed 30 minutes after drug treatment. Locomotor recovery was evaluated across a 21-day period, with additional tests of motor and sensory function conducted after day 21. Our results show that pretreatment with norBNI blocks the morphine-induced effects on recovery in a dose-dependent manner. At higher doses, norBNI eliminates morphine's adverse effects on recovery, but analgesia is also abolished. Conversely, at low doses, analgesia is maintained, but the adverse effects persist. This suggests that activation of the KOR system is necessary and sufficient for morphine-induced attenuation of recovery. However, as the protective dose of norBNI also diminished analgesic efficacy, simply blocking KOR activity is not sufficient for improving the efficacy and safety of clinical opioid use. Further understanding of the specific molecular changes induced by KOR activation is necessary to improve pain management strategies and facilitate functional recovery after SCI.

Disclosures: **M. Aceves:** None. **M.A. Hook:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.15/HH8

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH grant 1R01EB007615-01A1

Christopher and Dana Reeve Foundation

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NIH grant 8 P30 GM103507

Title: Lumbosacral spinal cord epidural stimulation enables full weight bearing standing in motor complete paraplegics

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[1]Neurolog. surgery, Univ. of Louisville, Louisville, KY; [2]Frazier Rehab Institute, Kentucky One Hlth., Louisville, KY

Abstracts: Epidural stimulation of lumbosacral spinal cord, combined with the sensory input associated with weight bearing, enabled a motor complete paraplegic to progressively regain full weight bearing standing. The aim of this study was to investigate whether this same strategy was effective to regain full body weight bearing standing in three more individuals. The effects of different stimulation parameters on EMG pattern, spinal cord evoked potentials (scEP) characteristics and behavioral responses during standing were studied. Four motor complete SCI participants with a chronically implanted epidural electrode array over the segments L1-S1 of the spinal cord participated in this study. EMG, kinematics and ground reaction forces were recorded. In this study, we showed that four out of four individuals with a clinically motor complete spinal cord injury achieved full weight bearing standing with little assistance when the lumbosacral spinal cord was stimulated with individual-specific parameters optimal for standing. During sitting, little or negligible EMG activity of lower limb muscles was induced by such stimulation. The sit to stand transition and full body weight bearing standing promoted remarkable levels of continuous EMG activity in most of the lower extremity muscles. We assessed the individual specificity of stimulation parameters by testing the stimulation configurations optimal for standing across participants. In all cases, when an individual was stimulated using parameters specific for other participants, additional assistance was needed to stand; EMG patterns were also different than the one recorded when individual-specific parameters optimal for standing were used. EMG patterns were also different across individuals when the same stimulation parameters were tested in standing. Stimulation parameters selectively modulated the lumbosacral neural networks during standing. Different electrode configurations induced remarkably different behaviors and EMG patterns. EMG activity of several muscles changed from continuous to rhythmic above certain stimulation strengths, when higher frequencies (i.e. 25 Hz and 50 Hz) were used. Finally, higher stimulation frequencies induced scEP with greater variability and lesser amplitude than lower frequencies. These results have important implications with respect to: 1) how lumbosacral neural networks can be selectively modulated by varying the epidural stimulation parameters, and 2) identifying strategies that are likely to be most efficacious in enabling improved motor function for standing after motor complete paralysis.

Disclosures: **E. Rejc:** None. **C. Angeli:** None. **S. Harkema:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.16/HH9

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Alberta Innovate - Health Solutions

CIHR

NIH

DoD CDMRP-SCIRP

Project SMART

NSERC

Title: Intra-spinal microstimulation implants for humans

Authors: *A. TOOSSI[1], D. G. EVERAERT[2], R. C. BUTZ[3], C. R. DENNISON[3], V. K. MUSHAHWAR[4];
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Abstracts: The overall goal of this study is to develop an electrochemically safe and mechanically stable implant for chronic intra-spinal neural implants. Spinal cord injury (SCI) affects approximately 12000 people every year in the US. One of the important functions lost after SCI is the ability to stand and walk. Our lab has developed an electrical stimulation approach for restoring standing and walking called intraspinal microstimulation (ISMS). The technique involves implantation of ultrafine microwires in the ventral horn of the lumbosacral enlargement targeting the motoneuron pools. Low electrical currents (<0.1 mA) delivered through these wires produce coordinated movements of the legs. Extensive testing in cats (Mushahwar et al, 2000) and rats (Bamford et al, 2010) demonstrated that the wires remain stable in place and functional for long durations. These results suggested that ISMS may be a viable clinical approach for restoring leg movements in people with SCI. Preparing ISMS for clinical testing requires design adjustments to accommodate human anatomy and surgeons' needs. Of utmost importance are the stimulation safety of the new electrodes and the mechanical stability of the implant in large animals. To assess the stimulation safety of the new electrodes,

arrays consisting of 24 Pt/Ir (80% Pt) microwires with different diameters and extents of tip deinsulation were fabricated and implanted in 1 adult cat for acute testing. Voltage transient measurements (VTM) were obtained. The stimulation safety boundaries of the VTMs are determined by oxidation limits of water. VTMs indicated that electrodes made of 50µm-dia Pt/Ir with 200µm tip exposure can be used to deliver currents up to 50% higher than ISMS requirements for cats, while remaining within the safe limits. To determine the mechanical forces leading to electrode dislodgment, in-Fiber Bragg Grating (FBG) sensors (Dennison et al, 2010) were mounted in line with the microwires (50µm dia). The FBG sensors were calibrated using masses ranging from 1-200 g and were deemed reliable in the range of interest. Tofu was initially used as a surrogate model of the spinal cord (Snow et al., 2006) in bench-top testing. Force was applied in-line with 4.7 mm long Pt/Ir ISMS electrodes. In the 3 trials conducted, the average dislodgment force was 0.024 ± 0.03 N. The same procedure was applied to 3 freshly extracted pig spinal cords in which 18 trials were conducted. The average dislodgement force was 0.028 ± 0.019 N. In this study the stimulation safety of 50µm Pt/Ir ISMS electrodes was demonstrated and the minimal forces leading to electrode dislodgment were determined. This information will guide the design of a chronic ISMS implant.

Disclosures: A. Toossi: None. **D.G. Everaert:** None. **R.C. Butz:** None. **C.R. Dennison:** None. **V.K. Mushahwar:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.17/HH10

Topic: D.10. Spinal Cord Injury and Plasticity

Support: FINEP 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: Novel rehabilitative strategy to facilitate EEG-triggered locomotor training in chronic spinal cord injury patients: Preliminary results of an ongoing study

Authors: L. SAWAKI[1,2], A. C. DONATI[3,4], A. N. NOGUEIRA[3,4], C. GARABELLO[3,4], C. M. GITTI[3,4], D. CAMPOS[3,4], D. YOSHIHARA[3,4], G. A. PEREIRA[3,4], I. ARAÚJO[3,4], J. CAMPOS[3,4], L. FERREIRA[3,4], M. ARES[3,4], M. SANTOS[3,4], P. B. AUGUSTO[3,4], S. TRIPODI[3], *E. MORYA[5], M. A. L. NICOLELIS[5,6,7,8,9];
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Abstracts: Spinal cord injury (SCI) is one of the most prevalent, devastating neurological conditions. Less than 1% of SCI survivors will experience full recovery. This statistic highlights the need for further research to advance effective therapy during long-term SCI recovery, including interventions to facilitate locomotor function. To this end, we conducted a preliminary study of a novel intervention using robot-assisted locomotor training triggered by EEG in complete SCI. Eight participants (7 AIS-A, 1 AIS-B) participated in 4 hours of intervention, 3 times per week for 4 months. Intervention consisted of clinical conditioning and robot-assisted locomotor training on a body weight-supported treadmill with virtual reality features, tactile feedback, and brain-computer interface. To maximize participants' cortical processing, our multidisciplinary team structured intervention 1) to ensure intensive, progressive challenge to each participant; and 2) to elicit participants' mastery of internal and external feedback. Further in keeping with this neuroplasticity-based paradigm, training required highly repetitive performance of tasks. All participants were able to trigger EEG in the brain-computer interface with the robot-assisted treadmill training. Overall, our initial findings demonstrate that clinically informed robotic rehabilitation technology has enormous potential to advance restoration of locomotor function in complete SCI.

Disclosures: L. Sawaki: None. **A.C. Donati:** None. **A.N. Nogueira:** None. **C. Garabello:** None. **C.M. Gitti:** None. **D. Campos:** None. **D. Yoshihara:** None. **G.A. Pereira:** None. **I. Araújo:** None. **J. Campos:** None. **L. Ferreira:** None. **M. Ares:** None. **M. Santos:** None. **P.B. Augusto:** None. **S. Tripodi:** None. **E. Morya:** None. **M.A.L. Nicolelis:** None.

Poster

629. Spinal Cord Injury and Plasticity I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 629.18/HH11

Topic: D.12. Kinematics and EMG

Support: The Don and Linda Carter Foundation

The Crowley-Carter Foundation

Title: Effects of deep brain stimulation on short and long-latency stretch response in childhood generalized dystonia

Authors: *E. ARGUELLES, N. H. BHANPURI, T. D. SANGER;
USC, Los Angeles, CA

Abstracts: Dystonia is a movement disorder characterized by involuntary muscle contractions, overflow, and abnormal postures. Furthermore, there is evidence to suggest that the long latency stretch response (LLSR) is increased in dystonia, while the short latency stretch response (SLSR) remains normal. Although the pathophysiology of dystonia is still an active area of investigation, dystonic symptoms are associated with basal ganglia dysfunction and increased cortical excitability. Deep brain stimulation (DBS) of the internal globus pallidus (GPi) has been used to treat dystonia, despite an incomplete understanding of its mechanisms of action. A common proposal is that GPi-DBS reduces cortical excitability. Since the LLSR is thought to have motor pathways travelling through cortex and is abnormal in patients with basal ganglia dysfunction such as Parkinson's disease, Huntington's chorea, and hypertonic dystonia, it is possible that they are directly affected by GPi-DBS. SLSR, on the other hand, which do not directly involve cortical pathways, may not be influenced by dystonia. There is little research on the interaction between DBS and LLSR; therefore, we investigated the effects of GPi-DBS on LLSR in children with generalized dystonia. Since DBS is thought to reduce overall excitability, we hypothesized that the amplitude of the LLSR will be attenuated in response to DBS. To test this, we measured EMG activity of the first dorsal interosseous (FDI) during rapid index finger perturbation for two conditions: DBS-on and DBS-off. During this experiment, subjects viewed a display and were instructed to keep the finger in the start position to the best of their ability. In addition to FDI, we recorded EMG from the abductor digiti minimi (ADM) in order to observe changes in overflow. Our results indicate modulation of the LLSR during DBS-on compared to DBS-off. The SLSR was less affected and overflow was present in both conditions. These results suggest that GPi-DBS may cause an immediate change in the activity of efferent cortical pathways that drive muscle activation during rapid corrective movements, yet has little effect on the short latency response, which involve only spinal pathways. Interestingly, we observe changes in EMG just minutes after switching on/off the stimulation, in contrast to the observation that clinical benefits can take weeks to months to fully manifest. Thus, DBS may affect motor output on different timescales.

Disclosures: E. Arguelles: None. **N.H. Bhanpuri:** None. **T.D. Sanger:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.01/HH12

Topic: D.10. Spinal Cord Injury and Plasticity

Support: EU FP7 Grant NeuWalk CP-IP 258654

Nano Tera Switzerland, RTD Grant SpineRepair (20NA21_145923)

ERC Grant, Walk Again (ERC 261247)

Title: Selective stimulation of spinal sensorimotor circuits by multipolar stimulation of the spinal cord

Authors: *M. CAPOGROSSO[1,2], N. WENGER[1], A. MORTERA[1,3], J. GANDAR[1], N. PAVLOVA[1], S. MICERA[2,1], G. COURTINE[1];
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Abstracts: Epidural electrical stimulation (EES) of lumbosacral segments is a promising intervention to improve motor function after spinal cord injury (SCI). Recent experiments in rat models of SCI and in humans with motor complete paralysis showed that continuous EES applied over specific lumbosacral locations could facilitate distinct aspects of standing and walking and even enable supraspinal control of the paralyzed legs. In these experiments, electrode configurations and stimulation parameters were selected based on empirical observations during time-consuming mapping experiments. These restrictions are mainly due to the limited understanding of the mechanisms underlying site-specific facilitation of movement with EES. To remedy this issue, we developed and validated an advanced computational model of EES to uncover the optimal configurations and use of multi-electrode arrays to facilitate movement after SCI. Using combinations of computerized simulations and electrophysiological experiments, we identified unique configurations of electrodes to increase the selectivity of responses elicited by EES. We could induce side-dependent movement of extension versus flexion, which could not be achieved previously using monopolar EES. These simulations aim to activate afferent proprioceptive fibers, which recruit excitatory pre-motor interneuronal networks with multi-segmental projections to specific sets of synergistic motor pools. Our results define new strategies for the design and use of more effective multi-electrode arrays to facilitate specific limb movements in humans with SCI.

Disclosures: **M. Capogrosso:** None. **N. Wenger:** None. **A. Mortera:** None. **J. Gandar:** None. **N. Pavlova:** None. **S. Micera:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.02/HH13

Topic: D.10. Spinal Cord Injury and Plasticity

Support: VA RR&D 1I01RX000417-01A1

Title: Transcutaneous spinal cord stimulation to modulate spinal reflex excitability and locomotor output after SCI

Authors: ***B. FARRELL**[1], J. BRUCE[1], W. B. MCKAY[1], K. TANSEY[2,3];
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Abstracts: Spinal cord stimulation (SCS) has been shown to both modulate and generate locomotor like electromyographic activity as well as to alter spasticity in individuals with spinal cord injury (SCI). Recent evidence has shown that transcutaneous (t)SCS can generate reflex responses similar to those elicited by epidural SCS but with reduced cost and invasiveness. It remains unclear however if there are certain stimulation frequencies at which tonic tSCS is most effective at reducing spinal reflex excitability and/or spasticity and whether the addition of stepping related afferent feedback further augments motor output. Therefore the goal of this research was to examine the effects of tSCS frequency on posterior root motor reflexes (PRMRs) and electromyographic muscle activation patterns recorded from healthy non-injured individuals and individuals with incomplete spinal cord injury during stepping in a robotic gait orthosis. We hypothesized that as frequency increases from 10-50Hz, excitability will first rise and then fall as some previous evidence has suggested. To achieve this, tSCS was delivered through stimulating electrodes placed over the T11/T12 inter-vertebral space (source) and reference electrodes placed on the abdomen (sink) and was applied at sub-motor threshold levels in non-injured and incomplete SCI subjects for up to 30 minutes. The results from the four healthy non-injured subjects, indicate that resting-state excitability is significantly modulated by sub-motor threshold tSCS though the effects of the individual frequencies varied. In the individuals with incomplete SCI, tSCS at higher frequencies (50 Hz) demonstrated the ability to depress reflex excitability both during tonic stimulation and up to 5 minutes after the tonic stimulation was removed.

Furthermore, during robotic assisted stepping in individuals with SCI, tSCS brought significant reduction in ankle clonus during the stance phase. These results and previous evidence support the idea that transcutaneous spinal cord stimulation, like its epidural predecessor at or above 50 Hz tends to decrease responsiveness to afferent inputs and that future studies should focus on training with tSCS.

Disclosures: B. Farrell: None. **J. Bruce:** None. **W.B. McKay:** None. **K. Tansey:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.03/HH14

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Wings for Life Spinal Cord Research Foundation (WfL), Proj.Nr. WFL-AT-007/11

Vienna Science and Technology Fund (WWTF), Proj.Nr. LS11-057

Foundation for Movement Recovery, Oslo, Norway

Title: Altering spinal cord excitability by peripheral nerve stimulation

Authors: *M. KRENN[1], S. M. DANNER[1,2], C. SCHLAFF[1,2], U. S. HOFSTOETTER[1], K. MINASSIAN[1], W. MAYR[1], M. R. DIMITRIJEVIC[3,4];
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Abstracts: The effects of locomotor training and spinal cord stimulation depend on the central excitability of the networks caudal to a spinal cord injury [1]. Peripheral nerve stimulation allows targeting these networks to alter their excitability. Here, we studied the effects of peroneal nerve stimulation on the central state of the lumbar spinal network. The modifications of lumbosacral motoneuron elicitation were tested by non-invasive, transcutaneously elicitation of posterior root-muscle (PRM) reflexes simultaneously in lower limb muscles. In five subjects (3f.) with intact nervous system we applied a conditioning test paradigm. One-second trains with a pulse rate of 15, 30 and 50 pps peroneal stimulation were applied as a conditioning input at 1.2 and 1.5 times of the motor threshold (MT). Test PRM reflexes were elicited following 50, 100, 500 and 1000 ms through surface electrodes over T11-T12 vertebrae referenced to large paraumbilical

electrodes [2,3]. The major observed effect was a suppression of ipsilateral motoneuron pools that increased with increasing conditioning rate and intensity and persisted over at least 1 second. This suppression was more prominent in the distal than in the proximal lower limbs muscle groups, e.g. applying 30 pps and 1.5 MT conditioning the PRM reflexes were reduced significantly by 40 % and 80 % in the thigh and shank muscles, respectively. No significant group results were observed in the contralateral leg, although both excitatory and inhibitory tendencies were found. In individuals with intact nervous system peroneal nerve stimulation had a suppressive effect more expressed in the motor nucleus of stimulated nerve and least of not stimulated contralateral limb nerves of thigh muscles. Within our ongoing studies we are interested in the effects of the same conditioning on the altered central state of excitability after spinal cord injury and whether excitatory influences could be delivered via nerves with different innervation zones and profiles. 1. Dietz V and Fouad K, 2014, Brain, 137:654-667. 2. Minassian K et al., 2007, Muscle and Nerve, 35(3):327-336. 3. Danner SM et al., 2011, Artif Organs, 35(3):257-262.

Disclosures: **M. Krenn:** None. **S.M. Danner:** None. **C. Schlaff:** None. **U.S. Hofstoetter:** None. **K. Minassian:** None. **W. Mayr:** None. **M.R. Dimitrijevic:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.04/HH15

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Vienna Science and Technology Fund (WWTF), Proj.Nr. LS11-057

Wings for Life Spinal Cord Research Foundation (WfL), Proj.Nr. WFL-AT-007/11

Title: Short- and long-term effects of intermittent transcutaneous spinal cord stimulation on spinal spasticity and residual motor control

Authors: *U. HOFSTOETTER[1], M. KRENN[1], S. M. DANNER[1,2], B. FREUNDL[3], H. BINDER[3], F. RATTAY[2], W. MAYR[1], K. MINASSIAN[1];
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Abstracts: Spinal cord injury (SCI) leads to altered brain control over the spinal cord, manifested as paralysis or paresis below the lesion as well as spasticity, one of the most disabling secondary complications. Clinically, spasticity includes muscle hypertonus, hyperreflexia, clonus, and involuntary muscle contractions and may further diminish the functional utility of residual motor control. In a proof of concept study, we demonstrated the usability of 50-Hz transcutaneous lumbar spinal cord stimulation (tSCS), a non-invasive analog of epidural SCS, applied for 30 minutes to temporarily alleviate spinal spasticity and facilitate voluntary movements in motor-incomplete SCI individuals [1]. Here, we present the persistence of the therapeutic effects after a single application of tSCS in a cohort of seven subjects of our ongoing clinical study with different profiles of motor-complete and -incomplete SCI. To this end, clinical, functional, and neurophysiological methods were used to evaluate the different manifestations of spasticity and residual motor control capacities before, immediately after, and two hours after a 30-minute session of 50-Hz tSCS applied at a sub-motor level. Preliminary analysis of the various outcome measures indicates a reduction of spinal spasticity with preserved residual mobility. The primary parameter, the index of spasticity calculated on basis of the Wartenberg pendulum test, showed a significant improvement two hours after stimulation. In one of the subjects with motor-incomplete SCI, we tested potential summation effects of tSCS applied 5 times a week over a period of six weeks. Stimulation parameter settings were the same as used for single application. Spasticity and residual motor control were evaluated before the first stimulation as well as once a week, 24 hours after the last application of stimulation. We found that the tSCS-induced antispastic effects persisted for 24 hours and, over the course of time, were progressively increasing. After six weeks, the subject discontinued the stimulation and two further evaluations were conducted one and two weeks later, respectively. Persisting tSCS-induced effects could still be detected after one week. These interim findings suggest that short- and long-term changes of altered motor control after SCI can be induced with repetitive exposure to tSCS. Further analysis and recruitment of additional subjects are ongoing to identify SCI profiles of responders. Essential future steps include testing combinations of tSCS with other treatment modalities to further potentiate the obtainable therapeutic effects. [1] Hofstoetter et al. J Spinal Cord Med. 2014; 37: 202-11.

Disclosures: U. Hofstoetter: None. **M. Krenn:** None. **S.M. Danner:** None. **B. Freundl:** None. **H. Binder:** None. **F. Rattay:** None. **W. Mayr:** None. **K. Minassian:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.05/HH16

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Vienna Science and Technology Fund (WWTF), Proj.Nr. LS11-057

Wings for Life Spinal Cord Research Foundation (WfL), Proj.Nr. WFL-AT-007/11

Foundation for Movement Recovery, Oslo, Norway

Title: Long-latency spinal reflexes predict rhythmicity in response to epidural lumbar cord stimulation

Authors: *S. M. DANNER[1,2], M. R. DIMITRIJEVIC[3], U. S. HOFSTOETTER[4], M. KRENN[4], W. MAYR[4], K. MINASSIAN[4], F. RATTAY[2], J. C. ROTHWELL[5];
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Abstracts: It is known that repetitive lumbar epidural stimulation can give rise to rhythmic activity in leg muscles in individuals with clinically motor complete spinal cord injury. However, the effect can vary in different people, in different muscles, and at different frequencies. Here, we examined what factors might influence the appearance of rhythmic activity. We applied epidural stimulation to the posterior lumbar spinal cord in 10 individuals with clinically motor complete spinal cord injury. Motor unit activity was assessed by electromyography (EMG) recordings from thigh and leg muscle groups. Stimulation was applied at various stimulation frequencies (2-80 Hz) at motor threshold intensity. Rhythmicity occurred most often at 50 Hz, only above 20 Hz and most frequently in tibialis anterior. Rhythmic activities in single muscle groups occurred in all subjects, yet the frequency of occurrence differed substantially between the subjects ranging from 1.3% to 25.9% of the recordings. Thus, tonic activity was always dominant and besides stimulation frequency additional factors must influence the motor pattern. In response to low frequency stimulation (2-5 Hz), in addition to the well described monosynaptic reflex components, three types of long-latency responses (>50 ms post-stimulus) were found: i) Interference-like tonic as well as ii) phasic asynchronous EMG activities spanning the time of analysis, and iii) stimulus time-related compound muscle action potentials. Amplitude and quality of late responses could change independently of the short-latency responses during constant stimulation conditions. In one subject with a laterally located electrode, stimulation of up to 10.5 V induced monosynaptic reflexes in the ipsilateral leg only. Yet, starting with 5 V long-latency responses were recorded on the contralateral side. Thus, it is possible with unilateral stimulation to elicit a contralateral response. Finally, we found that the root-mean-square of the late responses (50-150 ms post-stimulus) observed to follow 2 and 5 Hz trains of test stimuli in all muscle group EMG recordings from one leg significantly predicted the

probability of occurrence of rhythmic activity at higher stimulation frequencies but unchanged impulse width and amplitude ($p<.001$; $R^2=.624$). The fact that long-latency responses predict rhythmicity at higher frequencies suggests that the state of the spinal networks, i.e., the relative excitability of different neural pathways, determines the response to stimulation. Externally modulating the central excitability would thus allow for better control of the outupt to high frequency epidural stimulation.

Disclosures: S.M. Danner: None. **M.R. Dimitrijevic:** None. **U.S. Hofstoetter:** None. **M. Krenn:** None. **W. Mayr:** None. **K. Minassian:** None. **F. Rattay:** None. **J.C. Rothwell:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.06/HH17

Topic: D.12. Kinematics and EMG

Support: TÁMOP-4.2.1./B-11/2-KMR-2011-0002

TÁMOP-4.2.2./B-10/1-2011-0014

Hungarian Society for Sport Science

Title: Muscle co-activation as function of crank angle when cycling on an ergometer with altered power output

Authors: *J. LACZKO[1,2,3], P. KATONA[4], A. VALY[3];
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Abstracts: Cycling on an ergometer is often applied in medical rehabilitation for individuals who have suffered a stroke or other CNS lesion. If pedaling cadence or crank resistance increases than larger total muscle work is required from the cycler. This might be ensured by reducing of muscle co-activation and/or by increasing individual muscle activities. We examined cycling movements of able-bodied individuals, in particular how co-activation of knee muscles alters if pedaling cadence or crank resistance changes. Methods: 15 participants (age 20-28 y.) were involved in the study. They performed cycling movements on a stationary bike (SCIFIT, Germany). Cycling was performed with 2 velocities (45 and 60 revolution per minute) against 3

crank resistances. Surface EMGs of vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), biceps femoris (BF) were measured with the ZEBRIS CMS-HS movement analyzing system (sampling frequency 900Hz). In parallel, ZEBRIS recorded the positions of markers placed on the lower limb and on the crank of the ergometer (100Hz). Crank direction (crank angle respect to the upward vertical direction) was computed from coordinates of markers placed on the ergometer's crank and on the participant's foot. We investigated the co-activation of BF with 3 parts of the quadriceps separately (BF-VL, BF-VM, BF-RF muscle pairs). Muscle co-activations were quantified by the range of crank angles in which both muscle in a flexor-extensor muscle pair was simultaneously active. Student's t-tests ($p<0,05$) were applied to compare the size of co-activation ranges (SCR) under different cycling conditions. Results: The size of co-activation range of BF-VL muscle pair significantly decreased when the crank resistance increased in a given cycling cadence. This was true for the BF-VM muscle pair too. The SCR decreased for the BF-RF muscle pair but this change was not significant. When cycling cadence was increased and crank resistance remained constant, than no significant change was observed in the SCR of BF-VM, neither in the SCR of BF-VL nor in the SCR of BF-RF. Conclusion: Knee extensor muscles are mainly responsible for generating sufficient force during cycling movements but if crank resistance is increased than neural control may help to generate higher power output by reducing the ranges where flexor-extensor muscles co-activate. In contrast, when cycling cadence increases and resistance remains constant than purely the increased individual muscle activities ensure higher power output. Thus, lower limb cycling against increased crank resistance may be advantageous in those rehabilitation procedures in which the aim is to improve neural adaptation.

Disclosures: J. Laczko: None. **P. Katona:** None. **A. Valy:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.07/HH18

Topic: D.10. Spinal Cord Injury and Plasticity

Support: The European Commission's Seventh Framework Programme (CP-IP 258654)

The European Research Council (ERC 261247)

NCCR Robotics

Nano-tera (20NA21_145923)

Title: Physiologically inspired multisite spinal cord stimulation improves locomotion after spinal cord injury

Authors: *N. WENGER[1,2], P. MUSIENKO[2], E. MARTIN-MORAUD[3], J. GANDAR[2], A. LARMAGNAC[5], I. MINEV[4], P. DETEMPLE[6], Q. BARRAUD[2], J. BEAUPARLANT[2], L. BAUD[2], M. CAPOGROSSO[3], N. DOMINICI[2], S. MICERA[3], J. VORÖS[5], S. LACOUR[4], G. COURTINE[2];
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Abstracts: Electrochemical neuromodulation of spinal sensorimotor circuits improves motor control in animal models and human patients with spinal cord injury (SCI). Currently, stimulation parameters are tuned manually and remain constant during movement, which is likely suboptimal to mediate maximum therapeutic effects. The potential of spinal neuromodulation therapies to improve locomotor performance through multiple electrodes at distinct locations and at specific time of the gait cycle remains poorly explored. Here we developed a neuroprosthetic system composed of a multi-electrode array and a real-time control system that enabled closed-loop tuning of multisite spinal cord stimulation during locomotion in rats. We leveraged these integrated developments for the design of multisite stimulation strategies that mimicked the oscillating activity of flexor and extensor motor neuron pools underlying locomotion in healthy rats. These algorithms were designed based on computational models and comprehensive mapping experiments. Detailed analysis of kinematic, kinetic and muscle activity revealed significantly increased modulations of gait features and more physiologically relevant stepping patterns when delivering physiologically relevant, phase-dependent multisite stimulation compared to continuous stimulation. Our integrated developments show the potential to markedly improve gait execution with real-time control of electrochemical neuromodulation after spinal cord injury. These results emphasize the need to develop similar conceptual and technological framework to facilitate rehabilitation in human patients with spinal cord injury.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

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Program#/Poster: 630.08/HH19

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NCCR Robotics

The Bertarelli Foundation

Title: Neuromodulation of motor cortex and spinal circuits facilitates locomotor training and promotes recovery following spinal cord injury in mice

Authors: *J. A. KREIDER, E. DE SAINT-EXUPERY, L. ASBOTH, Q. BARRAUD, G. COURTINE;
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Abstracts: Traumatic spinal cord injury usually preserves bridges of intact neural tissues. Spontaneous reorganization of spared circuits and residual connections mediate improvement of function after partial lesions, which is enhanced with rehabilitative training. These results stress the importance of developing interventions that take full advantage of activity-dependent plasticity to repair the injured central nervous system. Here, we hypothesized that concomitant neuromodulation of motor cortex and spinal circuits during robot-assisted gait training would promote ubiquitous remodeling of preserved neural systems, leading to superior recovery after a partial lesion. Mice received a severe thoracic spinal cord contusion that spared less than 20% of descending fibers. During robot-assisted gait training, the mice received electrochemical neuromodulation of spinal circuits to enable locomotor permissive states, and optogenetic neuromodulation in the leg area of the motor cortex to force the brain to regain control of locomotion. After a few weeks of training, all the mice showed coordinated, weight bearing locomotion overground during concomitant neuromodulation of motor cortex and spinal circuits. Some of them were even capable of initiating and sustaining locomotion without any stimulation. In absence of training, only a few mice were able to sustain locomotion with neuromodulation therapies, and all of them failed to initiate walking voluntarily. These preliminary results highlight the remarkable potential of neuromodulation therapies across the nervous system to take advantage of residual connections and spared circuits to regain locomotor capacities after spinal cord injury.

Disclosures: **J.A. Kreider:** None. **E. De saint-exupery:** None. **L. Asboth:** None. **Q. Barraud:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.09/HH20

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Bertarelli Fondation

NCCR Robotics

Title: Optogenetic activation of the motor cortex unmasks supraspinal access to locomotor circuits caudal to a severe spinal cord injury in mice

Authors: ***L. ASBOTH**, Q. BARRAUD, J. KREIDER, J. VON ZITZEWITZ, G. COURTINE; EPFL SV BMI UPCOURTINE, Swiss Federal Inst. of Technol., Lausanne, Switzerland

Abstracts: We previously demonstrated that robot-assisted training enabled by electrochemical neuromodulation of spinal circuits restored supraspinal control of locomotion in rats with a spinal cord injury (SCI) leading to permanent paralysis. Training encouraged the motor cortex to elaborate alternative relay pathways that restored graded access to electrochemically-enabled lumbosacral circuits. However, the mechanisms underlying the cortical control of leg movements remain unknown, if not controversial. To address this question, we performed experiments in mice expressing channelrhodopsin in motor cortex neurons. An optic fiber was chronically implanted into the region of the motor cortex that induced rhythmic leg movements in healthy mice. After baseline evaluations, mice received a contusion SCI that completely interrupted the corticospinal tract, and led to severe impairments of both legs. Locomotor capacities were evaluated using a high-fidelity robotic interface that provided bodyweight support against gravity, but did not facilitate walking in the forward direction. Photoactivation of the motor cortex during chemical neuromodulation of spinal circuits instantly triggered continuous, weight-bearing locomotion in mice that failed to initiate walking voluntarily. The degree of limb extension and overall locomotor output directly correlated with the intensity of photoactivation. These findings suggest that chemical neuromodulation of the spinal cord raises excitability of lumbosacral circuits to a level that allows the motor cortex to control and modulate leg movements following a severe spinal cord injury that abolishes direct corticospinal inputs and only spares 20% of original descending pathways. These preliminary results establish the settings to dissect and enhance the contribution of descending pathways to recovery after SCI.

Disclosures: **L. Asboth:** None. **Q. Barraud:** None. **J. Kreider:** None. **J. von Zitzewitz:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.10/HH21

Topic: D.10. Spinal Cord Injury and Plasticity

Support: The European Research Council (ERC 261247)

Nano-tera (20NA21_145923)

Title: Closed-loop control of multisite spinal cord stimulation to improve locomotion following spinal cord injury

Authors: ***J. GANDAR**, N. WENGER, E. M. MORAUD, P. MUSIENKO, S. MICERA, G. COURTINE;
EPFL, Lausanne, Switzerland

Abstracts: Epidural electrical stimulation of lumbosacral segments improved motor control after spinal cord injury in animal models and human patients. In these studies, electrical neuromodulation of spinal circuits has been delivered continuously based on empirical observations, which is suboptimal to facilitate walking with this intervention. Multi-electrode arrays provide the intriguing possibility to access specific subsets of sensorimotor circuits at specific time of the gait cycle in order to maximize facilitation of gait with neuromodulation therapies. However, the development of effective multisite stimulation algorithms rely on advanced control infrastructures that interface rapid movement feedback with instantaneous tuning of stimulation parameters. To support these developments, we established a real-time stimulation platform that allows triggering site-dependent stimulation at any specific time of the gait cycle. To define triggering-times over the entire cycle duration, we developed robust algorithms that derived gait timing from the virtual angle of the foot trajectory around its center of rotation. We exploited this platform to uncover the effects of site- and time-dependent stimulation on the production of gait in rats with complete spinal cord injury. We found that the timing of stimulation onset and end mediated adjustments of locomotor kinematics that depended on the location of the electrode. We leveraged this mapping to conceive a closed-loop control algorithm that triggered stimulation at distinct locations and specific times with the aim to improve locomotor performance. Compared to continuous stimulation paradigms, closed-loop control of multisite stimulations significantly ameliorated gait execution in all the tested rats. The

control platform and developed stimulation protocols provide tools and concepts for the personalization of neuromodulation therapies during rehabilitation in human patients.

Disclosures: **J. Gandar:** None. **N. Wenger:** None. **E.M. Moraud:** None. **P. Musienko:** None. **S. Micera:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.11/HH22

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Bertarelli Foundation

European Research Council (ERC 261247)

Nano-tera (20NA21_145923)

Title: Electrochemical stimulation of the spinal cord using a soft intrathecal interface

Authors: ***I. MINEV**, P. MUSIENKO, A. HIRSCH, Q. BARRAUD, J. GANDAR, N. VACHICOURAS, N. WENGER, N. PAVLOVA, E. MARTIN-MORAUD, S. DUIS, G. COURTINE, S. LACOUR;
Ctr. for Neuroprosthetics, École Polytechnique Fédérale De Lausanne (EPFL), Lausanne, Switzerland

Abstracts: Electrical and chemical neuromodulation of spinal circuits restored advanced motor control capacities in animal models and human patients with spinal cord injury (SCI). These developments have created an urgent need for an implantable neural interface capable of delivering electrical and chemical stimulation over multiple spinal cord locations for extended periods of time. For this purpose, we designed a biologically transparent neural interface that resides in the intrathecal space, in direct contact with the dorsal surface of the spinal cord. The use of soft, mechanically compliant engineering materials that match the mechanical properties of the dura mater enabled chronic implantation without detrimental damage to spinal tissue. Contrary to conventional epidural electrode arrays, this novel interface allows electrical stimulation currents to bypass the dura mater, which increased stimulation specificity for lower current amplitudes. Direct access to the cerebrospinal fluid enables local, micro-delivery of chemical agents through a microfluidic system integrated within the interface. Iterative

optimization of technological and surgical approaches allowed us to develop a highly stable, biologically transparent neural interface that restored full weight-bearing locomotion in paralyzed rats with a complete spinal cord injury.

Disclosures: I. Minev: None. **A. Hirsch:** None. **P. Musienko:** None. **Q. Barraud:** None. **J. Gandar:** None. **N. Vachicouras:** None. **N. Wenger:** None. **N. Pavlova:** None. **E. Martin-Moraud:** None. **S. Duis:** None. **G. Courtine:** None. **S. Lacour:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.12/HH23

Topic: D.10. Spinal Cord Injury and Plasticity

Support: The International Paraplegic Foundation

Title: Noradrenergic neuromodulation of spinal circuits facilitates locomotion following spinal cord injury

Authors: *Q. BARRAUD, P. MUSIENKO, K. A. BARTHOLDI, G. COURTINE;
EPFL - Ctr. For Neuroprosthetics, Lausanne, Switzerland

Abstracts: A large number of studies have investigated the ability of monoaminergic neural pathways to engage spinal locomotor networks after the interruption of descending pathways. In particular, alpha2 noradrenergic receptors have historically been amongst the first identified pathways capable of inducing robust locomotion in paralyzed cats with complete spinal cord injury (SCI). These results encouraged the development of noradrenergic neuromodulation therapies to facilitate locomotion and rehabilitative training in paraplegic patients. However, application of these pharmacological agents in humans with SCI has yielded conflicting results, with outcomes ranging from modest facilitation of stepping to a complete suppression of motor outputs. Surprisingly, similar effects were found in rats with complete SCI. These discrepancies emphasize the need to conduct in-depth studies to decipher the specific function of alpha2 noradrenergic receptors subtypes in the production of locomotion after SCI, and to dissect the circuits underlying these effects. To address this question, we performed a comprehensive series of pharmacological experiments in mice, rats, and cats with complete SCI. We combined functional analyses with advanced anatomical evaluations to deconstruct the neuronal circuits underlying the effects of noradrenergic pathway activation. These experiments allowed us to

identify novel targets to facilitate standing and walking with noradrenergic neuromodulation therapies in in humans with SCI.

Disclosures: Q. Barraud: None. **P. Musienko:** None. **K.A. Bartholdi:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.13/HH24

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation (315230_149902)

Title: Closed-loop control of dynamic trunk posture improves gait patterns during locomotor training after spinal cord injury

Authors: *S. M. WURTH[1], J. MIEHLBRADT[2], E. MARTIN MORAUD[2], J. VON ZITZEWITZ[3], S. MICERA[2], G. COURTINE[3];
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Abstracts: The quality of locomotor movements and amount of weight bearing play a central role in the outcome of gait rehabilitation after spinal cord injury (SCI). These results triggered the development of various exoskeletons that aim to reinforce kinematic consistency and increase loading during training. However, most of these support systems disregard the importance of trunk posture, which is often completely blocked or at least strongly impeded. To demonstrate the importance of trunk posture and dynamic trunk movement during gait execution, we designed and fabricated a robotic trunk neuroprosthesis that automatically optimizes dynamic trunk posture during locomotion in rats. The system employs a servo-controlled back-plate to which the rat is attached with a custom made jacket. Rotation of the back-plate around an adjustable center of rotation allows online control of trunk posture. Due to careful choice of components and materials, the robot exhibits dimensions and weight comparable to traditional adapters used for bodyweight support on a treadmill. To adjust trunk posture dynamically during locomotion, we developed a closed-loop interface that tuned the back-plate position based on whole-body kinematic performance. The evaluation of the robot was achieved by testing rats

with complete SCI and severe contusion SCI during locomotion enabled through electrochemical neuromodulation of spinal circuits. In both models of SCI, closed-loop control of dynamic trunk movement increased the level of weight bearing in both legs during stance, and improved the consistency and symmetry of bilateral leg movements. These results stress the importance of incorporating dynamic trunk control into traditional gait rehabilitation for patients suffering from different neuro-motor disorders.

Disclosures: S.M. Wurth: None. **J. Miehlbradt:** None. **E. Martin Moraud:** None. **J. Von Zitzewitz:** None. **S. Micera:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.14/HH25

Topic: D.10. Spinal Cord Injury and Plasticity

Support: ERC Grant WALK AGAIN 261247

NCCR/SNF Grant 565236

NCCR/SNF Grant 513347

Title: Translational robotic platforms to evaluate, enable, and train locomotion and balance after neuromotor disorders

Authors: *J. VON ZITZEWITZ, L. ASBOTH, G. COURTINE;
EPFL - SV - Upcourtine, Lausanne, Switzerland

Abstracts: We previously introduced a robotic interface providing motor impaired rats with multi-dimensional bodyweight support during over-ground locomotion. This robotic interface immediately enabled motor control after partial spinal cord injury, and restored refined locomotion in combination with training and electrochemical neuromodulation therapies after more severe lesions. We aimed to establish similar conditions for mechanistic studies in mice, and to translate this robotic training into an effective rehabilitation environment for patients. Compared to rats, mice showed a 10-fold reduction in bodyweight, which imposed increasing the precision of force control by a similar factor of magnitude. To achieve this high-level of performance, we developed new solutions based on deformable elastic structures and high-precision 3D force sensors. When attached to the trunk, the robotic interface did not interfere

with quadrupedal locomotor movements of healthy mice, even when progressing along complex paths combining sinuous turns and slopes. The robotic interface was also capable of providing spinal cord injured mice with finely-adjustable support in the vertical and walking directions. We evaluated this enabling mode in mice that had received a severe thoracic spinal cord contusion. In the presence of chemical stimulation, the otherwise paralyzed mice showed coordinated, weight bearing steps with vertical oscillations of the body, resulting in continuous locomotion over-ground. To establish similar conditions in humans, we contributed to the development of a novel cable-based robotic body-weight support system. This low-inertial, cable-based robot creates a large 3D workspace in which versatile force fields can be applied to the user along the vertical, medio-lateral, and antero-posterior directions. This robotic portfolio opens new avenues for translational research in gait rehabilitation across species.

Disclosures: J. Von Zitzewitz: None. **L. Asboth:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

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Program#/Poster: 630.15/HH26

Topic: D.10. Spinal Cord Injury and Plasticity

Support: European Community's Seventh Framework Programme (CP-IP 258654)

European Research Council (ERC 261247)

International Paraplegic Foundation

Nanotera Swiss program (SpineRepair)

Title: Closed-loop neuromodulation of spinal circuits improves bilateral control of locomotion during training after spinal cord injury

Authors: *E. MARTIN MORAUD[1,2], N. WENGER[3], J. DIGIOVANNA[1], G. COURTINE[3], S. MICERA[1,4];
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Abstracts: The level of weight-bearing, walking speed, interlimb coordination, and kinematic consistency play essential roles to maximize recovery with rehabilitative training after spinal cord injury (SCI). To optimize these parameters, current strategies rely on manual assistance. Closed-loop electrical neuromodulation of spinal circuits provides an alternative strategy to promote the best possible patterns of leg movements during training. To test this hypothesis, we designed robust algorithms that adjust stimulation parameters in real-time based on movement feedback. The control structure integrates two independent layers operating in parallel. Each control layer tunes stimulation parameters during the appropriate phase of gait in order to maintain left and right foot heights within a desired window. We found that closed-loop electrical neuromodulation of spinal circuits automatically restored interlimb coordination, increased kinematic consistency, and improved balance in rats with complete or severe contusion SCIs . The control policies instantly corrected side-specific gait deficits that otherwise would require skilled manual assistance. Moreover, closed-loop neuromodulation of spinal circuits allowed training under more demanding conditions, including increased weight-bearing levels and faster treadmill belt speeds, while maintaining gait quality regardless of idiosyncratic deficits. Closed-loop neuromodulation of spinal circuits holds promises to augment activity-dependent plasticity and recovery after SCI in human patients.

Disclosures: E. Martin moraud: None. **N. Wenger:** None. **J. DiGiovanna:** None. **G. Courtine:** None. **S. Micera:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.16/HH27

Topic: D.10. Spinal Cord Injury and Plasticity

Support: The European Commission's Seventh Framework Programme (CP-IP 258654)

The European Research Council (ERC 261247)

Title: Multi-electrode arrays for chronic spinal cord stimulation in freely behaving mice

Authors: *S. E. DUIS[1], A. LARMAGNAC[2], J. GANDAR[1], E. MARTIN MORAUD[3], N. PAVLOVA[1], N. WENGER[1], J. VOROS[2], G. COURTINE[1];
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Switzerland; [2]Inst. for Biomed. Technique, ETH, Zürich, Switzerland; [3]Ctr. for Neuroprothetics, Sch. of Bioengineering, EPFL, Lausanne, Switzerland

Abstracts: Electrochemical neuromodulation of spinal circuits restored motor control after spinal cord injury, but the underlying mechanisms remain unclear. Transgenic mice provide the unique opportunity to investigate the neural structures and circuits recruited with this treatment paradigm. However, these experiments rely on the development of multi-electrode array for chronic spinal cord stimulation in freely behaving mice. The small size of the mouse spinal cord and extensive spine motion during movement create a highly challenging environment for neural interfaces. Here, we exploited conductive Polydimethylsiloxane (PDMS) to design stretchable conductive leads that can survive such high mechanical stress during chronic implantation. This all-elastomeric technology provides high compliance to the implant, thus allowing the array to act as a second skin in close contact with the spinal cord. Optimization of surgical techniques ensured long-term stability and bio-compatibility of the implant, which we demonstrated histologically and functionally. Despite the small size of the mouse spinal cord, the multi-electrode array was capable of mediating site-specific facilitation of left and right leg movement. Electrical neuromodulation of spinal circuits induced continuous, weight bearing locomotion in mice with complete spinal cord injury. This novel multi-electrode array provides the opportunity to conduct mechanistic studies in mice in order to guide and optimize the translation of spinal neuromodulation therapies in human patients with spinal cord injury.

Disclosures: S.E. Duis: None. **J. Gandar:** None. **N. Pavlova:** None. **N. Wenger:** None. **G. Courtine:** None. **A. Larmagnac:** None. **J. Voros:** None. **E. Martin Moraud:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: EU 7th Generation Framework CP-IP 258654

WFL CHE-018/13 81

Marie Curie IIF 587504

Title: Neuromodulation of spinal locomotor circuits in the freely moving rhesus monkey

Authors: *D. A. BORTON[1], E. MARTIN-MORAUD[1], N. WENGER[1], J. LAURENS[1], P. MUSIENKO[1], J. BLOCH[2], P. DETEMPLE[3], E. BEZARD[4], G. COURTINE[1];
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Abstracts: Epidural electrical stimulation of lumbosacral segments has restored supraspinal control over refined leg movements in animal models of spinal cord injury, and in paraplegic patients. Real-time adjustment of the timing, location, and parameters of electrical neuromodulation has achieved high fidelity control of leg kinematics during locomotion in rats. Experiments in non-human primates are essential along a roadmap for eventual human implementation. To this aim, we have developed a polyimide-based multi-electrode array that is specifically optimized for epidural implantation over the dorsal aspect of primate lumbosacral segments. Electrode positions, array configuration, and surgical procedures were established based on functional and dissection studies, high-resolution computed tomography (CT) scans, and motor-neuron tracing experiments. The stimulation array was connected to a RestoreSensor® implantable pulse generator (IPG) designed by Medtronic (MN, USA), which is controlled externally in closed-loop via custom software package. The IPG integrates a new firmware enabling real-time control of complex stimulation patterns based on external signals. This innovative technology provided us with the unique opportunity to deliver multisite patterns of electrical spinal cord stimulation that are tuned based on ongoing muscle activity, movement feedback, or neuronal modulations. We integrated this new stimulation interface within an advanced neuromotor analysis platform that enables wireless recording of motor cortex population dynamics, muscle synergies, and whole body kinematics in freely moving, unconstrained and untethered non-human primates. Using this unconventional platform, we mapped adjustment in electrical spinal cord neuromodulation with specific changes in leg kinematics, muscle activity, and neural states of the motor cortex during continuous locomotion in non-human primates. These technological developments establish the settings for the translational design of neuromodulation therapies after spinal cord injury.

Disclosures: D.A. Borton: None. **E. Martin-Moraud:** None. **N. Wenger:** None. **J. Laurens:** None. **P. Musienko:** None. **J. Bloch:** None. **P. Detemple:** None. **E. Bezard:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: International Paraplegic Foundation

Wings for Life

Adelson Medical Research Foundation

European Research Council (ERC 261247)

Title: Polypeptide hydrogel depot delivery of bioactive molecules after spinal cord injury

Authors: *M. ANDERSON[1], M. PELLISSON[2], S. ZHANG[3], T. J. DEMING[4], M. V. SOFRONIEW[5], G. COURTINE[2];
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Abstracts: Many molecules have been identified that can influence tissue repair, neural plasticity or axon growth by acting on glial cells, neurons, axons or extracellular matrix. Clinical application of these molecules requires neurosurgical intervention and delivery directly into the brain or spinal cord. Injectable biomaterials have the potential to serve as depots and scaffolds for *in vivo* delivery of bioactive molecules and cells. We have been developing di-block copolypeptide hydrogels (DCH) as fully synthetic biomaterials that can safely and easily be injected into specific tissue sites after spinal cord injury (SCI) to deliver molecules in order to study, dissect, and manipulate mechanisms of repair, plasticity and recovery. Using a mouse model, we found DCH are biocompatible after injection into the brain or spinal cord. The hydrogels self-assemble into depots with finely controllable properties that can provide prolonged release of protein growth factors and hydrophobic small molecules. Current work shows that DCH depots can safely and routinely be implanted into the lesion core at 2 days after SCI in mice, and can simultaneously deliver multiple growth factors that (i) stimulate production of growth permissive extracellular matrix and (ii) directly stimulate axon growth through the lesion core. We are now testing DCH depot delivery of bioactive molecules and stem cells in a rat model of crush SCI with the objective of combining this regenerative strategy with neuroprosthetic neurorehabilitation. Funding: International Paraplegic Foundation, Wings for Life, Adelson Medical Research Foundation, European Research Council (ERC 261247)

Disclosures: **M. Anderson:** None. **M. Pellisson:** None. **S. Zhang:** None. **T.J. Deming:** None. **M.V. Sofroniew:** None. **G. Courtine:** None.

Poster

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Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: The European Commission's Seventh Framework Programme (CP-IP 258654)

Title: Multidirectional robotic support enables gait rehabilitation under natural conditions in individuals with neuromotor disorders

Authors: *J.-B. MIGNARDOT[1], J. VON ZITZEWITZ[1], C. LE GOFF[1], R. VAN DEN BRAND[1], J. BLOCH[2], S. CARDA[2], G. COURTINE[1];
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Abstracts: We previously introduced a robotic interface providing motor impaired rats with multi-dimensional bodyweight support during overground locomotion. This robotic interface immediately improved motor control after partial spinal cord injury and stroke. We aimed to translate this robotic training into an effective rehabilitation environment for patients with neuromotor disorders. For this purpose, we leveraged a new robotic support system, named Free Levitation for Overground Assisted Training (FLOAT). This low-inertial, cable-based robot creates a large 3D workspace in which versatile force fields can be applied to the user along 3 independent directions. The FLOAT provides adjustable bodyweight support without impeding gait movements while the subject progresses overground within a safe environment. To evaluate the transparency of the FLOAT, we conducted kinematic, kinetic and muscle synergy recordings in healthy individuals during the execution of complex locomotor tasks including passing obstacles, steering curves, and climbing staircases. Principal component analysis applied on all the computed gait parameters demonstrated the absence of interferences between the robotic motion and gait movements. In turn, the robot was capable of delivering well-controlled postural perturbations, which allowed evaluation of dynamic balance control during walking. We also assessed whether the FLOAT mediated improvements of locomotor execution in individuals with spinal cord injury and stroke, as previously observed in rodents. Preliminary analyses suggest that optimized levels of robotic bodyweight support significantly improved the production of gait in all the tested patients. These results validate our novel robotic platform to enable gait rehabilitation under natural conditions in individuals with neuromotor disorders.

Disclosures: J. Mignardot: None. **J. von Zitzewitz:** None. **C. Le Goff:** None. **R. van den Brand:** None. **J. Bloch:** None. **S. Carda:** None. **G. Courtine:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Support: The Swiss National Science Foundation (31003A_146925)

The International Paraplegic Foundation

The European Research Council (ERC 261247)

The European Commission's Seventh Framework Programme (CP-IP 258654)

Title: Reticulospinal neurons play a key role in the recovery of voluntary locomotion in response to neuroprosthetic rehabilitation after a severe spinal cord contusion

Authors: *C. MARTINEZ GONZALEZ, L. FRIEDLI-WITTLER, J. BEAUPARLANT, G. PIDPRUZHNYKOVA, L. BAUD, S. DUIS, G. ULRICH, G. COURTINE;
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Abstracts: We found that neuroprosthetic rehabilitation combining electrochemical neuromodulation of spinal circuits and robot-assisted training re-established supraspinal control of locomotion after a severe mid-thoracic contusion of the spinal cord. Here, we sought to identify the mechanisms underlying functional recovery. Combination of anterograde and retrograde neuronal tract tracing revealed that the degree of functional recovery correlated with the amount of spared tissue and the extent of corticospinal, reticulospinal, and serotonergic fiber reorganization. To demonstrate the essential role of descending fiber reorganization in the recovery of supraspinal control of locomotion, we performed two sets of complementary experiments. First, we delivered deep brain stimulation in the midbrain locomotor regions to activate reticulospinal fibers that were spared by the lesion. Continuous stimulation of the midbrain locomotor region near-instantly triggered coordinated leg movements. This ability emerged and improved conjointly with voluntary motor control capacities. Second, we exploited a virus-mediated inactivation technique to reversibly silence reticulospinal neurons with spared synaptic projections to lumbar spinal circuits located below the injury. Inactivation of reticulospinal neurons in rats that had regained voluntary locomotion induced a marked decline in gait performance. Our results demonstrate that neuroprosthetic rehabilitation promotes

activity-dependent reorganization of spared reticulospinal fibers and that these synaptic projections significantly contribute to supraspinal control of locomotion during electrochemical neuromodulation of spinal circuits.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research

Christopher & Dana Reeve Foundation

Alberta Paraplegic Foundation

Rick Hansen Institute

University of Alberta Rehabilitation Medicine Students' Association

Title: Changes in spasticity-related reflexes after two forms of walking retraining in individuals with incomplete spinal cord injury

Authors: *A. KHAN[1,2], S. PATRICK[3], F. ROY[4,2], M. GORASSINI[5,2], J. YANG[6,2];
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Abstracts: Spinal cord injury (SCI) results in reduced mobility and spasticity. Spasticity manifests in various ways, such as repetitive and sustained muscular contractions (clonus and spasms, respectively), and hyperactive withdrawal reflexes (a cutaneomuscular reflex). Exercise has been shown to reduce some manifestations of spasticity and improve walking ability after incomplete SCI. Here, we examine the effects of two forms of training on spasticity and walking ability after incomplete SCI: 1) Endurance training (i.e., treadmill walking) and 2) Precision training (i.e., precise walking over obstacles and on targets overground). Twenty participants

with motor-incomplete SCI were randomized to undergo Endurance or Precision training (1hr/day, 5x/week) for two months, then crossed over to the other form of training (2 mo.) with a two-month rest period in between. A cutaneomuscular reflex (CMR) and clonus were recorded using electromyography (EMG) from the soleus muscle during treadmill walking before and after each form of training. The CMR was induced by surface electrical stimulation of the posterior tibial nerve at the ankle during walking. EMG responses were averaged as a function of time of stimulus arrival during the step cycle. EMG from undisturbed walking was then subtracted to extract the response. Time window of the average inhibitory response was determined on an individual basis, beginning and ending anywhere between ~35 to 200ms after the first stimulus. Clonus was quantified using spectral analysis of the soleus EMG during undisturbed walking. The signal power in the clonus frequency range of 4-10Hz was determined, and normalized to overall power from 0-40Hz. The 10-Meter Walk Test and 6-Minute Walk Test were also used to quantify walking speed and distance, respectively. There was a significant increase in reflex inhibition after Endurance (-2.1 to -4.9μV, p=0.032), but not Precision training (-3.5 to -2.3μV, p=0.39). There was a trend for reduction in clonus after Precision (1.1 to 0.94, p=0.36), but not Endurance training (1.0 to 0.96, p=0.79). There was a significant correlation between: 1) increase in walking speed and reflex inhibition after Endurance training (comfortable speed: p=0.025, r=0.64; fast speed: p=0.084, r=0.61), and 2) increase in walking distance and reduction in clonus after Precision training (p=0.013, r=0.59). Overall, reduction in spasticity after both types of training was related to improvements in walking ability. Changes in CMR hyperexcitability and clonus did not always change in the same way; considerable individual differences were seen. The findings will need confirmation with larger studies or meta analyses.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Topic: D.10. Spinal Cord Injury and Plasticity

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NSFC Grant 81171152

Title: Studying motor compensation and spinal motoneuron regeneration in mice with genetic absence of the corticospinal tract

Authors: *L. ZHOU, Y. DING, C. CAO;
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Abstracts: The corticospinal tract (CST) makes direct and indirect connections with spinal motoneurons invovled limb movement, particular skilled movement control. We previously generated *Celsr3|Emx1 mice*, in which corticospinal axons fail to course through the internal capsule and to innervate the spinal cord. In the present work, we used those mice to carry out two studies: (1) The compensation of motor components and its mechanisms. Results: Mutant mice had no paresis, but displayed hyperactivity in open-field tests and disabled skilled movement in food pellet-taking tests. Both retrograde and anterograde labeling shows projections from red nuclei to the spinal cord are significantly increased in mutants compared to control animals. In contrast, no difference in labeling of vestibulospinal and reticulospinal projections was noted. Contrary to control mice, mutants developed severe disability of forelimb use following section of the rubrospinal tract (RST) at the C4 spinal level. In the mutant, the number of motoneuronswas reduced and terminal axon arbors at the neuromuscular junction (NMJ) displayed atrophy, which was accompanied with NMJ functional deficits. Conclusion: The RST spontaneously compensate to play a crucial role in spinal motor control following genetic ablation of the CST, but not in skilled movements, and genetic absence of the CST affects locomotion rhythm, the maturation of spinal cord and NMJs. (2) Spinal motoneuron regeneration and functional recovery following root avulsion and reimplantation. Results: In adult mice, we tore off right C5-C7 motor and sensory roots and re-implanted the right C6 roots. Behavioral studies showed impaired recovery of elbow flexion in *Celsr3|Emx1* mice compared to controls. Five months after surgery, a reduced number of small axons, and higher G-ratio of inner to outer diameter of myelin sheaths were observed in mutant versus control mice. At early stages post-surgery, mutant mice displayed lower expression of GAP-43 in spinal cord and of myelin basic protein (MBP) in peripheral nerves than control animals. After five months, mutant animals had atrophy of the right biceps brachii, with less newly formed neuromuscular junctions (NMJs) and reduced peak-to-peak amplitudes in electromyogram (EMG), than controls. However, quite unexpectedly, a higher motoneuron survival rate was found in mutant than in control mice. Conclusion: Following root avulsion/re-implantation, the absence of the CST hampers axonal regeneration and remyelination, as well as target re-innervation and formation of new NMJ, resulting in lower functional recovery, while fostering motoneuron survival.

Disclosures: **L. Zhou:** None. **Y. Ding:** None. **C. Cao:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.23/II2

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Treadmill training reduces mechanical allodynia in a mouse model of spinal cord contusion injury

Authors: *T. A. NEES[1,2], M. MOTSCH[1], A. TAPPE-THEODOR[2], R. KUNER[2], N. WEIDNER[1], A. BLESCH[1];
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Abstracts: Below-level central neuropathic pain (CNP) affects a large proportion of spinal cord injured subjects. Patients suffering from CNP experience sensory disturbances including hyperalgesia, mechanical or thermal allodynia and spontaneous pain. Effective pharmacological means for treating this type of pain are limited. Despite the clinical relevance of spinal cord injury (SCI)-induced CNP, animal models have mostly been studied in the context of regenerative plasticity and recovery of motor function, and not in the context of pain plasticity. To better define the dynamic changes in the spinal cord contributing to the development of central neuropathic pain after SCI, we characterized the behavioral correlates of CNP in female C57BL/6 mice following a moderate T11 spinal cord contusion injury (50 kDyn) between 10 and 35 days post-injury. We further examined the influence of moderate physical activity on pain behavior and associated morphological changes. Spinal cord injured mice developed significant mechanical allodynia two weeks after injury when tested with small-diameter von Frey hair filaments ($p < 0.001$ for 0.16g and $p < 0.01$ for 0.4g filaments) but presented significant hypo-responsiveness to normally noxious stimuli ($p < 0.001$ for 1.4g filament). The mechano-sensory alterations lasted until the end of the experiment, 35 days post-lesion. Compared to sham operated animals, the response latency to heat stimuli already decreased significantly ($p < 0.05$) ten days post injury reaching a plateau three weeks after the spinal cord contusion. In contrast, injured mice developed a 1.67 fold increase in the time to respond on a 2°C cold plate demonstrating remarkable hypo-sensitivity to cold stimuli. To examine the influence of physical activity on CNP, animals underwent moderate treadmill training twice a day for 15 minutes, five days per week over a total of 25 training days. The training intensity was adjusted increasing from 0.12 m/s to 0.19 m/s. Treadmill training led to a significant reduction in the response rate to light mechanical stimuli. The positive training effect was already detectable after 6 days of

training (=day 14 post-lesion; $p < 0.05$) and remained evident for the rest of the experiment. In contrast to mechanical allodynia, thermal abnormalities were not influenced by physical activity. Treadmill training had also no influence on white matter sparing or functional recovery measured by BMS scoring. To define potential underlying mechanisms, structural changes in the spinal cord including sprouting of peptidergic and non-peptidergic nociceptive fibres are currently analyzed.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: International Spinal Research Trust (ISRT)

Medical Research Council (MRC)

Title: Regaining over-ground locomotor function following severe contusion injury with epidural stimulation and treadmill training

Authors: ***Y. D. AL'JOBOORI**, R. M. ICHIYAMA;
Sch. of Biomed. sciences, Univ. of Leeds, Leeds, United Kingdom

Abstracts: Spinal contusion injuries result in a loss of motor, sensory and autonomic function. Electrical epidural stimulation (ES) of the lumbar spinal cord (L2 to S1) has previously been shown to improve locomotor function in complete transection models of rat spinal cord injury in conjunction with monoaminergic and serotonergic agonists and bipedal locomotor training; however, ES has never been assessed in incomplete contusion lesions where some descending and ascending pathways remain. Here we demonstrate that the use of epidural stimulation (40 Hz; L2) and locomotor training following severe spinal contusion injury (T9/10) leads to improved locomotor function. Adult Sprague-Dawley rats received a severe spinal contusion injury (T9/10) and epidural implantation at segmental levels L2 and S1 and were randomly assigned to one of four groups: cage control, training only, ES only or ES+training. Rats in either trained group stepped bipedally on a body weight supported treadmill (5-16 cm/s) (5 days/week,

20 mins/day) for 8 weeks. By the end of the 8-week period rats in the ES+training group showed improvements not only in supported treadmill stepping ability but also in open field locomotion, which was not previously observed in the complete transection models. Therefore these results suggest that a combination of step training and epidural stimulation in an incomplete model of SCI successfully improved locomotor function further than either therapy administered alone.

Disclosures: Y.D. Al'Joboori: None. **R.M. Ichiyama:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: HIN NS069909-01

Dana Foundation

Title: Longitudinal assessment of spinal cord injury in monkeys by multi-parametric mri at 9.4t

Authors: F. WANG[1], A. MISHRA[1], Z. ZU[1], H. QI[2], C. TANG[1], J. GORE[1], *L. CHEN[1]; [1]Radiology, [2]Psychology, Vanderbilt Univ., Nashville, TN

Abstracts: This study aims to monitor quantitatively the spontaneous recovery of injured spinal cord (SC) using structural, cellular and molecular biomarkers obtained from serial multi-parametric MR images including MT (Magnetization Transfer), DTI (Diffusion Tensor Imaging) and CEST (Chemical Exchange Saturation Transfer) imaging. High-resolution MR images were acquired using a saddle-shaped surface transmit-receive coil in anesthetized squirrel monkeys at 9.4T. Three monkeys underwent surgery to introduce unilateral dorsal column lesions at C4-C5 level, and exhibited behavioral impairments on a food reaching and retrieval task within the first two-weeks after lesion. MT, DTI and CEST images were collected repeatedly at different time points for up to 24 weeks after the surgery. By comparing measures of ADC (Apparent Diffusion Coefficient), FA (Fractional Anisotropy), MTR (Magnetization Transfer Ratio), and MTRasym from CEST obtained before and after lesions, we observed dynamic changes in spinal cord evident from MT contrast images, in the number of tracked white matter bundles (by DTI tractography), water content and cell density (by ADC), integrity of white matter microstructure (by FA), and levels of macromolecules (e.g. proteins) by MTR and metabolites (e.g. amides,

amines or hydroxyls) by MTRasym of the SC tissue at and surrounding the injury site. We found that as early as two weeks after the lesion, a cyst formed rostral to the lesion site. By eight weeks post-lesion, the lesion cavity shrank while the cyst enlarged, exhibiting high water content and low cell density. The white matter surrounding the lesion and cyst was disrupted, with a reduced number of white matter tract bundles compared to the normal side. Concentrations of specific macromolecules and metabolites within the cyst peaked. By 24 weeks post-lesion, the lesion cavity continued to shrink, as did the cyst, which at this time showed increased cell density, sustained mobile protein levels, and decreased levels of amides, amines or hydroxyls. The number of tractable white matter bundles increased, indicating a recovery. Subsequent post-mortem histological evolution of the spinal cord tissue with GFAP and HE staining confirmed the nature of the cysts. In conclusion, our data demonstrate the power of multi-parametric MRI for the longitudinal and comprehensive assessment of the spontaneous recovery from SC injury, and highlight the potential of *in vivo* MRI for the evaluation of outcomes of therapeutic interventions in preclinical animal models.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Program#/Poster: 630.26/II5

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Eunice Kennedy Shriver National Institute Of Child Health & Human Development Grant T32 HD057845

Title: Spinal cord motor tract integrity, muscle architectural alterations, and volitional motor control following motor incomplete spinal cord injury

Authors: ***A. C. SMITH**[1], T. B. PARRISH[1], M. WASIELEWSKI[1], H. E. KIM[2], T. G. HORNBY[2], J. M. ELLIOTT[1];
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Abstracts: Background: In humans following motor incomplete spinal cord injury (iSCI), prognosis for motor recovery is a challenging yet important issue (Burns et al, 2012). Magnetic resonance imaging (MRI) is a valuable tool used to augment prediction of motor return after SCI,

but there exists a variety of pulse sequences reported to use for this purpose, each with differing levels of efficacy. A majority of studies utilized conventional sagittal T1 and T2 weighted imaging, with the American Spinal Cord Injury Association (ASIA) motor impairment scale as the motor outcome measure of choice, and report a range of correlation strengths (R values = 0.53-0.83) (Boldin et al, 2006, Lundell et al, 2011, Miyanji et al, 2007). Moving beyond standard clinical imaging, 1 study used both diffusion weighted and magnetization transfer MRI techniques to characterize white matter tract integrity in SCI, and found correlation with the ASIA motor scale (Cohen-Adad et al, 2011). The purpose of this ongoing study is to quantify and establish relationships between tract integrity, muscle alterations, and volitional motor control following human SCI. Methods: 1 subject with C5-7 iSCI completed the study so far (28 year old male, 2.5 years s/p injury). White matter integrity was quantified using a MRI magnetization transfer application (Cohen-Adad et al, 2011). Fat infiltration in the neck and lower extremity muscles was assessed using a MRI chemical shift application (Smith et al, 2014, Elliott et al, 2013). Volitional control was quantified using a central activation ratio (CAR) (Hornby et al, 2009), a value obtained by comparing maximum voluntary isometric plantarflexion torque production with a superimposed peak electrically elicited torque. Values were compared to previously collected control data. Results: The magnetization transfer ratio (MTR) was 40% of control value (38) at the level of the spinal lesion, indicating a disruption of myelin along the axonal projections of the cord. There was 63% greater fat infiltration in multifidus muscles (control = 19%), and 166% greater value in plantarflexor muscles (control = 6%), compared to controls. The central activation ratio was 25% of control (0.96), demonstrating decreased voluntary motor recruitment. Conclusions: Preliminary results from 1 subject with iSCI show feasibility for the quantification of integrity, muscle alterations, and volitional control in this population. As expected, MTR and CAR values were less than controls and corresponded to greater fat infiltration values in musculature. Next steps will be to collect data from additional subjects and establish relationships between these variables.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: The Craig H. Neilsen Foundation (#224125)

Title: Acute exercise after spinal cord injury modulates the regenerative profile of axotomized neurons

Authors: *R. SACHDEVA[1], A. KALINSKI[2], S. SAVANT[1], J. TWISS[2], J. HOULE[1]; [1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Dept. of Biol. Sci., Univ. of South Carolina, Columbia, SC

Abstracts: Peripheral nerve grafts (PNGs) support significant regeneration of central nervous system (CNS) axons after spinal cord injury (SCI) in rats, yet many more injured axons fail to regrow. CNS regeneration can be enhanced by a combination of treatment strategies, such as neural tissue transplantation, matrix modulation and use of exogenous neurotrophic factors. Work from our lab and others has utilized post-SCI exercise (Ex) to promote neuronal survival and regeneration after CNS trauma but the mechanism by which sensorimotor stimulation mediates this effect is poorly understood. We examined whether passive cycling exercise is associated with modulating the mRNA levels of growth associated molecules in CNS neurons post SCI. In addition to examining the overall changes in gene expression in neuronal soma, axonal compartments were analyzed for the presence of regeneration-associated mRNA transcripts and markers of protein synthetic machinery. All rats received a lower thoracic (T12) transection (Tx) and some received peripheral nerve grafts (PNG) to support the regeneration of ascending sensory axons. Retrograde tracing using True Blue (TB) was used to identify injured and regenerating neurons, in Tx only and Tx-Ex rats, respectively. Rats received exercise using motorized bikes for one or three weeks, starting five days after injury. Control rats were not exercised. Using fluorescent *in situ* hybridization (FISH) to identify mRNAs of growth-associated protein 43 (GAP43), β-actin and Neuritin/CPG15 in TB positive neurons, we show that acute exercise upregulates growth associated genes in axotomized neurons in spinal cord. Analysis of mRNA expression in neurons that are regenerating their axon is continuing. Confocal analysis of regenerating axons in PNGs using FISH/Immunofluorescence was utilized to examine axonal localization of these mRNAs and key molecules involved in protein synthesis [4EBP1, phosphorylated-4EBP1, phosphorylated ribosomal protein S6]. We show that in non-exercised animals CNS axons localize growth associated mRNAs and protein synthetic machinery as they regenerate within PNGs, suggesting the possibility of intra-axonal protein synthesis. Whether exercise is associated with alteration of axonal mRNA trafficking is currently under investigation. Our results show that acute exercise after spinal cord injury is associated with upregulation of growth promoting genes in injured neurons. Ongoing studies using combined analysis of axonal and somal compartments of injured and regenerating neurons will provide a better insight on the effect of exercise in modulating the overall regenerative profile of injured CNS neurons.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.28/II7

Topic: D.10. Spinal Cord Injury and Plasticity

Support: UK Medical Research Council

International Spinal Research Trust

Henry Smith Charity

International Foundation for Research in Paraplegia

Title: Chondroitinase gene therapy as a treatment for spinal cord injury

Authors: *N. D. JAMES[1], K. BARTUS[1], K. D. BOSCH[1], J. H. ROGERS[2], B. L. SCHNEIDER[3], J. VERHAAGEN[4], E. M. MUIR[2], E. J. BRADBURY[1];
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Abstracts: Spinal cord extracellular matrix is densely packed with growth inhibitory chondroitin sulphate proteoglycans (CSPGs), which become more abundant after injury. Thus, matrix modification has become a leading experimental strategy for promoting repair following spinal cord injury. Despite the beneficial effects that have been achieved by digesting CSPGs with the bacterial enzyme chondroitinase ABC (ChABC), the potential for achieving long term efficacy in traumatic injuries that mimic a human spinal cord injury has been limited, due to suboptimal delivery methods and issues of enzyme instability. However, we have recently demonstrated that gene therapy, using a mammalian compatible ChABC gene, offers a route to achieving stable and continuous delivery of ChABC, resulting in a dramatic reduction in pathology and significant improvements in functional recovery when used to treat a clinically relevant spinal contusion injury model in adult rats. Following on from these findings we now demonstrate the efficacy of chondroitinase gene therapy in contusion injury models at differing spinal levels (cervical and thoracic). When used to treat a contusion injury at either C5 or T10 spinal level, ChABC gene therapy resulted in increased spinal conduction through the injury epicenter, improved functional performance in skilled locomotion, significant neuroprotection and enhanced plasticity of intact spinal circuitry. Further to this, we present findings from recent experiments in which we have assessed the efficacy of different viral vectors (both adeno-

associated viral vectors and lentiviral vectors) containing the modified ChABC gene in order to determine the optimal vector structure to be used for ChABC gene therapy. We find that the use of different promoters results in differing patterns of ChABC expression, due to which cell types are transduced. Furthermore, this change in expression pattern affects the efficacy of ChABC gene therapy. The use of a PGK promoter primarily leads to transduction of neuronal cells, resulting in widespread CSPG degradation throughout the spinal cord and the most dramatic improvements in functional and anatomical outcome measures. Thus, we demonstrate the therapeutic potential of ChABC gene therapy to treat clinically relevant injury models at different spinal levels and present findings on optimizing the delivery mechanism for chondroitinase gene therapy.

Disclosures: **N.D. James:** None. **K. Bartus:** None. **K.D. Bosch:** None. **J.H. Rogers:** None. **B.L. Schneider:** None. **J. Verhaagen:** None. **E.M. Muir:** None. **E.J. Bradbury:** None.

Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 630.29/II8

Topic: D.10. Spinal Cord Injury and Plasticity

Support: MRC

Title: Investigating functional plasticity and synaptogenesis following experimental spinal cord injury and chondroitinase gene therapy

Authors: ***E. R. BURNSIDE**[1], K. D. BOSCH[1], F. GRILLO[1], S. B. MCMAHON[1], J. S. CARP[2], J. BURRONE[1], E. J. BRADBURY[1];
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Abstracts: The CNS has a poor intrinsic capacity for regeneration, although some functional recovery does occur. This is mainly in the form of sprouting, dendritic remodelling and changes in neuronal coding, firing and synaptic properties; elements collectively known as plasticity. Following spinal cord injury (SCI), an important approach to repair the injured CNS is therefore to harness, promote and refine plasticity. This is partly limited by some components of the extracellular matrix, key inhibitory molecules of which may be manipulated by therapeutic delivery of chondroitinase ABC. The corticospinal tract (CST) is an important descending motor

pathway involved in locomotion, posture and voluntary skilled movements. Therefore regeneration and anatomical reorganisation of this projection is often examined in studies of experimental SCI. Techniques such as anterograde tracing have been combined with immunolabeling for synaptic proteins or electron microscopy to elucidate connectivity; however whether active synaptogenesis occurs following SCI and potential therapeutic manipulations has not been studied. Genetically encoded reporters of presynaptic vesicular release represent novel tools to assess synaptogenesis and gain insight into the anatomical and functional status of new connections. Here we use an adeno-associated viral (AAV) vector encoding SynaptopHluorin (SpH), as a tool to measure functional synaptogenesis resulting from therapies which promote plasticity following experimental SCI. We utilise an ex-vivo acute cervical spinal slice preparation from adult rats in which we electrophysiologically stimulate the CST and real-time image fluorescence indicative of vesicular release. We are currently performing this ex-vivo preparation on rats which have undergone a unilateral pyramidotomy lesion and in which the intact contralateral CST is transduced by AAV-SpH in order to investigate the functional synaptogenesis resulting from plasticity of a known, spared population of fibres following injury. Furthermore we are investigating how this changes following therapeutic delivery of chondroitinase via gene therapy.

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Poster

630. Spinal Cord Injury: Repair, Training, and Rehabilitation I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Department of Veterans Affairs

Department of Defense

New York State Spinal Cord Injury Research Program

Title: Electro-magnetic stimulation over T2 spinal level combined with transgene delivery of neurotrophin NT-3 and exercise training improved synaptic transmission and locomotor function after contusion spinal cord injury in rats

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Abstracts: Locomotor training is widely used to facilitate recovery after spinal cord injury (SCI). Overall, the best results of training have been seen in young animals and children. This has been attributed to the higher level of plasticity in the spinal cord of young mammals. Our previous studies revealed that repetitive spinal electromagnetic stimulation over intact T2 vertebrae (sEMS) induces an LTP-like facilitation of synaptic responses in damaged spinal cord, thus promoting synaptic plasticity in spinal circuitry following chronic lateral hemisection SCI. In the current study we examined whether sEMS alone or combined with transgene delivery of neurotrophin NT-3 (AAV-NT3) will reinforce beneficial effects of exercise following contusion SCI. Adult rats received T10 150 kdyn contusion and were divided into five groups. 1) Control (SCI, no treatment), 2) sEMS only (over T2; 0.2Hz, 30min, 2.8Tesla), 3) Exercise (training in exercise ball and swimming) only, 4) Exercise plus sEMS, 5) Exercise combined with sEMS and AAV-NT3. Behavioral testing, multiple electrophysiological recordings (i.e. intracellular and extracellular from lumbar spinal cord and MEP from hindlimb muscles), immunocytochemistry analysis, and anatomical tracing were performed to assess the effects of treatments. We found that a single train of sEMS (30 min duration) induced transient facilitation of transmission in neuro-muscular circuitry in chronically contused rats. Facilitation sustained for at least 2 hours and amplitude of synaptic responses returned to the initial low levels after termination of stimulation. These physiological changes were associated with the increased immunoreactivity of GluR1 and GluR2/3 glutamate receptors in lumbar neurons. In contrast to a transient facilitation of synaptic responses evoked by a single train of sEMS, administration of sEMS every other day for 5 weeks, followed by exercise training, induced sustained and cumulative strengthening of transmission. These physiological improvements were associated with (i) improved anatomical plasticity (fluoro-ruby retrograde tracing) and (ii) improved locomotor function (BBB, challenging behavioral tests and CatWalk analysis). Chronic administration of sEMS alone or exercise training alone, however, did not induce significant changes of transmission or locomotor function. Importantly, improvements lasted for at least 4 weeks after chronic treatments were stopped. The best improvements were seen in the group that received exercise training in combination with sEMS and transgene delivery of NT-3 (AAV10-NT3). The potential of translating these results to clinical applications is discussed.

Disclosures: H.A. Petrosyan: None. **V. Alessi:** None. **S.A. Sisto:** None. **V.L. Arvanian:** None.

Poster

631. Afferent and Descending Control

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 631.01/II10

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ANR

Title: Computer-aided neurophysiology with applications to embryonic respiratory circuit development

Authors: ***J. A. HAYES**[1], E. PAPAGIAKOUMOU[2], P.-L. RUFFAULT[1], V. EMILIANI[2], G. FORTIN[1];
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Abstracts: The need for increased integration between imaging and physiological data has become increasingly important in experimental evaluation with advancing technological approaches in recent years. Many experimental protocols would benefit from rapid feedback on the quality and substance of data acquired at the physiological setup during, or immediately after, data acquisition. This is especially true in applications using optogenetic approaches to study activity within a population of neurons, when it is important to quickly know how light stimulation is affecting the system. To improve beyond the current state of conventional approaches, we have extended the open source program, ImageJ, to provide functionality that allows easier integration of imaging/electrophysiological data in tandem with the image-acquisition plug-in Micro-Manager. This offers a substantial improvement beyond the previous patchwork approach to analyses and interpretation using multiple tools, which is time-consuming and laborious, and often requires substantial time off-line to evaluate the quality of data. Furthermore, the software aids post-hoc high-throughput analysis of physiological data. The latter heretofore being a major challenge for the neurophysiological discipline despite being greatly advanced in other areas of neuroscience. We have utilized this software to begin investigating the intercellular interactions of embryonic respiratory circuits using holographic laser stimulation patterns on reduced subsets of a population of neurons.

Disclosures: J.A. Hayes: None. **P. Ruffault:** None. **G. Fortin:** None. **E. Papagiakoumou:** None. **V. Emiliani:** None.

Poster

631. Afferent and Descending Control

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish Medical Research Council

ERC advanced Grant

Ragnar Söderberg's foundation

Title: Spinal excitatory circuits as hub for the descending control of hindlimb motor activity

Authors: *A. E. TALPALAR, O. KIEHN;
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Abstracts: Locomotion is normally initiated by descending commands originating in the brainstem. In various vertebrate species, excitatory interneurons in the spinal cord are necessary for generating the locomotor-rhythm. It remains unknown, however, if excitatory descending commands are acting directly through spinal excitatory circuits for generating motor tasks, or if the excitatory descending systems are able to induce movements without the contribution of excitatory spinal neurons. We experimentally tested these ideas using the *in vitro* lumbar spinal cord of Vglut2-KO mice are that devoid of excitatory neurotransmission. Locomotor-like activity (LLA) - mediated by reciprocally connected inhibitory Ia-interneurons (Ia-IN) - can be evoked in spinal cords from these mice by neuroactive drugs (Talpalar et al. Neuron 2011). When Vglut2 is selectively eliminated from the spinal cord by crossing Hoxb8::Cre with Vglut2lox/lox mice, resulting crosses breath and elicit spontaneous motor activities in the forelimbs but not in the hindlimbs (n=11). Electrophysiological studies showed that the isolated lumbar spinal cord from Hoxb8::Cre; Vglut2-KO mice exposed to neuroactive drugs was able to elicit LLA with similar characteristics as the full Vglut2-KO mice (n=5). DC recordings from the ventral roots showed that motor neurons received descending excitatory inputs that were able to generate action potentials (n=5). However, stimulation of descending pathways (and afferents) with protocols that elicit LLA in wild type mice were inefficient to evoke LLA in Hoxb8::cre; Vglut2-KO mice (n=5). Stimulation of putative vestibulospinal tract in Hoxb8::cre; Vglut2-KO was able to activate motor neurons directly, to depress Ia-IN - mediated synaptic potentials in motor neurons, and to inhibit the ongoing rhythmic activity elicited by drugs in the lumbar spinal cord. These results indicate that Hoxb8::cre; Vglut2-KO mice display normal glutamatergic descending brainstem systems and that in normal animals descending excitatory commands evoke locomotion through activation of excitatory spinal neurons. Our study also shows that spinal intrinsic inhibitory pattern-generating networks can be directly inhibited by descending excitatory commands thereby short-circuiting the on-going rhythmic activity. Supported by the Swedish Medical Research Council , ERC advanced and Ragnar Söderberg's foundation.

Disclosures: **A.E. Talpalar:** None. **O. Kiehn:** None.

Poster

631. Afferent and Descending Control

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ONR N00014-0910352

NSF IGERT 0903637

NIH R90 DA023425

Title: The role of proprioception in stability control during aquatic station-holding

Authors: *M. E. HALE, R. WILLIAMS, IV;
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Abstracts: The limbs of vertebrates play central roles in postural stability. Fine-tuned movements adjust to subtle changes to the external or internal environment of the body. Proprioceptive feedback from the limbs is fundamental to an organism's sense of body position relative to its environment. Here we address the role of limb-based proprioceptive feedback for body stabilization in the aquatic environment. Our work focuses on the bluegill sunfish (Lepomis macrochirus), a model system for understanding the biomechanics of swimming and for bio-inspired design of engineered swimming devices. The bluegill beats its pectoral fins rhythmically, and in coordination with pelvic and median fin movement to maintain a stationary position while hovering. We have previously shown that pectoral fins are proprioceptive as well as propulsive structures. To determine how pectoral fin proprioceptive feedback is used in bluegill fin-based hovering, we performed a series of behavioral experiments in which we transected the sensory nerves innervating the pectoral fins and examined the effect on the hovering behavior of the bluegill. We hypothesized that the rhythmic movement of the pectoral fin would be maintained despite the absence of proprioceptive feedback, as these movements are believed to be driven by a central pattern generator (CPG). Two control groups were included in the experiments, a non-operated condition and sham-operated condition in which the full surgery was performed except for nerve transection. Full transection experimental conditions involved a transection of all sensory nerves innervating the fin rays either unilaterally or bilaterally. We found that transections of all the pectoral fin sensory nerves innervating a fin caused a decrease in overall fin displacement over the course of a fin beat cycle during hovering. Bilateral transections of pectoral fin sensory nerves resulted in a general increase in fin beat frequency. Our data also show that the amplitude of median fin movement increases as a consequence of

pectoral fin sensory nerve transection. Results of this study show that the complete absence of proprioceptive feedback alters movement patterns but does not abolish rhythmic movement altogether. These findings indicate a contribution of proprioceptive feedback to the rhythmic pectoral fin behaviors involved in maintaining a stable hovering position.

Disclosures: M.E. Hale: None. **R. Williams:** None.

Poster

631. Afferent and Descending Control

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF RCN-DBI 1062052

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Title: The stability of the locomotor rhythm in the lamprey central pattern generator

Authors: N. MASSARELLI[1], A. YAU[2], K. A. HOFFMAN[1], T. KIEMEL[3], *E. D. TYTELL[2]; [1]Dept. Math. Stat., Univ. Maryland, Baltimore County, Baltimore, MD; [2]Dept. of Biol., Tufts Univ., Medford, MA; [3]Dept. Kinesiology, Univ. Maryland, Col. Park, College Park, MD

Abstracts: The central pattern generator (CPG) circuit is a conserved neural circuit, present in the spinal cord of all vertebrates, that can generate the basic muscle activation pattern for locomotion and can respond to proprioceptive sensory information. For example, regular rhythmic stimuli are well known to entrain the CPG rhythm, causing the circuit to burst at the same frequency as the stimulus. Pulsatile stimuli can also advance or delay the cycle. It is not well understood, however, how the CPG might respond to more natural stimuli and perturbations, which contain both a sinusoidal component and an irregular perturbation. Moreover, the CPG functions as part of a feedback loop: a change in sensory input might cause it to change its output, but the change output affects muscle activity, which may change the sensory inputs. We have developed a new theoretical framework based on harmonic transfer functions (HTFs) for understanding how the CPG responds perturbations as a function of the

phase of the cycle, and what effect those responses might have as part of a feedback loop. We conducted experiments on the lamprey spinal cord. The cord was bent back-and-forth to entrain the CPG's rhythm, and then a Gaussian band-limited white-noise perturbation was added to the sensory stimulus. We computed the HTF, which describes how a sensory perturbation at given frequency produces a change in the CPG's output at multiple frequencies. The HTF characterizes the open-loop response of the CPG to bending and allows us to predict stability and the effect of perturbations on the closed-loop system. We also developed mathematical models of the CPG circuit, including phase models, other simple oscillator models, and more complex neural circuit models. By using the same stimuli in the mathematical models, we can examine which features of the CPG circuit are necessary for the results we observe.

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Poster

631. Afferent and Descending Control

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Vaugnie Lindsey New Investigator Grant - SIUe

Title: Masticatory and brux-like motor patterns in the freely behaving rat:Electromyography and phase analysis

Authors: *D. B. WELCH[1], J. TAYLOR[2], J. WALL[2], P. WANDA[2];
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Abstracts: In humans, bruxism has been classically defined as an involuntary rhythmic or spasmodic non-functional gnashing and grinding of the teeth. Unfortunately, the multifactorial etiology of bruxism prevents a full understanding of its control. Clinical cases suggest that normal suppression of brux-like movements might become deregulated during certain pathological conditions, anxiety/stress, and/or by commonly prescribed medications. However, anxiety/stress-induced brux-like behavior seems to be functionally important in rats, as they have to wear down their continuously growing incisors. The central pattern generating (CPG) circuits in the brain stem producing rhythmic brux-like movements might be shared with those that

produce normal masticatory movements. Fine-wire electromyographic (EMG) recordings from the temporalis, masseter, and digastric muscles of freely behaving laboratory rats (Rattus norvegicus), were collected during masticatory and brux-like behaviors in order to characterize the task-related motor patterns (n=21). We found a significant difference in the cycle periods of each of the respective muscles between rhythmic brux-like and masticatory episodes ($p<0.05$, Student's t-tests). Referent phase analysis was used to measure the level of coordination between the same subset of jaw opening and closing muscles. Overall, the mean phase relationships are significantly different between masticatory and brux-like motor patterns ($p<0.001$, Watson Williams F-Test). Our results suggest that this experimental platform can be used to examine the brainstem commands, and trigeminal neural networks that underlie the activation and switching of masticatory and brux-like motor patterns of the jaw.

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Poster

631. Afferent and Descending Control

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ERC

Söderberg's Foundation

EMBO

Title: Genetics, connectivity and function of brainstem inputs to spinal lumbar interneurons

Authors: *J. BOUVIER, C. BELLARDITA, A. MUNOZ-MANCHADO, Y. XUAN, K. MELETIS, J. HJERLING-LEFFLER, O. KIEHN;
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Abstracts: In mammals, locomotion is a crucial motor behavior that requires a precisely timed and coordinated activation of a large number of muscles in a recurrent manner. The neuronal elements sufficient to produce and pattern the rhythm are contained within neuronal networks of the spinal cord termed a Central Pattern Generators (CPG). The activation of the CPGs relies on supra-spinal structures that send locomotor commands and/or modulating signals to the spinal cord. Little is known about the precise nature of the locomotor descending drives and their

integration within the CPG. Yet, this question is of upmost importance to provide a unified understanding of the locomotor network with promising benefits for neurorehabilitation and repair strategies for motor defects and after spinal cord injury. Here we are addressing the organization of the descending motor command system with a particular focus on reticulospinal neurons, cellular contingents of the reticular formations of the medulla oblongata and the pons that send direct projections to the spinal cord. Using retrograde and mono-transsynaptic restricted labelling approaches from molecularly-identified neuronal populations in the lumbar spinal, we show that both excitatory (Vglut2-expressing) and inhibitory (VGAT-expressing) spinal interneurons receive direct inputs from overlapping medullary and pontine brainstem areas. We further demonstrate that descending neurons located in the ventral medullary reticular formation express the transcription factor Chx10, a signature of V2a neuronal subtype. In contrast, serotoninergic neurons of the raphe nucleus and glutamatergic contingents of the rostral medulla and the caudal pons are non-V2a but express the transcription factor Shox2. Using this segregation of molecular markers within distinct reticulospinal populations, we are investigating their role in locomotor control with targeted optogenetic activations on brainstem-spinal cord preparations *in vitro*. Altogether, these data provide the genetic first identification of brainstem populations standing immediately upstream the spinal cord interneuronal networks that controls hindlimb movements, and paves the way of functional studies aiming at bridging cellular fates to function in coordinating hindlimb mouvements. This work is supported by Söderberg's Foundation and ERC grant to OK.

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Poster

631. Afferent and Descending Control

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Support: Christopher and Dana Reeve Foundation

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Nencki Institute

CIHR (FRN 115147)

Title: Theta frequency oscillations predominate in the mesencephalic locomotor region during voluntary treadmill locomotion

Authors: *B. R. NOGA[1], F. J. SANCHEZ[1], C. O'TOOLE[1], L. VILLAMIL[1], S. STASIENKO[2], S. KASICKI[2], U. SLAWINSKA[2], L. M. JORDAN[3];
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Abstracts: Oscillatory rhythms in local field potentials (LFPs) are thought to coherently bind cooperating neuronal ensembles to produce behaviours, including locomotion. A variety of frequencies occur at LFPs recorded during locomotion, and have been used as a basis for identification of appropriate targets for deep brain stimulation (DBS) to enhance locomotor recovery in patients with gait disorders. The pedunculopontine nucleus (PPN) has been targeted to improve locomotor activity in Parkinson's disease (PD), for example, and the presence of gamma band activity (20-100 Hz) has been a basis for identification of this part of the presumed mesencephalic locomotor region (MLR). It is also evident that theta band activity (6-12 Hz) in the hippocampus as well as in the locomotor nuclei of the hypothalamus is associated with locomotor activity. There is sparse information available, however, about the LFPs that occur in the MLR during locomotion, the primary targets for DBS to improve locomotion in PD and after spinal cord injury. Here we use electrodes implanted in the MLR of rats to induce locomotion, and then to monitor the oscillatory activity that is associated with spontaneous locomotion. Thresholds for inducing locomotion were determined in awake rats sitting motionless in an open field. LFPs were then recorded from the same electrodes during treadmill locomotion. Here we show that the predominant oscillatory rhythms recorded from the most effective MLR stimulus sites (thresholds: 15-40 μA) are within the theta range of frequencies (n=5). Theta activity was minimal at rest, but it appeared at the onset of locomotion, and the amplitude was correlated with the speed of locomotion. In animals (n=5) with higher thresholds (70-300 μA), the correlations between locomotor speed and theta LFP oscillations were less robust. Changes in the gamma band were rather small and inconsistent. These results support the suggestion that the oscillatory rhythm responsible for the selection of locomotor output through the MLR is within the theta range of frequencies, with other frequencies, including the gamma band, being less important. These results indicate that theta and not gamma oscillations are suitable characteristics for identifying the functional MLR for DBS.

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Poster

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Program#/Poster: 631.08/II17

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: University of Leeds

Title: Modulation of monosynaptic reflexes during development of spinal motor systems

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Abstracts: In the rat, the maturation of locomotion is relatively rapid, with near mature gait patterns observed by 21 days postnatally (PN 21). At PN14-16 there is an almost instant transition from a slow clumsy gait pattern to a more swift and accurate pattern, suggesting key changes during this period that leads to maturation of motor patterns. The monosynaptic reflex arc (MSR) provides a relatively simple, easily accessible circuit, providing insight into these developmental changes. This pathway, which is the fundamental unit of the central pattern generator (CPG), is established prenatally but there is evidence of postnatal adaptation and refinement. In order to track developmental changes in the MSR arc, the present study used an artificially perfused whole rat preparation to elicit MSRs in rats aged PN7, 10, 14 and 21. Rats were decerebrated under anaesthesia and then perfused with a modified, oxygenated ringer's solution containing 1.25% ficcol with the temperature maintained at 32°C. The tibial nerve was isolated and stimulated using a bipolar hook electrode and both EMG and ENGs were recorded from the gastrocnemius-soleus (Gs) muscle and its nerve branch at each age. Results show that this preparation is viable at all ages and therefore suitable for studying the development spinal networks responsible for movement control. MSRs could be elicited in animals of all ages; however at PN7-10 EMG responses could only be evoked at intensities of 6-8 times greater than threshold for ENG responses. The amplitude of monosynaptic responses was found to increase with age. Additionally, the excitability of the MSR was assessed using homonymous paired pulse stimuli ranging from 1-700ms administered to the tibial nerve. At PN14-21, decreasing inter stimulus intervals (ISIs) produced a depression at shorter intervals (<50ms), a response which is typical of mature animals. Importantly however, depression was still not at the level expected of a mature animal. In comparison, younger animals were relatively resistant to short

interval depression. We show that the decerebrate, artificially perfused whole rat allows the physiologically relevant study of the development of spinal networks throughout the post natal period. The results suggest that PN14 is indeed an important time point for spinal network development as is suggested by behavioral observations at this age. Furthermore, functional maturity observed in the open field at PN21 was not corroborated by mature circuit function in this study, suggesting additional subsequent post natal refinement.

Disclosures: C.C. Smith: None. **S. Chakrabarty:** None. **J.F.R. Paton:** None. **R.M. Ichiyama:** None.

Poster

631. Afferent and Descending Control

Location: Halls A-C

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Program#/Poster: 631.09/II18

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R01 NS-048844

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Title: Removal of ankle extensors group Ia and Ib afferent feedback differentially affects walking mechanics and muscle activity in the cat: A computer simulation study

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Abstracts: It is well established that proprioceptive feedback modulates muscle activity during locomotion. However, relative contribution of length-dependent (Ia) and force-dependent (Ib) muscle afferents to control of locomotor activity is difficult to establish experimentally due to similar activation thresholds of these afferents and simultaneous increases in muscle length and force during muscle stretch. The goal of this study was to determine the relative contribution of Ia and Ib afferents of triceps surae to control of locomotion using computer simulations. We used a comprehensive neuromechanical model of cat locomotion (Markin et al. 2012) that included a

model of spinal circuits with the locomotor central pattern generator (CPG; Rybak et al. 2006) controlled by afferent signals from 18 hindlimb muscles modeled in the Hill-type style (Prilutsky et al. 2014). The spinal network incorporated basic circuits involved in the monosynaptic stretch reflex, Ia reciprocal inhibition, recurrent inhibition via Renshaw cells, and disynaptic excitation of extensor muscles via Ia and Ib afferents. Three groups of model parameters were tuned independently: (1) CPG parameters that allowed us to reproduce the effects of deletions and afferent stimulations observed during fictive locomotion in the cat (Rybak et al. 2006); (2) parameters of the musculoskeletal model of the hindlimbs that allowed reproduction of the recorded changes in joint angles, joint torques, and ground reaction forces during cat locomotion (Prilutsky et al. 2014); (3) parameters that defined weights of sensory feedback to the spinal circuits and allowed reproduction of realistic patterns of muscle activity during locomotion The combined neuromechanical model is able to qualitatively reproduce the activity patterns of Ia, Ib and II muscle afferents as well as the activities of paw pad cutaneous afferents described in the literature. To investigate the specific contribution of Ia and Ib afferents of triceps surae to locomotor activity, the gain of each afferent pathway was reduced to zero. Selective removal of Ia feedback did not substantially change the activity patterns of this and other muscles and the model maintained stable locomotion. When the Ib afferent feedback from triceps surae was reduced to zero, the model was unable to produce stable locomotion and failed. Our analysis has shown that Ib afferents from triceps surae are critical for providing stable locomotion due to their specific inputs affecting spinal circuits and locomotor CPG.

Disclosures: S.N. Markin: None. **N.A. Shevtsova:** None. **I.A. Rybak:** None. **A.N. Klishko:** None. **B.I. Prilutsky:** None. **M.A. Lemay:** None.

Poster

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Support: IDeA CTR support – NIH/NIGMS Award Number U54GM104942

Title: Do humans use limb velocity signal to control locomotion?

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Abstracts: Vertebrates have the innate ability to precisely control locomotion to meet ever-changing demands of the environment. The neural pathways controlling locomotion are hierarchical, highly integrated, and well-characterized. The control of locomotion is achieved with feedforward and feedback signals converging on the rhythmogenic spinal networks responsible for regulation of timing and magnitude of muscle activity. We have previously developed accurate models of these control elements (Yakovenko et al. 2004; Prochazka & Yakovenko, 2008); moreover, we have recently proposed that the essential signal driving spinal networks is expressed in the modality of desired velocity (Yakovenko 2011). If the modality of locomotor control signal is indeed velocity then, according to the classical control theory, limb velocity should also be accurately sensed. We tested this consequent hypothesis by probing human ability to detect minute changes in the velocity of each leg. Healthy volunteers with no previous history of neurological conditions or serious musculoskeletal damage to the lower extremities were recruited to walk on a split-belt treadmill with separately controlled belt speeds. Subjects were exposed to randomized asymmetric speeds of left and right legs for approximately 4 steps. A high-pitch cue instructed subjects to report the fastest leg. In addition, we tested velocity discrimination skills in two other conditions when subjects were either supported or loaded by 10% of their body weight. The perception threshold defined as the velocity detected with better than chance probability (above 50%) was 0.02±0.02 m/s or Weber's fraction (dV/V) of 2%, which is equivalent to load detection in muscles. The accurate velocity discrimination ability supports the idea that the velocity signal is represented within the locomotor control pathways.

Disclosures: K. Galbreath: None. **E. Olesh:** None. **S. Yakovenko:** None.

Poster

631. Afferent and Descending Control

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: KAKENHI 24500612

Title: Reciprocal functional interactions between the respiration/circulation center, the upper spinal cord, and the trigeminal system

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Abstracts: Decerebrate and arterially perfused *in situ* rat preparations and electrophysiological techniques were used to investigate: (i) whether fictive locomotion in the forelimbs can be autonomously generated by a certain sympathetic tone resulting from an increase in perfusion flow rate. (ii) Whether rhythm coupling of locomotion and respiration/circulation can be produced during locomotion. (iii) What rhythm coupling of respiration and opening movements of the mandible can be produced during locomotion. (iv) The central neural mechanisms of the entrainment of respiratory and locomotor rhythms. The results indicate that: (i) modulated sympathetic tone generates fictive locomotion in the forelimbs. (ii) The activated spinal cord, producing a left/right alternating discharge, affects the respiration/circulation center and the trigeminal system via ascending pathways and increases systemic pressure and respiratory rhythm during locomotion. (iii) Simultaneously, it generates opening movement in the mandible in both the inspiratory and expiratory phases, generated by increasing peripheral and central chemoreceptor discharges. Respiration during locomotion is autonomously performed, not only through nasal breathing but also mouth breathing, so improving ventilation. The modulated sympathetic tone triggers the forelimb pattern generator via descending pathways and generates fictive locomotion in the forelimbs and the locomotor rhythm in the cervical cord, while a left/right alternating activity occurs in the forelimbs, which entrains via ascending pathways both the respiratory rhythm and the rhythm of the opening movements in the mandible. (iv) The central mechanisms of the entrainments of respiratory and locomotor rhythms are spinal feedback mechanisms. Supported by KAKENHI (24500612).

Disclosures: I. Yazawa: None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Primary somatosensory cortical area inducing jaw-opening in the rat

Authors: K. UCHINO, K. HIGASHIYAMA, R. TAKEDA, *F. SATO, A. YOSHIDA;
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Abstracts: Our previous study has shown that the dorsal part of the juxtatrigeminal region (dorVjuxt) and the dorsal part of the trigeminal oral subnucleus (dorVo) contain premotoneurons to the trigeminal jaw-closing (JC) and jaw-opening (JO) motoneurons, and receive direct

projections from the rostral part of the primary somatosensory cortex (S1). Short-train stimulation of the S1 is known to induce EMG activities or twitches of JO muscles with a JO in the rat. However it is unclear in the rat whether long-train stimulation of the S1 can induce jaw-movements or EMG activities or twitches of JO or JC muscles, and whether the distribution of effective long-train stimulation sites corresponds to that of effective short-train stimulation sites and also corresponds to that of S1 neurons projecting directly to the dorVjuxt or dorVo. Therefore we examined these issues by means of intracortical microstimulation techniques in rats anaesthetized with a combination of ketamine hydrochloride and xylazine hydrochloride. We intracortically stimulated the rostral S1 through a monopolar, glass-insulated Elgiloy electrode with low-frequency long-train stimuli (450 pulses of 0.5 msec duration and 30 Hz, 20 to 80 μA) and high-frequency short-train stimuli (three pulses of 0.1 msec duration and 500 Hz, 30 to 80 μA). Long-train stimulation of the rostroventral part of S1 (rvS1) induced a sustained JO and EMG activities of bilateral anterior digastric muscles (Dig); most effective sites were located in the rostralmost region of the rvS1. Short-train stimulation of the rvS1 induced EMG activities of the bilateral Dig and a twitch-like JO; the most effective sites were located in the rostralmost region of the rvS1. These effective sites and most effective site were very similar in long-train stimulation cases and in short-train stimulation cases. Importantly, these effective sites were well confined in the S1 which has projections to the dorVjuxt or dorVo, and most of them were in the S1 which has projections to both the dorVjuxt and dorVo. The present findings suggest that not only short-train stimulation but also long-train stimulation of the rvS1 induces a JO by activation of the Dig, and that the activation of the Dig is intimately involved in the direct pathways from the rvS1 to the dorVjuxt or dorVo.

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Poster

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Program#/Poster: 631.13/II22

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR

University of Calgary

Title: A11 neurons in the mouse project to the spinal cord, are dopaminergic and lack expression of dopamine transporter

Authors: *K. KOBLINGER[1], T. FÜZESI[2], J. E. EJDRYGIEWICZ[2], A. KRAJACIC[2], L. M. YOUNG[2], J. S. BAINS[2], P. J. WHELAN[2];
[2]Hotchkiss Brain Institute, Univ. of Calgary, [1]Univ. of Calgary, Calgary, AB, Canada

Abstracts: The A11 cell group has been suggested to be the primary source of dopaminergic projections to the spinal cord. However, it remains an understudied dopaminergic nucleus within the brain. The A11 region has been identified in several species including rats, mice, cats, monkeys, zebrafish, and humans where it may contribute to the control of pain, spinal locomotor network modulation, restless leg syndrome, and cataplexy. Given the importance of mouse dopaminergic models it is important to fully understand the phenotype of A11 cells. Our goal with this work was to identify and characterize the A11 in the adult mouse and examine whether A11 neurons contain the full complement of enzymes to produce and release dopamine. First we confirmed that A11 cells directly project to the spinal cord using tracing methods. Next we were able to show that neurons within the A11 of the mouse contain tyrosine hydroxylase as well as aromatic L-amino acid decarboxylase (AADC), the enzyme that converts L-DOPA to dopamine. Furthermore, we show that the A11 neurons contain vesicular monoamine transporter 2 (VMAT2), the vesicular transporter necessary for packaging DA into vessels. On the contrary A11 neurons in the mouse lack the dopamine transporter (DAT). In conclusion, our data suggest that A11 neurons in the mouse are dopaminergic and the lack of DAT could lead to prolonged DA actions within the spinal cord.

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Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant F32-DC011249

NSF Grant BCS-0926196

NIH Grant R01-DC010145

Title: Sensorimotor adaptation in speech and its effects on auditory monitoring

Authors: *C. A. NIZIOLEK, S. S. NAGARAJAN, J. F. HOUDE;
Univ. of California San Francisco, San Francisco, CA

Abstracts: How does speech motor learning influence speech error monitoring? During speech, talkers monitor their auditory feedback, adjusting their speech output to counteract deviations from what they intend to say. Evidence for this monitoring and adjustment comes primarily from two types of experimental paradigms: the first, often described as sensorimotor adaptation, probes *short-term speech learning* by applying consistent manipulations to auditory feedback, causing a temporarily remapping of articulatory commands that persists after feedback is returned to normal ("adaptation"). The second, described as feedback alteration, probes *rapid error correction* by employing sudden changes to auditory feedback that are sparse and random, such that they cannot be learned. In two complementary experiments, we used real-time auditory feedback alteration to examine how these two mechanisms, adaptation and error correction, interact. In each experiment, subjects produced 800 repetitions of the word "Ed". Their speech signal was recorded and delivered to insert headphones (< 12 ms delay), serving as a primary source of air-conducted auditory feedback. Unbeknownst to the subjects, the first and second formants (F1 and F2) of this feedback were altered, causing the vowel to sound different from what was intended. Experiment 1 employed a feedback alteration design in which a pseudorandom 25% of utterances were altered to sound like "id" and a pseudorandom 25% were altered to sound like "add." Experiment 2 employed a sensorimotor adaptation design in which feedback was gradually and consistently shifted toward "id" over the course of the experiment, inducing a short-term remapping of motor commands in the opposite direction, towards "add"; once this steady state adaptation was reached, a pseudorandom 25% of utterances were altered even further towards "id", while a pseudorandom 25% had the alteration removed. Preliminary evidence suggests that overall compensatory changes in output in Experiment 1 were no different than those in Experiment 2, despite the large differences in magnitude of absolute discrepancy between produced and heard formants. That is, responses to an unexpected vowel shift in the context of normal feedback were no larger than responses to a return to normal feedback in the context of a constant, learned vowel shift. Neural data recording using magnetoencephalography (MEG) is ongoing. These results inform models of sensorimotor adaptation in which the learned remapping of vowel articulatory movements become integrated into the auditory target being monitored under conditions of unexpected feedback alteration.

Disclosures: C.A. Niziolek: None. **S.S. Nagarajan:** None. **J.F. Houde:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NS21135

NS40596

F31NS065656

Title: Redefining the role of Broca's area in speech production

Authors: *A. FLINKER[1], A. KORZENIEWSKA[2], A. SHESTYUK[3], R. T. KNIGHT[3,4], N. E. CRONE[2];
[1]Psychology, New York Univ., New York, NY; [2]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Helen Wills Neurosci. Inst., [4]Psychology, Univ. of California at Berkeley, Berkeley, CA

Abstracts: The neural organization subserving the production of speech has been debated for over a century. While Broca's area is considered critical for orchestrating articulation, it remains unclear during which stage of speech production it is recruited as well as what type of neural representations it coordinates. We used direct cortical recordings in neurosurgical patients together with novel effective connectivity measures to elucidate the temporal evolution of neural activity in Broca's area and its causal influence on peri-sylvian cortex during speech production. Seven patients with electrode implantations over peri-sylvian language regions, including Broca's area, consented and participated in the study during lulls in clinical management. Subjects participated in a battery of overt articulation tasks including word repetition and word reading. During auditory word repetition, cortical activation exhibited a systematic temporal propagation of high frequency power (high gamma: 70-150 Hz) from auditory cortices to Broca's area and culminating in motor cortices. Critically, by the time of articulation, processing in Broca's area was complete. Using measures based on granger causality we found that Broca's area communicates with temporal cortex during lexical access and then transforms cortical activity into an articulatory plan that is forwarded to motor cortex prior to articulation. Furthermore, production of pronounceable pseudowords significantly increased the load on Broca's area compared with real words controlled for sublexical properties (phonotactic

neighborhood density, transitional probability, positional probability). These unique electrophysiological data provide direct evidence that Broca's area coordinates the transformation of information into an articulatory code across large-scale cortical networks involved in speech production. In this role, Broca's area prepares motor cortex for articulation but is not engaged during the actual act of speaking. Supported by NINDS Grants NS21135, NS40596, and F31NS065656

Disclosures: **A. Flinker:** None. **A. Korzeniewska:** None. **A. Shestyuk:** None. **R.T. Knight:** None. **N.E. Crone:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ROI-DC010145

Title: Investigating the role of the cerebellum in sensory processing during vocal behavior

Authors: ***Z. K. AGNEW**[1,2], J. GILL[2], S. NAGARAJAN[2], R. IVRY[3], J. F. HOUDE[2];
[1]UCL Inst. of Cognitive Neurosci., London, United Kingdom; [2]Otolaryngology, Head and Neck Surgery, UCSF Med. Sch., San Francisco, CA; [3]UC Berkeley, Berkeley, CA

Abstracts: Background: It has been proposed that the cerebellum serves to generate predictions about the sensory consequences of future movements. Complete or over reliance on sensory feedback results in unstable movements. Patients with cerebellar ataxia are known for their deficits in visually guided movement. Tellingly, their movements are known to improve in the absence of visual feedback. Thus it is suggested that patients with damage to the cerebellum are less able to make accurate predictions about the sensory consequences of movements and have to rely on reafferent information which ultimately leads to unstable movements. Whilst the majority of this work has been done in relation to visually guided movements of the upper limbs, speech and voicing are both strongly affected in cerebellar ataxia yet relatively little is known about the nature of vocal behaviour in this patient population. The present study aimed to investigate the nature of auditory feedback processing in patients with cerebellar degeneration by measuring various aspects of vocal behaviour. Methods: Patients were tested on a battery of vocal assessments designed to probe different aspects of vocalisation: we investigated ability to

produce spontaneous voicing, pitch tracking of a moving pitch target and pitch perturbation. Results: We confirmed previous findings that patients with cerebellar damage display increased variability in spontaneous pitch resembling vocal tremor. We then investigated the hypothesis that reducing auditory feedback during vocalisation would improve vocal stability. We report that under auditory masking conditions, variability in vocal pitch is significantly reduced. In order to investigate this idea further, a third experiment was carried out where we investigated how patients responded to perturbations in pitch production whereby auditory feedback is pitch shifted during vocalisation. As predicted, patients with cerebellar damage displayed significantly altered responses to the pitch shift compared to healthy age matched controls indicating an alteration in the way reafferent information is utilised. Conclusions: Together, these three experiments provide compelling evidence in favour of the idea of the cerebellum as a prediction system, the dysfunction of which leads to over reliance on sensory feedback and hence unstable auditorily guided vocal movements. These data will be discussed in relation to the function of the cerebellum in the neural control of vocal behaviour and current models of speech production.

Disclosures: Z.K. Agnew: None. **J. Gill:** None. **S. Nagarajan:** None. **J.F. Houde:** None. **R. Ivry:** None.

Poster

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Program#/Poster: 631.17/II26

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Jaw muscle activity profile during individual chewing cycles in people lacking periodontal mechanoreceptors

Authors: *M. G. TRULSSON, A. GRIGORIADIS;
Karolinska Inst., Huddinge, Sweden

Abstracts: Periodontal mechanoreceptors, located around the roots of the teeth, signal detailed information about the contact state between food and dentition during chewing. An earlier study examining the time-varying activation of the masseter muscle during natural chewing in young adults found that the increase in the excitatory drive of the masseter muscle was biphasic, showing an early component before tooth-food contact and a late component during tooth-food contact. We hypothesize that sensory signals from periodontal mechanoreceptors are required for the formation of the late increase in the excitatory drive of the masseter muscle during tooth-food

contact. Thirteen participants with implant-supported bridges in both jaws, thus lacking periodontal mechanoreceptors, and 13 with natural dentition chewed and swallowed model food of different hardness. Electromyographic (EMG) activity of the masseter muscle was recorded together with the position of the mandible. The muscle activity and the jaw kinematics were analyzed for different phases of the chewing cycles. Throughout the masticatory sequence, the increase in the excitatory drive of the masseter muscle during jaw closing was biphasic for the dentate participants, whereas for the implant participants a biphasic elevation was only observed during the middle and last segments. Dentate participants showed a significantly stronger boosting of the EMG activity during late jaw closing compared to the implant participants, irrespective of food hardness and segment of the masticatory sequence. On the contrary, the elevation of muscle activity early during jaw closing was similar between groups throughout the masticatory sequence. For both groups of participants, the adaptation of mastication to food hardness took place by proportional scaling of EMG amplitude without noticeably affecting the temporal structure of the muscle's activation profile. In conclusion, sensory information from periodontal mechanoreceptors is necessary for boosting the increase of the masseter muscle activity during tooth-food contact. However, this information is not needed for regulation of muscle activity before tooth-food contact. Furthermore, periodontal mechanoreceptors are not required for magnitude scaling of EMG activity to food hardness.

Disclosures: **M.G. Trulsson:** None. **A. Grigoriadis:** None.

Poster

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Strategic Research Program in Neuroscience at the Karolinska Institute

Västerbotten County Council TUA 7000664

Title: Task dependent control of the jaw during food splitting

Authors: A. S. JOHANSSON[1], *K.-G. WESTBERG[2,3], B. B. EDIN[1];
[1]Integrativ medical biology, [2]Univ. Umea, Umea, Sweden; [3]Physiol., Integrative Med. Biol., Umea, Sweden

Abstracts: Splitting food items between the incisors often requires high bite forces but rarely do the teeth harmfully collide when the jaw quickly closes after food split. The prompt dissipation of the jaw closing force required to prevent harmful collisions has in previous studies been explained by the force-velocity relationship of the jaw closing muscles. If this mechanism was the only one, biting through heterogeneous materials could not be made in a smooth manner which is in contrast to every day experience. We present results from an experiment where we address the underlying control mechanisms in play when biting through such materials. The results suggest that the CNS regulates both bite force and jaw closing force dissipation by taking advantage of the anatomy in the jaw-closing muscles. Finally, we combine our results with previous work and present a model on how the dissipation of jaw closing force may be controlled according to task demands.

Disclosures: A.S. Johansson: None. **K. Westberg:** None. **B.B. Edin:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NRF-2008-0062282

Title: Ultrastructural analysis of the vesicular glutamate transporters VGLUT1- and VGLUT2-expressing axon terminals on the rat trigeminal motoneurons

Authors: *Y. BAE, S. PAIK;
Dept. Anat. and Neurobio., Sch. of Dentistry, Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstracts: Excitatory neurotransmission is critical for the control of trigeminal motoneuron output and production of oral-motor activity. Recent study showed that projection pattern of the two subpopulations of glutamatergic neurons, i.e., vesicular glutamate transporters VGLUT1- and VGLUT2-expressing neurons, are different in each dorsolateral and ventromedial regions of

the trigeminal motor nucleus, suggesting differential glutamatergic regulation between jaw closing (JC) and jaw opening (JO) motoneurons. In the present study, to investigate the glutamatergic control of the JC and JO motoneurons, we analyzed VGLUT1-immunopositive (+) and VGLUT2+ boutons that make synaptic contact with the JC and JO motoneurons by retrograde labeling of JC and JO motoneurons, electron microscopic immunocytochemical staining of VGLUT1 and VGLUT2, and quantitative analysis. VGLUT1+ boutons frequently synapsed with JC motoneurons, while they were rare on the JO motoneurons. Frequency of VGLUT1+ boutons was significantly higher on the dendritic shafts than on the soma or primary dendrites of the JC motoneurons. VGLUT2+ boutons were far more frequently observed than VGLUT1 in the JC and JO motoneurons: Their frequency was not different between in the JC and JO motoneurons, and also among their soma, primary dendrites and dendritic shafts. Bouton volume, mitochondrial volume and active zone area, which are related to synaptic strength, were significantly larger in the VGLUT1+ boutons than VGLUT2+ boutons. These findings suggest that JC and JO motoneurons are differently regulated by VGLUT1- and VGLUT2-expressing glutamatergic neurons, and which may contribute to the production and maintenance of oral-motor activity.

Disclosures: Y. Bae: None. **S. Paik:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR

NIH

Title: Neuroplasticity of rat orofacial sensorimotor cortex induced by dental manipulations: Are glial cells involved?

Authors: V. VARATHAN[1], M. SOOD[2], H. PUN[1], L. AWAMLEH[1], D. CHOCRON[1], P. BHATT[1], A. THAKORE[1], *B. J. SESSLE[3], L. AVIVI-ARBER[1];
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Abstracts: Rationale and Aims. Previous studies have documented that injury or other manipulations of orofacial tissues can induce neuroplastic changes in the adult rat orofacial sensorimotor cortex (oSMCx). Since glial cells have been shown to be critical for the manifestation of neuroplasticity in other parts of the orofacial sensorimotor system, the aims of this study were to determine (i) if oSMCx neuroplasticity occurs following dental manipulations involving orthodontically-induced tooth movement (OTM), maxillary molar teeth extraction, or acute noxious (mustard oil, MO) dental stimulation, and (ii) if glial cells are involved in any such oSMCx neuroplastic changes. Methods. In male adult Sprague-Dawley naïve rats and in rats receiving one of these manipulations or sham procedure, intracortical microstimulation (ICMS) was used to map oSMCx and test for any OTM or extraction-induced changes in oSMCx jaw (anterior digastric) or tongue (genioglossus) motor representations and to test for any MO-induced changes in oSMCx excitability. We also tested if any such oSMCx excitability changes could be attenuated by oSMCx application of the astroglial inhibitor methionine sulfoximine (MSO; 0.1mM), and if there was immunohistochemical evidence of astroglial (GFAP labelling) or microglial (Iba1 labelling) activation following any of these dental manipulations. Results. Compared with sham or naïve rats, rats receiving OTM or molar teeth extraction showed significant decreases in jaw or tongue motor representations (25-50%; ANOVA, $p<0.05$) that lasted up to 28 days, and acute noxious stimulation of the molar tooth pulp produced a significant decrease in oSMCx excitability (50%; ANOVA, $p<0.05$) that lasted for at least 60 minutes and that could be reversed following oSMCx application of MSO. Furthermore, these oSMCx changes induced by each of the 3 dental manipulations were associated with significant increases in both astroglial and microglial labelling in oSMCx (15-50%; ANOVA, $p<0.05$). Conclusions. These novel findings reveal that several different types of dental manipulations can induce oSMCx neuroplastic changes that may be involved in adaptation of orofacial sensorimotor behaviours in clinically related situations involving the teeth, and that glial cells may be integrally involved in the mechanisms underlying the induction and consolidation of these oSMCx neuroplastic changes.

Disclosures: V. Varathan: None. **M. Sood:** None. **H. Pun:** None. **L. Awamleh:** None. **D. Chocron:** None. **P. Bhatt:** None. **A. Thakore:** None. **B.J. Sessle:** None. **L. Avivi-Arber:** None.

Poster

631. Afferent and Descending Control

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 631.21/II30

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: KAKEN 26870547

Title: Glycine-mediated jaw-opening reflex excitability in rats

Authors: *K. ADACHI[1,2,3], S. HINO[4], R. ODAI[5], T. SHIMOYAMA[4], H. SAKAGAMI[1], G. J. LAVIGNE[6], B. J. SESSLE[7];
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Abstracts: Recently we reported that the excitability of the jaw motor system (e.g., tongue stimulation-evoked jaw-opening reflex [JOR] and intracortical microstimulation-evoked rhythmical jaw movements) is depressed during quiet sleep (QS) in monkeys and that, consistent with these data, the electrical stimulation threshold for evoking the JOR in rats is significantly increased in quiet sleep (QS) compared with quiet awake (QW). Further, the systemic administration of glycine (75-150 mg/kg, i.p.) increased the JOR threshold in both QW and QS to a similar extent that was not, however, significantly different from that in QS of glycine non-treated rats. Glycine also reduced sleep latency (68.9-74.8%) and the incidence of micro-arousals (60.6-71.9%) without affecting the distribution of electroencephalographic (EEG) frequency bands (δ, θ, α and β). These results suggest that the central glycinergic system is involved in maintaining JOR excitability and improving sleep quality. In the present study, we investigated the effects of brain microinjection of glycine on JOR excitability and sleep quality in rats. Under general anaesthesia with isoflurane, male Sprague-Dawley rats (6 weeks old) received wire implantations for electrocardiographic (EKG) recording, electromyographic (EMG) recording of bilateral anterior digastric (AD) and masseter (MA) muscle activities, EEG and electrooculographic (EOG) recording, and electrical stimulation of the tongue (genioglossus muscle). Each animal also received guide cannula implantation bilaterally in the AD region of the trigeminal motor nucleus (AP -9.7 and lateral 1.7 mm from bregma) for microinjection of glycine (0.1 or 0.4 M, 0.2 μl). A week after the surgery, during QW before sleep, the threshold for evoking the JOR by tongue stimulation (200 μs) was defined. Then the animal was allowed to sleep and the JOR threshold was determined during QS as well as QW after sleep. After the microinjection of glycine, the JOR excitability and sleep-related physiological activity were determined again across sleep-awake states. The lower dose (0.1 M) of glycine failed to alter any physiological features, but the higher dose (0.4 M) of glycine reduced the JOR threshold during QS (96.2% of that before glycine) without affecting the JOR excitability during QW and, similar to its effect with systemic administration, also significantly reduced sleep latency (14.1% of that before glycine) and the incidence of micro-arousals (58.3% of that before glycine). These

findings suggest that the glycinergic system in the region of the trigeminal motor nucleus may have a role in maintaining jaw motor function during sleep and improving sleep quality.

Disclosures: **K. Adachi:** None. **S. Hino:** None. **R. Odai:** None. **T. Shimoyama:** None. **H. Sakagami:** None. **G.J. Lavigne:** None. **B.J. Sessle:** None.

Poster

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Program#/Poster: 631.22/JJ1

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR Grant MOP-4918

NIH R01DE023816

Title: Spike-spike and spike-field coherence reveal mutual intercortical communication in the orofacial sensorimotor cortex

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Abstracts: We have shown recently that motor learning induces transient coherent firing of neuronal pairs within the orofacial motor (MIo) and in the somatosensory (SIo) cortices (Arce-McShane et al, J Neurosci 2014). However, it is unknown whether neurons or local field potentials (LFPs) exhibit functional connectivity across the two cortical areas. Here, we examined the intercortical spike-spike and spike-field coherence between pairs of MIo and SIo neurons or LFPs as monkeys (*Macaca mulatta*) learned to generate tongue-protrusive force. Coherence between the spiking of MIo and SIo neurons (MIo_{spk}-SIo_{spk}) was dynamically modulated within-trial for low frequencies (2-6 Hz) as seen in significant peaks in coherence around 50 ms after force onset. Spike-field coherence (MIo_{spk}-SIo_{lfp} and SIo_{spk}-MIo_{lfp}) was similarly modulated for low frequencies. Peak and time-to-peak coherence differed significantly between MIo_{spk}-SIo_{lfp} and SIo_{spk}-MIo_{lfp} (Mann Whitney, $p<0.01$); they were lower and earlier in SIo_{spk}-MIo_{lfp} (0.11 vs. 0.15 and 70 ms vs. 160 ms after force onset). The time-to-peak coherence in MIo_{spk}-SIo_{lfp} was coincident to the time that monkeys reached the required force level and the

time difference with SIo_{spk}-MIo_{lfp} corresponds to 90 ms or roughly a half-cycle at 6 Hz. Moreover, MIo_{lfp} showed significantly earlier time-to-peak coherence with the spiking of its own neurons (MIo_{spk}-MIo_{lfp}) compared to SIo neurons (50 ms vs. 70 ms after force onset, Mann Whitney, $p<0.01$). In contrast, time-to-peak coherence of SIo_{spk}-SIo_{lfp} did not differ from MIo_{spk}-SIo_{lfp}. The phase-at-peak coherence taken at 6 Hz exhibited a bimodal distribution with means at ~0° and ~180° (Rayleigh Test, $p<0.001$). Intracortical spike-field coherence also showed a bimodal phase distribution which did not depend on interelectrode distance (Circular correlation, $p>0.10$). Early in learning, the phase relations of MIo LFP oscillations to spiking in MIo and SIo were similar (Common median multi-sample test, $p>0.10$), suggesting that MIo LFP oscillations might have driven spiking in MIo and SIo neurons. On the other hand, phase relations of SIo LFP oscillations to spiking in MIo and SIo were significantly different ($p<0.01$), suggesting that MIo_{spk} and SIo_{spk} were driving the LFP oscillations in SIo. With increasing spike-spike coherence as monkeys learned, spike-field phase relations were altered transiently and became similar at the end of learning. The results provide evidence of mutual communication between MIo and SIo in the slow cortical rhythms and their reliance on inputs from within each of their own neuronal pool and those of the other during motor learning.

Disclosures: F.I. Arce-Mcshane: None. **C.F. Ross:** None. **J. Lee:** None. **B.J. Sessle:** None. **N.G. Hatsopoulos:** None.

Poster

631. Afferent and Descending Control

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Program#/Poster: 631.23/JJ2

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Support: NIH 5P20-RR-016463

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NSF IOS-1121973

HHMI Undergraduate Science Program

Paller Fund of Bowdoin College

Doherty Fund of Bowdoin College

Title: Mechanisms and effects of stretch feedback in the heart of the American lobster, Homarus americanus

Authors: *K. HARMON, M. CHIN-PURCELL, E. S. DICKINSON, T. M. HARTLEY, O. ELLERS, A. S. JOHNSON, P. S. DICKINSON;
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Abstracts: Although central pattern generators (CPGs) can produce rhythmic outputs in isolation, their outputs *in vivo* are often altered by both sensory feedback and neuromodulators. The interactions of these components are not well understood. We examined the neurogenic heart of the lobster, *Homarus americanus*, which is controlled by a small CPG, the cardiac ganglion (CG). Previous research suggests that crustacean heart muscle provides feedback to the CG about the degree of cardiac filling, mediated by stretch sensitive dendrites emanating from the CG neurons. We thus investigated the effects of stretch on CG output and the mechanisms underlying stretch sensitivity. Dendrites of CG motor neurons were identified using intracellular dye fills. To determine the role of stretch-sensitive dendrites, isolated hearts were stretched while heart contractions and CG motor output were recorded. Tonic stretches of the heart significantly increased contraction frequency in most preparations; the proportion that increased depended on direction of stretch. Frequency in the remaining preparations decreased or did not change. Removing stretch feedback either by severing the branches of the CG that are thought to contain the dendrites or by cutting the CG motor nerves decreased or eliminated the response to stretch, but increased the variability of the heartbeat. Additionally, both frequency and amplitude of heart contractions decreased slightly when the CG motor neuron dendrites were severed; frequency likewise decreased when the motor nerves were severed. Our data suggest that feedback is mediated largely by dendrites branching from the main CG trunk, and is triggered primarily by active muscle contractions. This feedback appears to stabilize the heartbeat and simultaneously enhance the activity of the CPG. To determine the cellular mechanism that underlies stretch sensitivity, we recorded intracellularly from CG motor neurons while manually stretching small bundles of muscle fibers. Stretch of a single muscle bundle did not alter cycle frequency, and we saw no changes in the membrane potential between bursts. Surprisingly, the amplitude of the driver potentials that underlie bursting in the CG neurons decreased when the attached muscle was stretched. Both the changes in heartbeat frequency in response to removal of the dendrites and the responses of the membrane potential to stretch differ considerably from those previously recorded in other species, suggesting a different mechanism underlying stretch sensitivity in this crustacean species. Experiments examining the effects of neuromodulators on this CPG-effector system are underway.

Disclosures: K. Harmon: None. **M. Chin-Purcell:** None. **E.S. Dickinson:** None. **T.M. Hartley:** None. **O. Ellers:** None. **A.S. Johnson:** None. **P.S. Dickinson:** None.

Poster

631. Afferent and Descending Control

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Program#/Poster: 631.24/JJ3

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: KAKENHI Grant 2529001

KAKENHI Grant 23650202

JST RISTEX

Title: Spinal interneuronal organization involved in the control of postural muscle tone in the cat

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Abstracts: In the decerebrate cat preparation, microinjection of carbachol into the pontine reticular formation (PRF) induced muscular atonia by modulating the activities of reticulospinal neurons in the medullary reticular formation (MRF) (Takakusaki et al., 1993, 1994). The present study was designed to understand spinal interneuronal mechanisms involved in the control of postural muscle tone. For this purpose, extracellular activities of 105 interneurons were recorded from the lower lumbar segments (L6-L7) of acute decerebrate cats (n=28). Interneurons were divided into 3 types following inputs from the inhibitory region of the MRF and from peripheral sensory afferents. Type I cells (n=39) received excitation from the MRF and inhibition from volleys in flexion reflex afferents (FRA). They were located in the ventromedial part of the grey matter (lamina VII and VIII of the Rexed). Type II cells (n=38) received inhibition from the MRF. Type III cells (n=28) did not receive input from the MRF. Type II and III cells were located in the intermediate region (lamina IV~VII) and dorsal horn (lamina III) and exclusively received excitation from FRA. Firing rates of these groups of cells were further examined before and after injections carbachol (1.6~4.0 μg/0.1~0.25 μl) into the PRF. Before carbachol injection, or a state with higher level of muscle tone due to decerebrate rigidity, Type I cells were silent or had low firing rates (2.0 ± 2.7 Hz; n=18). On the other hand, spontaneous firing was observed in Type II cells (38 ± 11.5 Hz; n=13) and Type III cells (21.4 ± 10.0 Hz; n=7). During motor inhibition or muscular atonia induced by pontine carbachol inhibition, firing rates of the Type I cells was increased to 33 ± 11.7 Hz, while those of the Type II and III cells were reduced to 7.1 ± 5.2 Hz and 10.1 ± 5.8 Hz, respectively. Spike-triggered averaging revealed that some Type I cells exerted postsynaptic inhibitory effects upon α-motoneurons and interneurons mediating

reflex pathways, and presynaptic inhibitory effects upon primary afferents. These findings suggest the presence of functional topographical organization of spinal interneurons with respect to the control of postural muscle tone. Interneurons in the ventromedial grey matter (Type I cells) may be involved in muscular atonia, while those in dorsal horn and intermediate region (Type II and III cells) may contribute to maintenance of muscle tone. Because muscular atonia induced by pontine carbachol injection resembles to that of rapid eye movement (REM) sleep, the Type I cells may be responsible for motor inhibition during the preiod of REM sleep.

Disclosures: K. Takakusaki: None. **R. Chiba:** None. **T. Nozu:** None. **T. Okumura:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.01/JJ4

Topic: D.14. Cerebellum: Central Physiology

Support: NIH Grant HD63071

Title: REM sleep twitches drive Purkinje cell activity in the developing cerebellum

Authors: A. M. PLUMEAU[1], *G. SOKOLOFF[2], D. MUKHERJEE[2], M. S. BLUMBERG[2]; [1]Neurosci., [2]Psychology, The Univ. of Iowa, Iowa City, IA

Abstracts: One of the primary behavioral states in infancy is sleep. During active, or REM sleep, the spontaneous myoclonic twitches of the skeletal muscles provide discrete sensory feedback to the brain. It has been proposed that this information is important for the development and refinement of neural circuits involved in sensorimotor integration. One structure crucial for sensorimotor integration is the cerebellum, which in rats develops extensively after birth. Although the anatomical changes that occur in the first few weeks of postnatal life have been thoroughly characterized, less is known about the cerebellum's functional development. Despite the immaturity of the cerebellar circuit, recent work in our lab has shown that Purkinje cells in rats at 6 days of age show patterned activity, including the presence of complex and simple spikes, both of which show more activity during sleep immediately after twitches. Using 16-channel silicon electrodes, we recorded extracellularly from Purkinje cells in unanesthetized, head-fixed rats at 4, 8, and 12 days of age as they cycled between sleep and wake. EMG activity was recorded from nuchal and hindlimb muscles and behavior was scored concurrently. Complex and simple spikes were apparent at all ages, with firing rates for both increasing with

age. Additional age-related changes were observed in complex and simple spike auto-rhythmicity, both of which peaked at 8 days of age. Finally, state- and twitch-dependent Purkinje cell activity was also observed at all three ages. These results suggest that sleep-related twitching plays a key role in refining cerebellar circuits and strengthens the argument that activity-dependent development of the cerebellum is driven in part by twitching.

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

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.02/JJ5

Topic: D.14. Cerebellum: Central Physiology

Support: Searle Scholars

MH093727

JSPS Fellows

Title: Firing rate modulation of two antagonistic Purkinje cell populations during motor timing in mice

Authors: *S. OHMAE, K. OHMAE, D. SUBRAMANIAN, J. F. MEDINA;
Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstracts: To examine the contribution of the cerebellar cortex to motor timing, we recorded the simple spike activity of individual Purkinje cells while mice performed well-timed conditioned eyelid movements. Mice were trained to make conditioned blinks in response to a visual stimulus (LED light) that was repeatedly paired with a periocular airpuff at one particular interstimulus interval (ISIs range: 220-370 ms for different mice). Regardless of the ISI used during training, all mice learned to adaptively regulate the speed of the conditioned blink and achieve maximum eyelid closure around the expected time of the airpuff. We found two types of Purkinje cells with movement-related activity: the firing rate of Type I cells was strongly suppressed and was negatively correlated with the speed of the conditioned eyelid movement, i.e. the firing rate was reduced soon after the presentation of the LED but returned to baseline by the time of the airpuff, when the eyelid speed was close to zero. In contrast, the firing rate of Type II cells ramped up

gradually and, similar to the eyelid position, reached maximum around the time of the airpuff. We have started making focal electrolytic microlesions and high-pressure dye injections to mark the locations of Type I and Type II cells in the cerebellar cortex of individual mice. Preliminary data suggests that Type I cells are clustered in a very small "hotspot" near the bottom of the primary fissure, while Type II cells can be found more widely distributed along the walls of the primary fissure. We are currently assessing the locations of Type I and Type II Purkinje cells relative to zebrin stripes.

Disclosures: S. Ohmae: None. **K. Ohmae:** None. **D. Subramanian:** None. **J.F. Medina:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH MH093727

Title: Dynamic modulation of anterior interpositus neuron activity during the performance of conditioned eyelid movements in mice

Authors: *S. A. HEINEY, J. F. MEDINA;
Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstracts: We examined the firing properties of neurons in an identified a region of the anterior interpositus (AIP) nucleus that is essential for the expression of learned eyelid movements in mice. Mice were head-fixed on top of a cylindrical treadmill and trained to blink in response to a light or vibrissal conditioned stimulus (CS) that was repeatedly paired with an airpuff to the eye. After the mice had learned to make well-timed conditioned eyelid movements we used electrical microstimulation to map out the AIP and localize putative eyeblink controlling regions. We found a small "hotspot" within the AIP from which discrete, graded, ipsilateral eyelid movements could be reliably evoked with low current stimulation. Functional disconnection of this small region in AIP, through reversible pharmacological inactivation or permanent electrolytic lesions, completely abolished learned eyelid movements with little or no effect on reflex blinks. Neurons recorded within and around this "hotspot" showed a variety of firing rate modulations during the performance of conditioned eyelid movements. However, a large

proportion of the neurons exhibited an idiosyncratic response profile starting with a transient pause in firing at a fixed latency after the onset of the CS, followed by an increase in firing rate to a level that was well above baseline and remained elevated until the time of the airpuff. On individual trials, the firing rates of many neurons showed strong correlations with the kinematics of the eyelid movement, such that the presence or absence of a conditioned response, and often its size, could be predicted based on the firing of a single neuron on a single trial. Our results demonstrate that neurons in this region of AIP are a critical component of the circuitry mediating conditioned eyelid movements, and that their activity is dynamically regulated to precisely shape motor output.

Disclosures: S.A. Heiney: None. **J.F. Medina:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

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Program#/Poster: 632.04/JJ7

Topic: D.14. Cerebellum: Central Physiology

Support: Grant-in-Aid for JSPS Fellows Number 263364

Title: Timed pauses of simple spikes and up-and-down patterns of deep cerebellar nucleus activity code cerebellar temporal processing during voluntary movement tasks

Authors: *K. YAMAGUCHI[1], S. TAKAHASHI[2], Y. SAKURAI[1];
[1]Kyoto University, Grad. Sch. of Letters, Dept. of Psychology, Kyoto, Japan; [2]Doshisha University, Grad. Sch. of Brain Science, Dept. of Neural Circuitry, Kyoto, Japan

Abstracts: Time is a fundamental and critical factor in daily life. Millisecond timing, which is the underlying temporal processing for speaking, dancing and other activities, is reported to rely on the cerebellum. Some studies have reported the relationship between temporal processing of several hundred milliseconds and the cerebellar neuronal spikes that may directly organize behavioral timing. As an example of the notable contribution of the cerebellar cortex activity to temporal processing, eyeblink conditioning studies using electrophysiological recording demonstrated that the simple spikes of Purkinje cells exhibited excitatory or inhibitory responses before the presentation of unconditioned stimuli in well-trained animals. On the other hand, evidences from the experiments that controlled some of the temporal properties in voluntary-movement tasks are limited although the contribution of the cerebellum to voluntary movements

is well documented. Here, we suggest that precise temporal control in the cerebellum may be realized by regular pauses of simple spikes of Purkinje cells and up-and-down activity pattern of deep cerebellar nucleus neurons. We used behavioral tasks that require the rats to touch a switch continuously at regular intervals of a few hundred milliseconds with their paw. When the rats fail to pause for a fixed interval between behavioral responses, the current trial is canceled and restarted. Consequently, the rats need to correctly perceive the intervals between the touch responses to successfully complete the trials. Behavioral timing in the task that requires only one interval to complete one trial is defined as 'duration-based timing', and the other requiring multiple intervals was defined as 'beat-based timing'. As the results of multiple Purkinje cell recording during the beat-based timing task, we found that simple spikes paused near the time of the touch only when the rat paused for the fixed intervals. The results also showed that deep cerebellar nucleus neurons show increasing activity toward the time of touch and a sudden decrease of activity after the touch. The pattern may reflect that there is a pause of the simple spikes of Purkinje cells for temporal processing. This is in line with the notion that deep cerebellar nucleus activity reflects disinhibition from Purkinje cellular inhibitory inputs. The simple spikes display regular activity patterns including successive firing and short/long pauses, and such regularity controls downstream deep cerebellar nuclei. In conclusion, these results suggest that precise temporal control in the cerebellum may be realized by such regularity of pauses.

Disclosures: K. Yamaguchi: None. **S. Takahashi:** None. **Y. Sakurai:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.05/JJ8

Topic: D.14. Cerebellum: Central Physiology

Support: MH46904

MH74006

Title: Predicting conditioned response profiles on a single trial from Purkinje cells population activity

Authors: *A. KHILKEVICH, H. E. HALVERSON, J. PILLOW, M. D. MAUK;
Univ. of Texas At Austin, Austin, TX

Abstracts: As the principle neurons of the cerebellar cortex, Purkinje cells (PCs) are thought to control the timing properties of motor responses. Eyelid conditioning provides a cerebellar-dependent behavior (conditioned responses, CRs), and therefore an ability to establish how PCs population encodes eyelid trajectory during CR. We employed a generalized linear model (GLM) approach to study to what extent CR trajectory on a single trial can be predicted from spike trains of simultaneously recorded PCs. We used data from nine New Zealand albino rabbits trained with delay eyelid conditioning protocols with ISI=200ms, 250ms, 500ms, 700ms. Tetrode recordings from PCs were made from the left anterior lobe of the cerebellar cortex while animals were showing a robust expression of conditioned responses. PCs were classified as "eyelid" PCs based on the presence of complex spike(s) during the unconditioned stimulus presentation. Only eyelid PCs were included into this analysis. With GLM based analysis we explored how PC spike history is integrated and transformed into the behavioral response, as well as how nonlinearities contribute to motor output transformation. Our results indicate that: 1) Purkinje cells control the time profile of conditioned responses; 2) the largest behavioral response is achieved via increased inhibition followed by disinhibition of downstream DCN neurons, consistent with previous findings; 3) even a small subset of simultaneously recorded PCs is sufficient to explain a high amount of trial-to-trial variability of CR trajectory profiles. Additionally, we investigated how prediction accuracy scales with the number of simultaneously recorded PCs. Interestingly, squared prediction error scales as one over the number of PCs included, with the exception of the single PC case, which gives much higher accuracy than would be estimated from the trend. This provides evidence that PC population activity is highly correlated and that we can estimate some features of CR profile even from the activity of a single PC.

Disclosures: **A. Khilkevich:** None. **H.E. Halverson:** None. **J. Pillow:** None. **M.D. Mauk:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

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Program#/Poster: 632.06/JJ9

Topic: D.14. Cerebellum: Central Physiology

Support: FP7 / ERC

Title: Locomotion initiation and termination in mice following selective optogenetic activation of Purkinje cell ensembles

Authors: *T. M. HOOGLAND[1], N. A. FLIERMAN[1], H. HOEDEMAKER[1], J. R. DE GRUIJL[1], L. WITTER[2], C. B. CANTO[1], C. I. DE ZEEUW[1,3];
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Abstracts: Synchronous optogenetic activation of Purkinje cells (PCs) results in a transient suppression of firing in cerebellar nuclei neurons followed by rebound firing and correlated motor behavior in mice including twitches that occur with a short delay relative to the stimulus offset. We sought to determine if such stimulation in the anterior vermis of the cerebellum could interfere with ongoing locomotion patterns, or limb placements in head-fixed awake behaving mice that were placed on a transparent disc-based treadmill system. Our findings show that optogenetic activation of PCs during ongoing locomotion can either perturb locomotion causing the animal to slow down, or end locomotion altogether. When animals were resting, stimulation could trigger not only twitches relative to the stimulus offset, but also result in the initiation of locomotion that began with a fixed latency to onset of the optogenetic stimulus and resulted in a stereotyped stepping pattern. We will present data obtained using a three-fiber ferrule used for optogenetic stimulation of PC ensembles at distinct mediolateral locations and demonstrate the effects of stimulation at these sites on mouse locomotion patterns.

Disclosures: T.M. Hoogland: None. **N.A. Flierman:** None. **H. Hoedemaker:** None. **J.R. De Gruijl:** None. **L. Witter:** None. **C.B. Canto:** None. **C.I. De Zeeuw:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH Grant R01 NS39395

Title: Modulation of purkinje and cerebellar nuclear cell activity during running in awake mice

Authors: *R. SARNAIK, I. M. RAMAN;
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Abstracts: Corticonuclear synapses, between GABAergic Purkinje cells of the cerebellar cortex and projection neurons of the cerebellar nuclei, are central to both correction of motor errors and regulation of well-learned movements. To investigate signaling at these synapses during periodic, predictable, coordinated movements, we recorded spiking in Purkinje and nuclear cells in awake, head-fixed mice allowed to run freely on a linear treadmill, while simultaneously recording the hindpaw position ipsilateral to recording site. Loose cell-attached recordings from Purkinje cells in simplex and crus 1 (Pkj, n = 13) or cerebellar nuclear cells in the interpositus (CbN, n = 8) were made in mice expressing channelrhodopsin-2 (ChR2(H134R)) in Pkj cells (L7-cre x Ai27). The average stride duration during running was 209 ± 10 ms (n=21). Spiking patterns of both Pkj and CbN cells were clearly reorganized between rest and running epochs, suggesting that the recorded cells were involved in the task. In addition, when local illumination was applied through an optical fiber to increase spiking of Pkj terminals in the vicinity of the recorded CbN cell, long light pulses (1-2 sec, 1-2 mW/mm2) elicited strong inhibition of the CbN cells and also caused a missed step on the ipsilateral side by making the animal slip on the treadmill during silencing of the CbN cell (at least 2 repeats in each of 6 recordings in 3 mice). Independently of absolute firing rates, spiking rates of Pkj and CbN cells rose and fell reliably during specific segments of the stance and swing phases of the step cycle. The two cell types showed similar maximal firing rates during running epochs (Pkj: 101 ± 12, CbN: 96 ± 10 sp/s), but CbN cells showed lower minimal firing rates (Pkj: 61 ± 12, CbN: 24 ± 7 sp/s). As a consequence, CbN cells showed a significantly greater modulation of firing rates with the step cycle compared to Pkj cells, measured either as (max rate-min rate)/min rate (Pkj: 1.2 ± 0.67, CbN: 13.6 ± 9.8, $p<0.01$) or as the normalized net vector on polar plots of the spike rate vs. step cycle phase (Pkj: 0.1 ± 0.03, CbN: 0.3 ± 0.05, $p<0.01$). Although individual Pkj and CbN cells increased their firing rates at a specific phase of the step cycle, across the population all phases of the step cycle seemed to be represented. Our results show that nuclear cells modulate their firing rates greatly to specific phases of the step cycle, possibly by integrating several moderately modulated Purkinje cell inputs.

Disclosures: **R. Sarnaik:** None. **I.M. Raman:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.08/JJ11

Topic: D.14. Cerebellum: Central Physiology

Support: EC grant REALNET (FP7-ICT270434)

Title: Cerebro-cerebellar encoding of motor actions involves dynamic formation of network assemblies

Authors: Y. BAUMEL[1], *D. COHEN[2];
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Abstracts: The primary motor cortex (M1) and the deep cerebellar nuclei (DCN) are involved in the control and production of motor actions (Gross, 02; Williams, 09). Local field potentials (lfp) recorded simultaneously in the cerebrum and cerebellum display coherent activity in the low frequencies during anesthesia (Ros, 09) and in the high frequencies during expectancy and various motor tasks (Courtemanche & Lamarre, 05; Soteropoulos & Baker, 06). Yet, the interplay between these two structures and their synergistic contribution to the encoding of different actions in freely moving animals remains to a large extent unknown. By using chronically implanted microwire electrodes, we show in freely moving rats that the activity of single units recorded in M1 and the DCN correlated with the performed motor action. Analysis of the population activity showed that the neurons formed dynamic assemblies within and between structures that correlated with the performed action. In addition, lfps recorded in M1 and the cerebellar nuclei (DCN) displayed a significant power in the frequency range of 6-8Hz. The coherence between these lfps was modulated in relation to the preformed action. Also, the observed phase lag in the cross-correlation calculated between these lfps depended on (1) the performed action, and (2) whether the lfps were taken from the ipsilateral or the contralateral side. A Granger causality test suggested that the DCN drive M1 lfp oscillations during grooming and ambulation but not in the absence of overt movement. Surprisingly, the emerged single unit network assemblies were independent of the observed lfp cross-correlations. These data suggest a flexible, oscillations based, computation and provide a neuronal substrate for the performance of bi-manual movements.

Disclosures: Y. Baumel: None. **D. Cohen:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.09/JJ12

Topic: D.14. Cerebellum: Central Physiology

Title: Cross frequency interactions of oscillations in the cerebellum

Authors: *J. GROTH, M. SAHIN;
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Abstracts: The cerebro-cerebellar pathways have long been known to play an important role in motor planning and control. The cerebellar signals contain oscillations in various bands. The most prominent are the high frequency oscillations (HFO), in the 150-400Hz range, and the oscillations in the 4-25Hz range. The HFOs arise due to the simple spike synchrony in the Purkinje cells. The 4-25Hz oscillations arise out of the granular layer and the inferior olive. Another feature of the cerebellum is that the Purkinje cells, granular layer, and inferior olive all show a resonance in the theta range (4-8Hz). How these oscillations are related to each other and to the motor cortex oscillations is not known. To this end, we have recorded neural activity from the cerebellar and the primary motor cortices using micro-ECoG arrays (4x8 matrix with 300 micron pitch). This allows simultanious observation of activity in a large area of the cortex with a dense matrix of electrodes. The recordings were performed on male Long Evans rats. The ECoG arrays were placed over the paramedian lobule of the posterior cerebellum and the primary motor cortex. All recordings were performed during a lever press task. All data analysis was performed using MatLab. The field potentials in HFO and 4-25Hz bands were analyzed using time frequency methods available with the Chronux tool box. Phase synchrony was analyzed using Hilbert transform and Shannon entropy. Spectral analysis shows significant similarity in temporal changes between the two frequency ranges during the movement. Our analysis also demonstrates that when two electrodes are coherent in the high frequency range they are also coherent in the 4-25Hz range. Furthermore, synchrony in the two frequency bands overlaps in time. The synchrony analysis also shows three distinct bands in the 4-25Hz range that are consistent with the theta, Mu, and beta bands in the cortex. Coherence and synchrony analysis between the two cortices reveal that there is a relationship between the cerebral and cerebellar oscillations. Stimulation of the motor cortex in the 4-25Hz range produces high frequency components with resonance bands similar to those seen in the cerebellar synchrony. Our results show that there are significant cross frequency interactions during the movement in the cerebellum as well as to those in the motor cortex. The data also indicate that high frequency oscillations can be modulated by afferent inputs through cross frequency interactions.

Disclosures: J. Groth: None. **M. Sahin:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

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Program#/Poster: 632.10/JJ13

Topic: D.14. Cerebellum: Central Physiology

Support: SMIC ANR-12-BSV4-0027

Title: Neighboring Purkinje cells in the cerebellum are synchronized during voluntary movements

Authors: H. GAO[1,2], C. POUZAT[3], M. SPOLIDORO[1], *C. LENA[1];
[1]CNRS UMR 8197 / INSERM U 1024, Inst. de Biologie de l'Ecole Normale Superieure, PARIS, France; [2]East China Normal Univ., Inst. for Advanced Interdisciplinary Res., Shanghai, China; [3]CNRS, UMR 8118, Lab. de Physiologie Cérébrale, UFR biomédicale de l'Université René Descartes (Paris V), Paris, France

Abstracts: Cerebellar computations are performed within functional microzones that process information in parallel and form distinct output streams. In the cerebellar cortex, sets of neighboring Purkinje cells belonging to the same microzone are organized in parasagittal bands. How neuronal activity is coordinated within each microzone is currently unknown. In this study, we have recorded simultaneously sets of neighboring cells activated during fast forelimb voluntary movements. Neighboring Purkinje cells exhibit millisecond-timescale synchrony during motor execution. This synchrony is maintained, embedded in high-frequency oscillations, during sleep and active exploration, suggesting that recurrent inhibition continuously shapes the correlations between neighboring cells. Our results indicate that during a fast and complex movement, local assemblies of Purkinje cells, whose projections converge in the cerebellar nuclei, dynamically form at the millisecond time scale and will thus produce very transient episodes of inhibition in the cerebellar nuclei.

Disclosures: **H. Gao:** None. **C. Lena:** None. **M. Spolidoro:** None. **C. Pouzat:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

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Program#/Poster: 632.11/JJ14

Topic: D.14. Cerebellum: Central Physiology

Support: CereDySTim ANR-12-JSV4-0004

Title: Role of the cerebellum in the motor system dysfunctions in Parkinson's disease

Authors: A. BOUSQUET, F. MENARDY, C. LÉNA, *D. POPA;
CNRS UMR 8197 / INSERM U 1024, Inst. de Biologie de l'Ecole Normale Superieure, Paris, France

Abstracts: Parkinson's disease is associated with a substantial functional reorganization of the motor system, particularly of the cortical motor areas and within the two main subcortical afferents to these areas: the basal ganglia -where the dopaminergic degeneration takes place- and the cerebellum. The consequences of the alterations of the cerebellum are not fully understood, but several reports already point toward its involvement in motor dysfunction and in the abnormal involuntary movements in dyskinesia, the side effects of the levodopa treatment in the late phase of the disease. In our study, we combine pharmacological manipulations, optogenetics and *in vivo* electrophysiology in anesthetized and behaving animals to examine the changes of activity in the cerebellum and the compensatory reorganisation of the cerebello-cortical pathway in a 6-hydroxydopamine (6-OHDA) rodent model of Parkinson's disease. In particular, we assess the impact of cerebellar stimulation on dyskinesias that occur in the late stage of Parkinson's disease and we observe a reduction of abnormal involuntary movements following these stimulations, suggesting that the cerebellum could offer a gateway for corrective treatment of the motor dysfunctions in Parkinson's disease.

Disclosures: **A. Bousquet:** None. **D. Popa:** None. **F. Menardy:** None. **C. Léna:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.12/JJ15

Topic: D.14. Cerebellum: Central Physiology

Title: Plane specific zonal organization of Purkinje cell responses to vertical head rotations in the cat cerebellar nodulus and uvula

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Abstracts: Purkinje cells in the cerebellar nodulus and uvula have been shown anatomically to project topographically to the vestibular nuclei. However, the functions of topographically organized modules of Purkinje cells in the cat nodulus and uvula have not yet been demonstrated. In the present study, we investigated the neural responses of Purkinje cells during vertical head rotations, and their cellular distribution in the nodulus and uvula to clarify whether a modular organization exists in these regions. Four adult cats were chronically prepared for single-unit recording. All animal care and experimental procedures complied with SfN guiding policies and principles. We recorded simple spike (SS) and complex spike (CS) firing of Purkinje cells in the nodulus and uvula of awake head-restrained cats, during sinusoidal vertical rotation of the head in four stimulus planes: pitch, roll, and two diagonal planes, i.e., 45° clockwise, and 45° counterclockwise from the sagittal plane. The optimal response planes for SS and CS for each cell were estimated from the responses in the four stimulus planes using a sinusoidal function. Both SS and CS firing of the major population of cells were modulated by vertical but not horizontal head rotation. None of the sampled cells showed any sign of a response during either spontaneous saccades or quick phases of nystagmus (vestibular or optokinetic) in the horizontal or vertical planes. Individual cells have a preferred orientation of the head-rotation plane. In the best-response plane among the four that were explored, the phase of SS activity is close to that of either table (head) position or velocity. Two functional zones within the rostrocaudal extent of the nodulus and uvula can be recognized: a medially located band of cells with an optimal orientation in the pitch plane, and a lateral band located further than 1.0 mm from the midline with an optimal orientation in the roll plane. These findings suggest that the cat nodulus and uvula encode sensory information of head motion with regard to the vestibular signals through each plane-specific functional zone. These two zones are the same as previously reported zones in the cerebellar flocculus that are active during head rotation in the canal planes, in the point that both these cerebellar sagittal zones are plane specific functional zones. This suggests that anatomical sagittal zones serve as functional plane-specific zones, at least in the vestibulocerebellum.

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Poster

632. Cortex and Nuclei: In Vivo Studies

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Program#/Poster: 632.13/JJ16

Topic: D.14. Cerebellum: Central Physiology

Support: JSPS KAKENHI No.24650224

Title: Mossy fibers in the cerebellar hemisphere show activity during an instructed delay period

Authors: *T. ISHIKAWA[1], S. TOMATSU[2], D. S. HOFFMAN[3,4], S. KAKEI[1];
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Abstracts: The cerebellar hemispheres receive their primary inputs from the cerebral cortices via pontine nuclei (PN) and therefore are called the cerebrocerebellum. The ponto-cerebellar projection terminates as mossy fibers (MFs) in the granular layer of the cerebellar cortex. MFs in lobules IV-VI of the cerebrocerebellum receive predominant inputs from the primary motor cortex (M1) and premotor cortex (PM) (Kelly and Strick 2003). Because of this anatomical structure, examination of the pattern of MF activity in the cerebrocerebellum may be key for understanding what information the cerebral motor cortices provide to the cerebellum for motor control. However, little information is available about activities of MFs in the cerebrocerebellum in behaving monkeys. We recorded unit activity of MFs in the cerebrocerebellum of 2 monkeys that were required to make step-tracking movements of the wrist joint after an instructed delay period (1-2 sec). We found 155 MFs with significant task-related activity and most of them (n=148) had clear somatosensory RFs in distal part of the ipsilateral arm. We compared their activity with that recorded in M1 and PM neurons during the same task in previous studies (Kakei et al., 1999, 2001), and found that MFs had several features of task-related activity that were observed in M1/PM neurons. First, a part of MFs (n=70) showed a prolonged modulation change during the delay period (between instruction cue presentation and go signal). Both increases and decreases of activity were observed. Second, all MFs (n=155) showed a phasic or tonic modulation change at movement onset. Modulation onset of the movement-related activity preceded the movement onset by -67 ms on average, and lagged that for PM and M1 neurons (-124 ms and -97 ms, respectively). Third, most MFs showed directional tuning in the delay period (n=57) and/or at movement onset (n=120). Forty-eight MFs showed continuous directional activity that started before go signal and maintained until the end of movement, and preferred direction for most (n=41) showed little change. Overall, we found that patterns of task-related activity in M1/PM neurons were well-preserved in MF activity in the cerebrocerebellum. This result indicates that the cerebellum receives an efference copy of motor cortical activity that can serve to predict results of an action. Given that many MFs showed delay activity, the cerebellum may be involved not only in movement execution but also in planning and/or preparation for an upcoming movement.

Disclosures: **T. Ishikawa:** None. **S. Tomatsu:** None. **D.S. Hoffman:** None. **S. Kakei:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH R01 18338

NSF IGERT DGE-1069104

Title: Manipulations of visual feedback modulate Purkinje cell simple spike encoding of error signals during a manual random tracking task

Authors: M. STRENG, L. POPA, *T. J. EBNER;
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Abstracts: The view that the cerebellum functions as a forward internal model necessitates a role for the cerebellum in the processing of motor errors. Specifically, this requires the generation of opposing predictive and feedback related signals, whose summation results in a sensory prediction error (SPE). Recent results from our lab have implicated simple spike (SS) discharge of Purkinje cells (PCs) in the processing of prediction and feedback signals corresponding to motor errors. Many PCs have this dual representation of both kinematics and performance errors, and the prediction and feedback signals have opposing effects on the SS, consistent with an SPE. To further test this hypothesis, we are investigating the effects of disrupting sensory information pertinent to both predictive and feedback signaling on motor errors and their modulation of SS activity. Single unit recordings of PCs were obtained from rhesus macaques performing a manual tracking task, in which the animals track a randomly moving target using a manipulandum. Spike trains were analyzed using multiple linear regressions, where kinematic and performance errors were regressed against firing residuals in which variability associated with other parameters is removed. Feedback information during the task in the form of cursor position was either disrupted by an artificial delay of 100-200msec or reduced by making the cursor visible only when it was outside the target. Feedback delay resulted in a significant increase in motor errors, whereas visual feedback reduction results, somewhat surprisingly, in a significant decrease in motor errors. These manipulations and resulting behavioral changes are reflected in SS activity by a degradation of predictive signaling in the delay condition, and a degradation of feedback signaling in the hidden cursor condition. In PCs that exhibit bimodal R^2 error profiles, a reduction in magnitude of the predictive peak and an

increase in the magnitude of the feedback peak are observed in the delay condition, and vice versa for the hidden cursor condition. Kinematic R^2 profiles showed less overt changes, suggesting that SS signaling related to cursor movement is a combination of both visual and proprioceptive information. Results suggest that visual feedback delay causes in an invalidation of the feed forward components of the internal model, resulting in an increase in feedback-related signaling. Conversely, reducing visual feedback increases the dependence on the feed forward aspects of the internal model, thus increasing predictive signaling. Taken together, these results further support a role for SS discharge in the encoding of SPEs.

Disclosures: **M. Streng:** A. Employment/Salary (full or part-time):; University of Minnesota. **T.J. Ebner:** A. Employment/Salary (full or part-time):; University of Minnesota. **L. Popa:** A. Employment/Salary (full or part-time):; University of Minnesota.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.15/JJ18

Topic: D.14. Cerebellum: Central Physiology

Support: HHMI

NIH Grant EY003878

Title: Responses of Purkinje cells during pursuit eye movement place limits on the amount of spiking synchrony across the population

Authors: ***M. H. PHILLIPS**, S. G. LISBERGER;
Neurobio., Howard Hughes Med. Institute, Duke Univ., Durham, NC

Abstracts: Person & Raman (2012) showed that synchronous spiking across a population Purkinje cells (PCs) could cause increased PC firing to lead to increases in the firing rate of target neurons in the deep cerebellar nucleus (DCN), even though PC synapses onto DCNs are inhibitory. They posed synchrony as an explanation for why researchers have failed to observe a consistent inverse relationship between PC and DCN firing rates. They also found that the Purkinje cell IPSC in DCNs shows a very short (tau = 2.4ms) time constant of decay at physiological temperatures. The rapid decay would facilitate conversion of synchronous PC firing into excitation, through rebound firing in DCNs. Our recordings from Purkinje cells in the

floccular complex of the cerebellum provide data that may constrain the degree of synchronous firing across the Purkinje cell population. For example, we find modest "neuron-behavior correlations" between PC firing rate and eye movement. The correlations account for 40% of the trial-by-trial variation in the firing rate of individual floccular PCs during the initiation of pursuit (Medina & Lisberger, 2007). Here, we ask whether the observed neuron-behavior correlations constrain the amount of synchrony across the population. We simulated populations of PCs with synchrony ranging from 5-33%, where 5% corresponds to the amount of synchrony arising from fully independently generated firing rates, and we simulated behavior. Individual model neurons mimicked the firing rates of neurons in the pursuit task of Medina & Lisberger and had interspike interval variation drawn from the empirical distributions. We decoded the discharge of the population of model PCs and simulated behavioral output by convolving population output spike trains with a causal exponential filter having a time constant of 2.4 ms. We found that the neuron-behavior correlations in synchronized populations with as little as 15% synchrony were considerably higher than those measured from the Medina & Lisberger data when the neural data was filtered similarly. Correlation strengths in populations with 5-10% synchrony produced neuron-behavior correlations similar to those in the data. Longer decoder time constants (13 ms, 34 ms) substantially reduced or eliminated the difference between synchronous and non-synchronous populations. Our simulations suggest that large amounts of synchronous spiking across the population of PCs may not be an important feature of cerebellar output in the behaving animal.

Disclosures: M.H. Phillips: None. **S.G. Lisberger:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.16/JJ19

Topic: D.14. Cerebellum: Central Physiology

Title: Multiplexed coding by cerebellar Purkinje neurons

Authors: *S. HONG[1], M. NEGRELLO[2], M. JUNKER[3], A. SMILGIN[3], P. THIER[3], E. DE SCHUTTER[1];
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Abstracts: The cerebellar cortex processes various aspects of sensorimotor information and sends out its output via Purkinje cells (PC). Previous studies demonstrated good correspondence between their population rate and motion variables (Shidara et al., Nature, 1993) that can be facilitated by weak correlation between the PCs (Medina and Lisberger, J Neurosci, 2007). On the other hand, the spike trains of individual PCs have a non-trivial temporal structure that cannot be captured by a rate-modulated point process (Shin et al, PLOS One, 2007). Therefore, it has been a question how this temporal information in the PC spike train is represented at the population level and used for information transfer (de Zeeuw et al, Nat Rev Neurosci, 2011). To address this question, we analyzed simultaneously recorded single PC spiking and cerebellar local field potential (LFP), the latter serving as a proxy for the network activity, in addition to saccadic eye movements from three rhesus monkeys (Macaca Mulatta). We found that the correlation of single PCs to the local network activity is dynamic and depends on the neuronal and behavioral context. More specifically, the spike-LFP correlation is stronger and temporally more precise when the spike begins or ends "pauses" in the spike train that intermittently interrupts the typically fast and regular PC firing (Schonewille et al., Nat Neuroci, 2006; Shin and De Schutter, J Neurophys, 2006) while only a small fraction of the pauses was caused by complex spikes. This results in the pause spikes coupling to particular phases of the β/γ component of the LFP whereas a much weaker spike-LFP phase relationship is found for other spikes. Across the saccades, we observed that the β/γ LFP is significantly synchronized, and therefore becomes a reliable network signature for these fast eye movements. As the pause spike-LFP correlation predicts, the pause spikes have sharply peaked firing rates with respect to saccade onset with significant synchronization across saccades, again forming a reliable representation for the timing of motion. Our results suggest that the PC spike code is multiplexed: the population rate code is effective since the overall correlation between PC firing rates is small, but a small fraction of the spikes can strongly couple to the network activity and encode different behavioral information from the rate (Ratté et al., Neuron, 2013). Our study provides a unifying perspective that integrates the opposing views on PC population coding.

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Poster

632. Cortex and Nuclei: In Vivo Studies

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.17/JJ20

Topic: D.14. Cerebellum: Central Physiology

Title: Individual neurons in the caudal fastigial oculomotor region (FOR) convey information on both macro- and microsaccades

Authors: *Z.-P. SUN[1], M. JUNKER[2], D. ARNSTEIN[2], A. SMILGIN[2], P. DICKE[2], P. THIER[2]; [1]Dept. of Cognitive Neurol., Hertie Inst. for Clin. Brain Research, Univ. of Tübingen, Tuebingen, Germany; [2]Cognitive Neurol., Hertie Inst., tuebingen, Germany

Abstracts: Microsaccades are small (<1°) amplitude saccades made during attempted fixation in order to move the images of small objects of interest into the highest acuity center of the fovea (Putnam et al., J Vis. 2005). Behavioral studies have indicated that microsaccades and macrosaccades share most, if not all properties. Physiological studies of two major centers of saccade control, the superior colliculus (SC) (Hafed et al., Science. 2009) and the brainstem reticular formation (Van Gisbergen et al., J Neurophysiol. 1981) have established that they serve both macro- and microsaccades. The cerebellum is well known to ensure the high degree of precision of visually guided macrosaccades. As microsaccade amplitudes require a precision in the order of 10 um and less, a contribution of the cerebellum in fine tuning microsaccade amplitude may be expected as well. Two findings meet this expectation: 1. Purkinje cell simple spikes encode both macro- and microsaccades (Arnstein et al., this meeting). 2. Unilateral inactivation of the FOR, the major gateway for saccadic control signals destined for the brainstem causes dysmetria of macro- as well as of microsaccades. (Guerrasio et al., J Neurophysiol. 2010). However, there has so far been no electrophysiological evidence for microsaccade-related activity in the FOR which is why it is not clear if the integration of macro- and microsaccade related signals characterizing cerebellar cortex is perpetuated at the level of the FOR. In this study, we explored the deep cerebellar nuclei of 3 rhesus monkeys in order to delineate the FOR and to compare the responses of FOR neurons to macro- and to microsaccades. Forty saccade-related FOR neurons could be allocated to the FOR based on electrophysiological criteria and the availability of histological reconstructions of recording sites in 2 monkeys. All neurons tested showed qualitatively similar saccade-related bursts for macro- and microsaccades with earlier burst onset for contraversive saccades and vice versa. When pooling saccades of similar amplitudes, independent of their directions, the resulting population averages exhibited a monotonic dependence on saccade amplitude, spanning the full range of macro- and microsaccade amplitudes. Taken together, our study supports the notion of a continuous representation of saccade-related information, independent of saccade size, at all stages of cerebellar processing.

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Poster

632. Cortex and Nuclei: In Vivo Studies

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH Grant EY022788

Title: Lateral cerebellar activity correlated with microsaccades

Authors: *R. T. RAGHAVAN[1], V. PREVOSTO[2], M. A. SOMMER[2];
[1]Dept. of Neurobio., [2]Dept. of Biomed. Engin., Duke Univ., Durham, NC

Abstracts: Prior studies have demonstrated that medial cerebellar areas that participate in visually guided, saccadic eye movements also contribute to the execution of smaller amplitude, fixational microsaccades (Guerrasio et al. 2010). This is not necessarily surprising given that a main function of medial cerebellum is the fine control of saccade parameters. It remains unknown, however, if such findings extend to the lateral cerebellum, which is interconnected with association areas of cerebral cortex and is hypothesized to play a greater role in the planning and monitoring of saccades (Ashmore & Sommer 2013; Ohmae et al. 2013; Prevosto et al. 2013 SfN abstract) as opposed to saccade execution. To what extent is this lateral cerebellar activity related to fixational microsaccades? To answer this question, we recorded from 33 neurons in the oculomotor domain of the lateral cerebellar output nucleus (caudal dentate) in monkeys performing delayed saccade tasks. Comparing modulation in neural activity prior to microsaccade and saccade execution, we found that over half of the recorded neurons changed their activity during the execution of both kinds of movements (n=19). Typically, these changes in activity were perisaccadic bursts or pauses of activity aligned with the movement. The rest of the neurons were active for large saccades only. All of the neurons that were modulated by both microsaccades and saccades exhibited similar response profiles around the time of movement execution, though the vast majority (n=17) showed smaller amplitude modulations for microsaccades as compared to saccades. We hypothesize that the diversity of response profiles we record may be explained, in part, by the fact that caudal dentate neurons have different efferent projections to the frontal eye fields and the intermediate layers of the superior colliculus. These findings suggest that the lateral cerebellum, despite its putative role in higher-level motor planning and monitoring, maintains exquisite sensitivity to even the smallest of eye movements, thus underscoring its close relationship with motor acts.

Disclosures: **R.T. Raghavan:** None. **V. Prevosto:** None. **M.A. Sommer:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH Grant EY018585

NIH Grant RR-00166

Title: Estimated force during horizontal saccades controlled by monkey fastigial nucleus

Authors: ***F. R. ROBINSON**[1], Z. LINDBLOOM-BROWN[2,3], A. MUELLER[3,4], T. EGGERT[4], A. STRAUBE[4];
[2]Physiol. & Biophysics, [1]Univ. Washington, SEATTLE, WA; [3]Neurobio., Stanford Univ., Stanford, CA; [4]Neurol., Ludwig-Maximilians Univ., Munich, Germany

Abstracts: Cerebellar output modifies movement by changing the activity of motoneurons and thereby the force that agonist and antagonist muscles generate. By describing the size and timing of the force that the cerebellum controls during a movement we can characterize in detail how cerebellar output influences movement. No previous work characterizes the force contributed by the cerebellum to a voluntary movement. Here we estimate in 4 monkeys the force contributed by the cerebellum to 10° horizontal visual guided saccades. To do this we first estimated the force rotating the globe during a saccade by transforming eye position records into force using a model of the globe's mechanical properties (Anderson et al., '09). We then compared estimated force before and after we block saccade-related activity in the caudal fastigial nucleus (CFN) of the cerebellum. In all 4 monkeys the CFN contralateral to saccade direction contributes force in the direction of the saccade that increases from saccade start to a peak of ~16 grams at ~28ms later. In 3 monkeys the CFN ipsilateral to saccade direction contributes force in the saccade direction that peaks at ~6 grams ~18 ms after saccade start. From this peak force decreases and begins to oppose the movement by ~29 ms after saccade start. This opposing force increases to ~18 grams at saccade end. In the fourth monkey, the ipsilateral CFN contributes only opposing force that increases from saccade start to saccade end when it is ~31 grams. Net force resulting from activity in both the contra- and ipsilateral CFNs represents ~30-50% of the force on the globe during a 10° horizontal saccade. Finally, in one monkey we recorded 21 CFN neurons and compared the summed response of these neurons during 10° contraversive and ipsiversive a saccades to the force that we estimate is the result of this activity. The size, shape, and durations

of CFN activity and force records are similar but peak force does not lag peak activity by a fixed latency. Peak force from the CFN contralateral to saccade direction lags peak CFN activity by ~19 ms. Peak force from the ipsilateral CFN lags peak activity by ~30 ms.

Disclosures: F.R. Robinson: None. **Z. Lindbloom-Brown:** None. **A. Mueller:** None. **T. Eggert:** None. **A. Straube:** None.

Poster

632. Cortex and Nuclei: In Vivo Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 632.20/JJ23

Topic: D.14. Cerebellum: Central Physiology

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Title: Neural activity in the cerebellum during associative learning

Authors: *A. E. IPATA[1,2], N. N. ODEAN[2], Z. KRZYMINSKA[1], J. F. ZHANG[3], M. E. GOLDBERG[1,2,4];
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Abstracts: The role of the cerebellum in motor learning and motor control is well established. It is necessary for gain control, as demonstrated by its role in the adaptation of saccadic amplitude and the vestibule-ocular reflex, and for the classical conditioning of the eye blink reflex. Clinical

studies show that it contributes to the coordination, precision and accurate timing of movements. Although the cerebellum modifies movements it does not initiate them. However, the cerebellum has been shown to project to cortical areas not ordinarily associated with the control of movement (Strick, 2009 Buchner, 2011) and recent clinical studies suggest that patients with cerebellar disease exhibit frontal-like cognitive deficits (Schmamann, 2009). To see if the cerebellum were involved in the cognitive aspects of motor behavior, we recorded the activity of putative Purkinje cells from the lateral hemisphere of the cerebellar cortex in a Rhesus monkey performing an arbitrary visual motor association task. In this task the monkey had to learn to associate a novel abstract visual stimulus (for instance, a red square) with a well-learned right hand movement, and a second novel abstract stimulus (for example, a green square) with a left-hand movement. We used a new pair of stimuli for each learning session. Depending on the task the monkey had either to release its grasp on a bar by a finger extension or to release a lever by a wrist extension, both of which were well trained and required no new motor skill or gain learning. We found that the activity of putative Purkinje cells in the anterolateral cerebellar cortex correlated with the monkey's learning process in establishing the association of a well-trained movement with a new visual symbol. The activity of these cells changed gradually as the monkey's performance improved. The learning effect was apparent in different types of cell activity: some neurons' activity changed at the appearance of the stimulus, but did not continue to change during the movement, and other neurons' activity increased during the trial, peaking at the movement. Interestingly, this part of the cerebellum does not seem to be involved in the fine motor aspects of the behavior, but rather functions to associate a well-learned behavior with a new cue. It seems from these early data that the critical behavioral aspect for the cerebellum is the assignment of the cue to the movement: a cognitive decision, not a new skill or a gain change. Put differently, the question solved by this part of the cerebellum is not 'how can the brain control the movement so that it is smooth and accurate' but 'how can the brain choose what well-learned movement a new cue will trigger.

Disclosures: **A.E. Ipata:** None. **M.E. Goldberg:** None. **Z. Krzyminska:** None. **N.N. Odean:** None. **J.F. Zhang:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

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Program#/Poster: 633.01/JJ24

Topic: D.15. Basal Ganglia

Support: UCLA Edith Hyde award

RO1 MH070712

Title: Pharmacogenetic interrogation of a cortico-basal ganglia circuit that controls vocal-motor variability in zebra finch

Authors: *J. B. HESTON, J. SIMON, S. A. WHITE;
UCLA, Los Angeles, CA

Abstracts: Behavioral variability is posited to enable procedural learning. Indeed, studies in songbirds, rodents and humans has shown that variability positively predicts the rate, accuracy and even capacity for learning, and that variability is dynamically reshaped as a consequence of experience. The neural mechanisms responsible for generating this behavioral exploration remain unclear. In songbirds, a cortico-basal ganglia circuit is dedicated to the procedural learning of song, and plays an important role in generating song variability. However, the role of the song-dedicated basal ganglia region known as Area X is contentious, with lesions to Area X producing mixed results. We recently observed that experimental disruption of the dynamic, behaviorally-driven regulation of the FoxP2 transcription factor in Area X interferes with and even reverses transitions between states of low and high vocal variability that occur on the order of hours. This observation suggests that Area X may contribute to the control of vocal exploration which can be revealed on a relatively short time scale. Here, we test this hypothesis using viral-driven expression of designer receptors exclusively activated by designer drugs (DREADDs) to transiently and bidirectionally alter the activity of Area X neurons and measure the effect on song. These receptors only affect neural activity when activated by the otherwise biologically inert molecule, clozapine-N-oxide (CNO). Birds received bilateral stereotaxic injections to Area X of a virus that expresses one of the two types of DREADDS: one which increases neural excitability or one which decreases it. After recovering from surgery, song variability was measured following a systemic injection of CNO on a given day and compared to that following an injection of saline on another. Preliminary findings indicate that this manipulation birdirectionally alters song variability, supporting the idea that Area X can directly contribute to vocal-motor exploration, and offering a candidate neural mechanism that may be used for natural fluctuations in behavioral variability.

Disclosures: **J.B. Heston:** None. **J. Simon:** None. **S.A. White:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.02/JJ25

Topic: D.15. Basal Ganglia

Support: BMBF Bernstein Fokus 'Variable Tunes'

Title: Sensorimotor properties of different neuron types in basal ganglia song nucleus Area X

Authors: *L. KOLB[1], A. HANUSCHKIN[1], C. SCHARFF[2], R. HAHNLOSER[1];
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Abstracts: Young male zebra finches learn to produce their stereotyped song by vocal imitation of an adult conspecific 'tutor'. Using auditory feedback they transform an initially highly variable juvenile subsong into a stable copy of a memorized tutor song. This goal-directed motor learning requires a specialized anterior forebrain pathway (AFP), a basal ganglia-thalamocortical circuit, which is highly homologous to the mammalian counterpart. The AFP drives necessary motor exploration for vocal learning and includes song nucleus Area X that contains both striatal and pallidal neuron types. Several neurophysiological studies described the behavior of Area X neurons either in awake singing birds or in anesthetized birds exposed to playback of the bird's own song (BOS). However, the integrated sensorimotor properties of Area X neurons in awake birds have not yet been characterized. To address this issue, we recorded the firing patterns in different types of Area X neurons in awake and freely moving male zebra finches using chronically implanted single high impedance electrodes. Stable recordings were obtained from four striatal and two pallidal neuron classes while birds were singing and while they were subsequently exposed to their own songs that we broadcast in the dark through a loudspeaker. All six neuron classes were responsive during singing and mostly showed increased activity shortly before song onset. Two striatal neuron classes were almost exclusively spiking during song, whereas the remaining four classes were also tonically active when the bird was silent. Interestingly, three of these classes also responded to BOS playback, including the pallidal GPi-like neurons, the presumed only output neurons of Area X. With repeated BOS playback and starting roughly 0.5 s after playback onset these neurons developed a more structured, less tonic firing pattern, defined by increases in bursting density and duration of pauses. This sensory-evoked activity pattern qualitatively resembled the singing related activity. Our results demonstrate that Area X neuron firing is modulated by sensory stimulation. Understanding the structure of this auditory responsiveness may provide a better insight into how auditory feedback is used by Area X to guide vocal learning.

Disclosures: **L. Kolb:** None. **A. Hanuschkin:** None. **C. Scharff:** None. **R. Hahnloser:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

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Program#/Poster: 633.03/JJ26

Topic: D.15. Basal Ganglia

Support: Hereditary Disease Foundation (RM)

NIH Grant DC02524 (RM)

Astellas Foundation Fellowship (MT)

Title: Expressing mutant Huntingtin in the songbird basal ganglia increases song variability

Authors: *M. TANAKA[1], M. MURUGAN[2], R. MOONEY[1];
[1]Duke Univ. Sch. of Med., Durham, NC; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstracts: The basal ganglia (BG) play important roles in learning, initiating, and terminating complex motor sequences. Consequently, genetic mutations that impair BG function often produce serious motor disorders. Huntington's disease (HD), which is caused by mutations with expanded CAG repeats in exon 1 in the *Huntingtin* gene (*HTT*), induces degeneration of BG neurons and causes various motor disorders, which include increased involuntary movements and impaired speech. Although speech impairment can be a severe problem for individuals afflicted with HD, how this disease disrupts speech remains poorly understood. Here we made a model of Huntington's disease in songbirds, one of the few non-human animals that produce complex learned vocal sequences to communicate with others of their species. We injected a lentivirus containing *HTT* exon 1 with expanded CAG repeats (i.e., mutant *HTT*) into a region (Area X) of a songbird cortico-BG pathway necessary for juvenile song learning and adult modulation of learned songs. Although adult zebra finches normally sing songs comprising highly stereotyped sequences of syllables, expressing mutant *HTT* in Area X increased syllable sequence variability and the amount a bird would sing each day. In contrast, the spectral features of individual syllables remained stable, indicating that mutant *HTT* selectively disrupts the neural control of vocal sequences. Prior studies have shown that augmenting activity in the output of a cortico-BG pathway, the song premotor nucleus LMAN, which is downstream of Area X, can also increase syllable sequence variability. Here we found that pharmacologically silencing LMAN reversibly rescued the increased variability of syllable sequence in adult zebra finches

expressing mutant *HTT*. These findings advance the zebra finch as a useful model for understanding how mutant *HTT* alters BG circuitry to disrupt the generation and execution of complex motor sequences, including those necessary to speech.

Disclosures: M. Tanaka: None. **M. Murugan:** None. **R. Mooney:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.04/JJ27

Topic: D.15. Basal Ganglia

Support: NIDA Grant DA011064

Title: Effects of the 5-HT1BR agonist CP94253 on cocaine-induced locomotion before and after abstinence from repeated cocaine administration in C57BL/6 mice

Authors: *T. DER-GHAZARIAN[1], S. BRUNWASSER[2], K. DAI[2], N. PENTKOWSKI[2], J. NEISEWANDER[2];
[1]Sch. of Life Sci., [2]Arizona State Univ., Tempe, AZ

Abstracts: We previously showed that 5-HT_{1B} receptors (Rs) modulate cocaine abuse-related behavior in opposite directions depending on the addiction cycle phase. Specifically, either viral over-expression of 5-HT_{1B}Rs or a 5-HT_{1B}R agonist given during maintenance of cocaine self-administration enhances cocaine intake in rats. In contrast, following 21 days of forced abstinence these same manipulations decrease cocaine intake, as well as cocaine-primed and cue-elicited reinstatement of cocaine seeking, suggesting adaptations in 5-HT_{1B}R circuitry may be involved in the pathology of protracted relapse. This study investigated the effects of a 5-HT_{1B}R agonist on cocaine-induced locomotion before and after an abstinence period in C57BL/6 mice. To that end, we first treated mice daily with either saline or cocaine (15 mg/kg, IP) for 21 days. On the last day of treatment, after a 1- h habituation period in test chambers, mice received either vehicle or CP94253 (10 mg/kg, IP) and were returned to their home cage for 30 min. Next, mice were injected with saline or cocaine (5 mg/kg) and placed immediately into test chambers for 1 h. The same test session was repeated after 21 days of abstinence. During the treatment phase (i.e., test 1), CP94253 enhanced cocaine hyperlocomotion, whereas after a 21-day abstinence period (i.e., test 2) CP94253 reversed cocaine hyperlocomotion. These findings suggest that 5-

HT_{1B}Rs may offer a novel approach to treating cocaine dependence as the treatment produces anti-cocaine effects during abstinence from chronic cocaine administration.

Disclosures: T. Der-Ghazarian: None. **S. Brunwasser:** None. **K. Dai:** None. **N. Pentkowski:** None. **J. Neisewander:** None.

Poster

633. Systems Physiology and Behavior

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Topic: D.15. Basal Ganglia

Support: ANR-09-MNPS-028-01

ANR 2010-NEUR-005-01

Fondation France Parkinson

CECAP

Ministry of Education and Research

Title: The subthalamic nucleus keeps you high on emotion: Behavioral consequences

Authors: *Y. PELLOUX, J. MEFFRE, E. GIORLA, C. BAUNEZ;
Inst. de Neurosciences Timone, INT CNRS UMR7289, Marseille, France

Abstracts: The subthalamic nucleus (STN) belongs to the basal ganglia and is the current target for the surgical treatment of neurological and psychiatric disorders such as Parkinson's Disease (PD) and obsessive compulsive disorders, but also a proposed site for the treatment of addiction. It is therefore very important to understand its functions in order to anticipate and prevent possible side-effects in the patients. Although the involvement of the STN is well documented in motor, cognitive and motivational processes, less is known regarding emotional processes. Here we have investigated the direct consequences of STN lesions on emotional processing and reinforcement in the rat. We have used various behavioral procedures to assess affect for neutral,positive and negative reinforcers in STN lesioned and sham control rats. While STN lesions had no effect on responses for a neutral reinforcer (novelty induced place preference), they reduced affective responses for positive (sweet solutions) and negative (electric foot shock,

Lithium Chloride-induced sickness) reinforcers. Furthermore, when given the choice between a bland caloric glucose solution with sweet but non caloric saccharine solution, STN lesioned animals preferred glucose over saccharine, in contrast to sham animals. Taken altogether these results reveal that STN plays a critical role in emotional processing. These results, in line with some clinical observations in PD patients subjected to STN surgery, suggest possible side-effects of treatments targeting the STN. They could also be responsible for the decreased motivation for cocaine reported after STN lesions.

Disclosures: **Y. Pelloux:** None. **J. Meffre:** None. **E. Giorla:** None. **C. Baunez:** None.

Poster

633. Systems Physiology and Behavior

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Topic: D.15. Basal Ganglia

Support: NINDS Grant NS064984

NINDS Grant NS078435

Title: Optical tagging of striatal medium spiny neurons

Authors: ***A. V. KRAVITZ**[1,2], S. F. OWEN[3], A. C. KREITZER[3];
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[3]Gladstone Inst. of Neurolog. Dis., San Francisco, CA

Abstracts: The striatum integrates information from cortical, thalamic, and mesolimbic inputs to select and guide actions. There are two main projections from the striatum: one to the substantia nigra pars reticulata (SNr), comprised of direct pathway medium spiny neurons (dMSNs), and one to the external segment of the globus pallidus (GPe), made up of indirect pathway medium spiny neurons (iMSNs). Classic models of basal ganglia function suggest that dMSNs facilitate specific motor programs, whereas iMSNs inhibit competing motor programs. Many electrophysiological studies have examined the relationship between striatal activity and movement, particularly with respect to reward-directed movements. Historically, it was impossible to distinguish striatal dMSNs from iMSNs in such recordings using electrophysiological criteria alone; therefore most studies of the striatum have analyzed the two populations together. In recent years, the technique of "optical tagging" has been used to identify

specific cell types in *in vivo* recordings. Briefly, this involves expressing channelrhodopsin-2 (ChR2) in a specific cell type, and identifying ChR2-expressing cells by their response to light. Despite the apparent simplicity of this approach, we encountered multiple potential confounds when applying this approach to identifying dMSNs and iMSNS. Here, we discuss advances and difficulties in applying this technique to dMSNs and iMSNs in striatal recordings.

Disclosures: **A.V. Kravitz:** None. **S.F. Owen:** None. **A.C. Kreitzer:** None.

Poster

633. Systems Physiology and Behavior

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.07/JJ30

Topic: D.15. Basal Ganglia

Support: NIH Intramural Program

Title: Comparison of different powers and frequencies in optogenetic stimulation of striatal direct and indirect pathways

Authors: ***D. M. FRIEND**[1], A. KRAVITZ[2];
[1]Interdepartmental Program in Neurosci., NIH, Washington, DC; [2]NIDDK/NIDA, NIH, Bethesda, MD

Abstracts: Although optogenetic stimulation and inhibition has become a standard technique in behavioral neuroscience, few studies have quantitatively explored specific behavioral output in response to varying light powers or stimulation frequencies. Here, we examined motor output in animals with Channelrhodopsin-2 targeted specifically to striatonigral (direct pathway) or striatopallidal (indirect pathway) medium spiny neurons. Animals were placed in behavioral chambers outfitted with LED optical stimulation and tracking software. A range of light powers and frequencies were randomly presented. Following the completion of optogenetic stimulation movement parameters including speed were calculated for the range of light powers and frequencies. As previous work has demonstrated, we show that optical stimulation of striatonigral medium spiny neurons results in increased motor output whereas optical stimulation of striatopallidal neurons inhibits motor output. However, we expand the present knowledge by developing motor output response curves for a range light powers and stimulation frequencies. These data provide a possible mechanism though which optogenetic parameters can be optimized for behavioral experiments, providing a more complete description of behavioral output to a

range of light power and frequencies. Furthermore, these results may also provide novel insight into disease states where altered striatonigral and striatopallidal activity underlies symptoms of the disease.

Disclosures: D.M. Friend: None. **A. Kravitz:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.08/JJ31

Topic: D.15. Basal Ganglia

Support: NIH Intramural funding

Title: Optogenetic stimulation of dorsal striatal indirect pathway neurons increases anxiety

Authors: *K. H. LEBLANC[1], D. M. FRIEND[1], A. V. KRAVITZ[1,2];
[1]NIH/NIDDK, Bethesda, MD; [2]NIH/NIDA, Baltimore, MD

Abstracts: It has been well established that activation of the indirect pathway of the basal ganglia inhibits movement. However, indirect pathway medium spiny neurons (iMSNs) also play a role in negative affect. Stimulation of iMSNs in the dorsal striatum has been shown to be aversive, and a number of disorders involving the striatal dopamine system, such as Parkinson's disease, addiction, and obesity, are comorbid with anxiety. Based on this evidence, we hypothesized that iMSNs may respond to anxiety, and thus stimulating these neurons may have anxiogenic effects. Using *in vivo* electrophysiology we found that the firing rate of a subset of iMSNs in the dorsal striatum was substantially higher in either the open or closed arms of the elevated zero maze, suggesting that the firing of these neurons may be linked to anxiety state. Using optogenetic techniques, we have found that directly stimulating iMSNs in the dorsal striatum not only decreases movement but also induces anxiety as demonstrated by a reduction in the amount of time spent in the open arms of an elevated zero maze. This reduction of time in the open arms is separate from the movement effect, since limiting analysis to periods of movement still reveals a decrease in the percentage of time spent in the open arms. Our results indicate a potential role of indirect pathway neurons in the striatum in anxiety, which could have serious implications for a number of disorders, including Parkinson's disease, OCD, addiction and obesity.

Disclosures: **K.H. Leblanc:** None. **D.M. Friend:** None. **A.V. Kravitz:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.09/JJ32

Topic: D.15. Basal Ganglia

Support: DA006886

Title: Ivestigating activity of striatal Fast-Spiking Interneurons using single unit recordings in rat

Authors: *J. KULIK, K. COFFEY, J. STAMOS, M. WEST;
Rutgers Univ., Piscataway, NJ

Abstracts: The striatum is the largest structure of the basal ganglia and functions as its main input relay. The principal neurons of striatum, Medium Spiny Neurons (MSNs), constitute about 95% of striatal neural population. The remaining 5% of neurons are made up by striatal interneurons. Striatal Fast Spiking Interneurons (FSIs) are parvalbumin positive neurons that contribute less than 1% to the total striatal population. It is becoming increasingly evident that these striatal interneurons play an important role in mechanisms by which competing striatal output is selected. Striatal FSIs are hypothesized to be a main source of inhibition for MSNs. They display a strong medial-lateral distribution gradient, which suggest that they are important for functioning of dorsolateral MSNs which receive direct synaptic input from regions of cortex representing discrete body parts. The aim of the current study was to determine whether it is possible to identify FSIs recorded with extracellular chronically implanted microwires in awake behaving animals and assess whether these identified FSIs display body part sensitivity. Body part sensitivity of recorded neurons was assessed using the same sensorimotor exam that identifies sensitivity of striatal MSNs. Multi-label immunoflourescnece histology was performed to identify parvalbumin positive cells. It was found that staining for parvalbumin can serve as a useful tool for determining the type of neuron recorded with extracellular microwires. A proportion of neurons that expressed characteristics typical of FSIs also showed body part sensitivity. This suggests that striatal interneurons also receive direct cortical input and that dorsolateral striatal interneurons participate in generation of motor output by striatum

Disclosures: **J. Kulik:** None. **K. Coffey:** None. **J. Stamos:** None. **M. West:** None.

Poster

633. Systems Physiology and Behavior

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Topic: D.15. Basal Ganglia

Support: NIH EY07391

NIH F31NS078838

Thomas Hartman Center for Parkinson's Research

Title: The role of basal ganglia beta band oscillations in blinking

Authors: *L. EVINGER[1], J. KAMINER[2], P. THAKUR[3], P. ENMORE[3];
[1]Neurobio. & Behavior, [3]Program in Neurosci., [2]Stony Brook Univ., Stony Brook, NY

Abstracts: Synchronized, exaggerated beta band oscillations in the basal ganglia-cortical networks are characteristic of Parkinson's disease (PD). The correlations of movement planning and movement cues with beta oscillations make it difficult to establish a causal relationship between exaggerated beta band oscillations and the abnormalities in voluntary movements with PD. Because reflex movements occur independently of modulations in beta band strength, however, it is possible to determine whether frequency specific oscillations cause PD reflex abnormalities. We investigated the causal role of beta band activity in the abnormal reflex movements of PD by testing the effects of beta frequency subthalamic nucleus deep brain stimulation (STN DBS) on blink reflex excitability, amplitude, and plasticity in **normal** rats. Delivering 16 Hz STN DBS produced the same increase in trigeminal reflex blink excitability, reduction in reflex blink amplitude, and impairment in blink reflex plasticity in normal rats as occurs in PD patients and rats with 6-OHDA lesions. These deficits were not an artifact of STN DBS because 130 Hz STN DBS did not affect blink characteristics. To demonstrate that the blink reflex abnormalities created by beta frequency STN DBS stimulation were frequency specific, we tested the same rats with 7 Hz STN DBS, a theta band frequency typical of dystonia. In contrast to beta stimulation, 7 Hz STN DBS increased reflex blink excitability, elevated reflex blink amplitude, and exaggerated blink reflex plasticity as occurs in the focal dystonia benign essential blepharospasm. Unlike the frequency specific modifications in blink reflex behavior and motor learning, however, neither beta nor theta band STN DBS modified spontaneous blinking in normal rats. Beta and theta STN DBS can create PD- or dystonic-like abnormalities

in the blink reflex and motor learning circuits of normal rats, but spontaneous blinking must utilize a different circuit.

Disclosures: **L. Evinger:** None. **J. Kaminer:** None. **P. Thakur:** None. **P. Enmore:** None.

Poster

633. Systems Physiology and Behavior

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Topic: D.15. Basal Ganglia

Support: NIH grant 021074

NSF GRFP

Title: The role of the substantia nigra in licking behavior

Authors: ***M. A. ROSSI**, J. W. BARTER, H. H. YIN;
Duke Univ., Durham, NC

Abstracts: The substantia nigra pars reticulata, a major output nucleus of the basal ganglia, is thought to be involved in regulating a variety of behaviors including orofacial movements, but the specific relationship between neural activity in the substantia nigra and licking behavior remains unclear. To understand the role of basal ganglia outputs in licking and drinking behavior, we performed chronic, multi-electrode extracellular recordings from substantia nigra neurons as freely behaving mice licked for sucrose solution. We found two opposing populations of putative GABAergic projection neurons in the substatia nigra pars reticulata with activity reflecting the pattern of licks. One population of neurons was excited at the time of the lick, while the second population was excited just before each lick but inhibited at the time of lick. These two neuronal populations therefore show antiphasic activity during licking behavior. To test whether these neurons were involved in patterning the orofacial movements associated with licking, we selectively expressed channelrhodopsin-2 in GABAergic neurons of the substantia nigra. Stimulation of these neurons at the onset of a bout of licking disrupted licking behavior.

Disclosures: **M.A. Rossi:** None. **J.W. Barter:** None. **H.H. Yin:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.12/JJ35

Topic: D.15. Basal Ganglia

Support: Howard Hughes Medical Institute Janelia Group Leader

Title: Suppression of basal ganglia output selectively impairs the vigor of reaching movements in mice

Authors: K. MARTIN[1], J. BROWN[1], *J. T. DUDMAN[2];
[1]Janelia Farm Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; [2]HHMI, ASHBURN, VA

Abstracts: Animals learn to perform arbitrary, purposive actions with the appropriate timing and vigor to efficiently obtain desired outcomes. The basal ganglia are a collection of subcortical nuclei that have been proposed to play diverse roles in the selection and specification of purposive movements as well as processing signals about reward and timing. However, these aspects of purposive behaviors are frequently correlated making it difficult to disambiguate specific roles of basal ganglia function. Perturbation experiments in primates performing goal-directed reaching tasks have shown that suppression of the basal ganglia output nuclei, the internal globus pallidus (GPi) and substantia nigra pars reticulata (SNr), cause reductions in movement vigor. By contrast, analogous experiments in rodent models have tended to reveal changes in the abundance of movement. We reasoned that these differences could stem (1) from differences in the tasks that the subjects were asked to perform during perturbation; and/or (2) the manipulations performed in primates have been done with pharmacological inactivation whereas experiments in rodents have often confounded movement parameters. To address these issues, we developed a task in which reward receipt was a function of the vigor of lever pressing in head fixed mice. We then used cell-type specific expression of a pharmacogenetic inhibitor to specifically suppress activity in the SNr during behavior. Consistent with prior results in the primate we reliably observed a primary effect on the vigor of purposive, lever pressing movements with relatively little effect on the total amount of behavior or the ability to collect rewards efficiently. However, we also observed changes in the timing of movements unlike recent observations in the primate. We suggest that the observed changes in the timing of actions in our experiments could either reflect the necessity of cell-type specific manipulations that have not been possible in prior experiments or could reflect compensatory strategies to maintain stable reward rates in the face of perturbed motor function. Taken together our results are consistent

with a role for the basal ganglia in the specification of parameters of purposive behaviors when required to obtain stable, high reward rates.

Disclosures: **K. Martin:** None. **J. Brown:** None. **J.T. Dudman:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.13/JJ36

Topic: D.15. Basal Ganglia

Title: Manipulation of specific movement parameters through pathway selective optogenetic stimulation of the basal ganglia

Authors: *E. A. YTTRI, J. T. DUDMAN;
Janelia Farm - HHMI, Ashburn, VA

Abstracts: The basal ganglia have been proposed to be involved in diverse aspects of voluntary, purposive movements including initiation and termination, selection, valuation, and/or specification of movement parameters. Projections from many areas, including motor or prefrontal cortex, converge in the striatum, the main input nucleus of the basal ganglia. The output neurons of the striatum, medium spiny neurons (MSN) expressing either D1 or D2 receptors, give rise to the direct (dMSN) and indirect (iMSN) pathways, respectively. A common feature of all theories of basal ganglia function mentioned above is that the balance of indirect and direct pathway activity is critical for control of voluntary movement. Using cell type specific gene expression and optogenetics in dMSN and iMSNs we can selectively activate either basal ganglia pathway. We therefore sought to test if the pathway-specific activation could elicit the specification of a movement parameter - namely velocity. We developed a reaching task in which we could monitor multiple movement parameters of single, discrete reaches in a head-fixed mouse. We then used a closed loop paradigm to photostimulate dMSNs or iMSNs in real time during the subset of reaches with suprathreshold velocities. Photostimulation corresponding to only the quickest reaches led to a progressive shift in the mean velocity of subsequent, unstimulated reaches. dMSN stimulation induced an increase in reach velocities while iMSN stimulation induced a decrease of reach velocities. Upon removal of photostimulation, distribution of reach velocities gradually returned to baseline. Importantly, in an open loop paradigm we failed to elicit reach initiation or termination with stimulation of either pathway. These results demonstrate that the basal ganglia can specify a particular component of movement and strongly suggest a role for the basal ganglia in the regulation of movement vigor.

Disclosures: **E.A. Yttri:** None. **J.T. Dudman:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.14/KK1

Topic: D.15. Basal Ganglia

Title: A center-out joystick task for quantifying motor variability in mice

Authors: T. BOLLU, *J. H. GOLDBERG;
Dept. of Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstracts: Exploratory variability is a key component of trial and error learning, but the brain circuits that generate and/or control it remain poorly understood. Recent data from songbirds indicate that specific parts of dopamine-basal ganglia-cortical loops actively introduce variability into juvenile song [1,2], raising the possibility that homologous mammalian motor circuits are endowed with similar 'variability-generating' functionalities [3]. New genetic and optical methods make mice an ideal model system for dissecting mammalian circuits for motor control [4,5], but the expansion in experimental tools has not been matched by an expansion of behavioral paradigms for motor learning. To address this issue, we adapted a center-out joystick task, commonly deployed in primates [6,7], for use in mice. Mice learned to move custom-made joysticks for reward, allowing forelimb trajectories to be monitored and shaped with high (micron, sub-millisecond) spatiotemporal resolution. Because the joysticks were integrated into computer-controlled, rack-mountable home cages [8], our setup can be scaled up for high throughput behavioral experiments, and can thus be an effective platform for studying neural circuits for motor variability and learning. - 1. Kao MH, Doupe AJ, Brainard MS (2005) Contributions of an avian basal ganglia-forebrain circuit to real-time modulation of song. Nature 433: 638-643. 2. Olveczky BP, Andalman AS, Fee MS (2005) Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. PLoS Biol 3: e153. 3. Murugan M, Harward S, Scharff C, Mooney R (2013) Diminished FoxP2 Levels Affect Dopaminergic Modulation of Corticostriatal Signaling Important to Song Variability. Neuron 80: 1464-1476. 4. Gerfen CR, Paletzki R, Heintz N (2013) GENSAT BAC Cre-Recombinase Driver Lines to Study the Functional Organization of Cerebral Cortical and Basal Ganglia Circuits. Neuron 80: 1368-1383. 5. Azim E, Jiang J, Alstermark B, Jessell TM (2014) Skilled reaching relies on a V2a propriospinal internal copy circuit. Nature 508: 357-363. 6. Georgopoulos AP, Kalaska JF, Caminiti R, Massey JT (1982) On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex. J Neurosci 2: 1527-1537. 7. Slutzky

MW, Jordan LR, Bauman MJ, Miller LE (2010) A new rodent behavioral paradigm for studying forelimb movement. J Neurosci Methods 192: 228-232. 8. Poddar R, Kawai R, Olveczky BP (2013) A Fully Automated High-Throughput Training System for Rodents. PLoS One 8: e83171.

Disclosures: T. Bollu: None. **J.H. Goldberg:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.15/KK2

Topic: D.15. Basal Ganglia

Support: European Research Council Grant STG 243393

Title: Substantia nigra dopaminergic neurons are critical for the initiation of self paced actions

Authors: *J. ALVES DA SILVA[1], F. TECUAPETLA[2], V. PAIXÃO[1], R. M. COSTA[1];
[1]Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisbon, Portugal;
[2]Inst. de Fisiología Celular, Univ. Nacional Autónoma de México, Mexico City, Mexico

Abstracts: We frequently initiate new actions and transitions between actions. Although vital to our survival, most of the time we are not aware of this orchestration of action sequences. However for people with Parkinson's disease initiating movement when they want becomes specially daunting. Among other changes in the brain, these patients present with a loss of substantia nigra compacta (SNC) dopaminergic neurons. Recently, phasic activity of these same neurons has been found to be correlated with the start and stop of a learned action sequence. However, it is still not clear if this activity is necessary for action initiation. We are using an optogenetic approach to evaluate the role of SNC dopaminergic neurons in the initiation of self paced movement in mice. This strategy enables us to specifically inhibit or activate dopaminergic neurons, with millisecond resolution, while analysing mice behavior using 3-axis accelerometers and video recordings. We have found that inhibiting SNC dopaminergic neurons while mice were exploring an open field, lead to a decrease in motion and an increase in the time spent immobile. However if the mice were moving before inhibition started, their motion did not change until they stopped for the first time. Likewise, the latency to stop was not different during inhibition. On the other hand, if the mice were not moving before the inhibition, they stayed immobile or in a low acceleration state during the period of inhibition. Mice were also trained in a self-paced operant task and developed a particular sequence of actions to obtain an outcome.

We found that inhibiting SNC dopaminergic neurons just before action initiation, delayed the start of the action sequence. However, if the inhibition started immediately after the action sequence was initiated, the performance of the action remained unchanged. In preliminary experiments, a very brief activation of SNC dopaminergic neurons was sufficient to promote movement when the mice were immobile in the open field. These observations suggest that SNC dopamine neurons are specifically necessary for the initiation of movement but not for the maintenance of ongoing movements. To corroborate these findings, we are recording the activity of genetically identified dopamine neurons during movement/action initiation.

Disclosures: J. Alves Da Silva: None. **F. Tecuapetla:** None. **V. Paixão:** None. **R.M. Costa:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.16/KK3

Topic: D.15. Basal Ganglia

Support: NSF GRFP

NIH Grant 021074

Title: Beyond reward prediction errors: Basal ganglia output and dopaminergic signaling correlates with movement kinematics

Authors: *J. W. BARTER, S. LI, T. SUKHARNIKOVA, M. A. ROSSI, R. BARTHOLOMEW, H. H. YIN;
Psychology and neuroscience, Duke Univ., Durham, NC

Abstracts: Using wireless recording from single neurons, we measured basal ganglia output from the substantia nigra pars reticulata and dopamine neurons in the neighboring pars compacta in freely moving mice, while quantifying their behavior with motion tracking. We found that the firing rate of nigral GABAergic output neurons reflected the Cartesian coordinates of the animal's head position in space, while the firing rate of nigral dopamine neurons reflected the acceleration of the animal's head in Cartesian space. GABA cells can be separated into two major groups: x-coordinate neurons and y-coordinate neurons. Together the firing rates of these two groups of GABA neurons can be used to accurately determine the (x,y) position of the

animal's head, while the activity of dopamine neurons is correlated with movement acceleration and deceleration, and can be used to determine movement velocity. Such correlations with kinematics, independent of outcome valence (rewarding or aversive), are found in the majority of recorded neurons (r as high as 0.99). Our results reveal the functional role of basal ganglia in controlling body position and movement. They suggest that previous results in support of the reward prediction error hypothesis for dopamine function can be explained by movement kinematics during the behavioral tasks. They have significant implications for understanding and treating movement disorders involving the basal ganglia, e.g. Parkinson's disease, which is characterized by postural deficits and slowness of movement.

Disclosures: J.W. Barter: None. **S. Li:** None. **T. Sukharnikova:** None. **M.A. Rossi:** None. **R. Bartholomew:** None. **H.H. Yin:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

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Program#/Poster: 633.17/KK4

Topic: D.15. Basal Ganglia

Support: NIH Grant NS064577

NIH Grant 5T32GM008441-22

McKnight Endowment Neuroscience Award

Title: Striatal substrates for making and breaking habits

Authors: *J. O'HARE[1,2], K. ADE[1,2], T. SUKHARNIKOVA[3], H. YIN[3], N. CALAKOS[1,2]; [1]Neurobio., [2]Neurol., Duke Univ. Med. Ctr., Durham, NC; [3]Psychology & Neurosci., Duke Univ., Durham, NC

Abstracts: Habit formation is an adaptive form of motor learning employed broadly in the animal kingdom. Habitual behavior reduces attentional and computational demands for routine activities. However, it is also believed that mechanisms underlying habit formation may be co-opted by drugs of abuse and disrupted in neuropsychiatric diseases leading to harmful compulsive behavior. A mechanistic understanding of habit formation therefore stands to benefit a broad population by providing insights to a basic brain learning mechanism as well as informing development of treatments to facilitate adaptive habit learning and/or extinguish

pathological compulsions. Habit formation is known to require the dorsolateral striatum (DLS) and is accompanied by changes in firing activity in both DLS and cortical regions *in vivo*. Although DLS is required and its activity is altered *in vivo*, it is not known whether habit-related changes in DLS activity are a read-out of altered afferent activity or driven by plasticity local to the striatal microcircuit. To determine whether local striatal adaptations that alter striatal output are associated with habit formation, we examined action potential firing properties of striatal projection neurons (SPNs) in response to controlled activation of cortical inputs *ex vivo* in mice trained to varying degrees of habitualness using established lever press protocols. Firing properties were simultaneously measured in dozens of DLS projection neurons of defined projection type using a combination of BAC transgenics, calcium imaging and two photon laser scanning microscopy. We found that properties of DLS SPN firing correlated strongly with habitual behavior. Moreover, training aimed at extinguishing the learned habitual behavior eliminated habit-correlated activity changes. In some cases, inverse correlations emerged. Taken together, these findings indicate that the neural substrate for habit expression may reside locally in the striatal microcircuitry and provide general support for contemporary models of motor learning in the basal ganglia.

Disclosures: **J. O'Hare:** None. **K. Ade:** None. **T. Sukharnikova:** None. **H. Yin:** None. **N. Calakos:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.18/KK5

Topic: D.15. Basal Ganglia

Support: NIH grant AA021074

Title: Cell type specific coding of movement kinematics in the sensorimotor striatum

Authors: ***H. H. YIN**, N. S. KIM, J. BARTER;
Dept. of Psychology and Neurosci., Duke Univ., DURHAM, NC

Abstracts: Using wireless multi-electrode recording, we measured single-unit activity from neurons in the sensorimotor or dorsolateral striatum in mice, while monitoring their movement kinematics in real time with motion capture. We recorded from three types of neurons, which are classified according to their waveform, firing rate, and inter-spike-interval distribution: putative

medium spiny projection neurons, putative fast-spiking interneurons, and putative giant cholinergic interneurons. The firing rate of most recorded medium spiny neurons and fast-spiking interneurons reflects movement velocity, whereas the firing rate of most cholinergic interneurons reflects position in Cartesian coordinates (x and y). We are currently using 3D motion capture to study the correlations with movement in the additional z-axis. These results show for the first time cell-type specific "coding" of movement kinematics in the striatum, as proposed by a recent model (Yin, 2014). Yin HH (2014) Action, time and the basal ganglia. Philosophical Transactions of the Royal Society B: Biological Sciences 369:20120473.

Disclosures: H.H. Yin: None. **N.S. Kim:** None. **J. Barter:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.19/KK6

Topic: D.15. Basal Ganglia

Support: ANR-10IAIHU-06

ATIP-Avenir

Title: The mesencephalic locomotor region integrates motor, cognitive, and emotional information: An anatomical substrate for differential roles of the pedunculopontine and the cuneiform nuclei

Authors: C. KARACHI[1,2], *B. LAU[1], A. ANDRÉ[2], D. TANDÉ[1], E. C. HIRSCH[1], C. FRANÇOIS[1];
[1]Inst. du cerveau et de la moelle épinière, Paris, France; [2]Dept. of Neurosurg., AP-HP, Hôpital de la Pitié-Salpêtrière, Paris, France

Abstracts: The mesencephalic locomotor region, including the pedunculopontine (PPN) and cuneiform nuclei (CuN), is thought to have a central role in gait and postural control. The MLR is also implicated in functions including sleep, attention, and emotion. We used tract-tracing in monkeys to understand how the inputs and outputs of the MLR relate to these diverse functions. We show the PPN receives inputs from motor, associative and limbic cortices, and projects back to the motor, associative and limbic territories of the basal ganglia and thalamus, as well as the substantia nigra pars compacta. In contrast, the CuN receives inputs exclusively from associative

and limbic cortices, and projects back to the limbic territory of the basal ganglia and thalamus, as well as the ventral tegmental area, basal forebrain and amygdala. Our findings suggest the PPN integrates motor, cognitive, and emotional information, whereas the CuN integrates cognitive and emotional, but not motor, information.

Disclosures: C. Karachi: None. **B. Lau:** None. **A. André:** None. **D. Tandé:** None. **E.C. Hirsch:** None. **C. François:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.20/KK7

Topic: D.15. Basal Ganglia

Support: SNSF - SYNERGIA Programm

Labex - Cortex

FRM

Title: Active avoidance in non-human primate, a behavioural paradigm to investigate neural bases and serotonin modulation effects on anxiety related disorders

Authors: *G. DRUI, Y. SAGA, A. RICHARD, V. SGAMBATO-FAURE, L. TREMBLAY; CNRS UMR5229 - Ctr. of Cognitive Neurosci., BRON, France

Abstracts: Each day, beyond the behaviors associated with positive outcomes (approach towards appetitive stimuli), a lot of stimuli or events is associated to a negative outcome and induces aversive anticipation to produce avoidance behaviors. Serotonin has been linked to anxiety as well as impulsivity behaviors, thus highlighting this neuromodulator in the aversive processes. To study the neurobiological and pharmacological mechanisms implied in aversive information processing, we have developed an update version of the classical instrumental delay task. The monkeys were trained to recognize and associate conditioned visual stimuli (CS) with appetitive outcome (juice) and visual CS with aversive outcome (air puff) in two different conditions. In a choice condition, on every trial, two opposite CS are presented simultaneously and the animal could select an action between approaching the appetitive and actively avoid the aversive outcome. In opposite, for imperative condition, one of the CS is presented, so they must either approach the appetitive stimulus or actively avoid the aversive ones, depending on the CS

presented. Thus, this task will allow us to determine the cerebral structures involved in the value-based decision making in appetitive or aversive contexts and their specific roles during different processes (CS presentation, decision and the delay periods). Moreover, the intrinsic value of stimuli in the behavioral response is also evaluated by using different type of motivational domain (e.g natural, social and learned stimuli). We showed that monkeys performed approach for appetitive trials (in imperative and choice context) and avoidance for aversive trials. Progressively during the daily session, the strategy of the animals to the aversive stimuli is modified and they produce premature responses facing negative CS, which could be interpreted as an escape reaction. Interestingly, we can retard this type of response by pharmacological manipulations of the serotonergic system. This task could allow the investigation of the role of the cerebral structures as ventral striatum (see Richard et al., *SFN* 2014) and the neuromodulator systems involved in aversive processes lays the foundation for a better understanding of the pathophysiology of psychiatric disorders such as obsessive compulsive disorders, panic disorder, depression, and anxiety, where anticipation, detection, and avoidance of aversive events or contexts appear to guide decisions and behaviors.

Disclosures: G. Drui: None. **Y. Saga:** None. **A. Richard:** None. **V. Sgambato-faure:** None. **L. Tremblay:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

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Program#/Poster: 633.21/KK8

Topic: D.15. Basal Ganglia

Support: Fondation Recherche Médicale

Labex - Cortex

SNSF - Sinergia Programm

Title: Characterization of aversive-related neuronal activity and negative motivation disorders induced by local dysfunction inside ventral striatum in monkey

Authors: *A. RICHARD[1], Y. SAGA[1], G. DRUI[1], E. HOSHI[2,3], V. SGAMBATO-FAURE[1], L. TREMBLAY[1];

[1]Ctr. Des Neurosciences Cognitives, Bron Cedex, France; [2]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [3]CREST, JST, Tokyo, Japan

Abstracts: The ventral striatum, which belongs to a limbic circuit, is involved in reward processing. Human brain imaging and animal pharmacological studies have suggested that ventral striatum is also involved in aversive information processing. To investigate the striatum implication in aversive encoding and behavioral disorders that characterized negative motivation dysfunctions, we recorded its neuronal activities and disturbed its different parts. Two monkeys were trained to perform two versions of a delay task (see Drui et al., SFN 2014) in which they had to associate visual conditioned stimuli (CS) with appetitive (juice) or aversive outcome (air puff). In the choice context, on every trial, the monkey can choose between approaching the appetitive stimulus and avoiding the aversive one. In imperative context, only one CS is presented by trial, so the monkey must either approach the appetitive stimulus or actively avoid the aversive one. Monkeys performed approach in positive CS trials (imperative and choice task) and active avoidance in the majority of negative CS trials. Among anterior striatum neurons exhibiting a task related activity, a large percentage (5-15% depending on the analysis period) of them encoded selectively or preferentially negative value during CS presentation or aversive outcome delivery and also before the aversive CS, an aversive anticipation. Interestingly, these neurons could be expressed and dissociated from the appetitive ones only in imperative context when monkeys have to actively avoid aversive outcome, but not in choice context. Moreover, these negative value neurons were not only located in the ventral and central part of the striatum, as expected on the basis of local disturbances study (Worbe et al., 2009), but widely distributed in various parts of the anterior striatum. Finally, results from reversible perturbations into different part of the anterior striatum strongly support the involvement of this structure in the negative stimulus value encoding as well as in learning how to avoid predicted negative outcomes. Indeed, the majority of local perturbations into the anterior striatum induced difficulties only to perform active avoidance in the aversive context. The monkeys produced premature responses or omissions which seemed to be underpinned by hypersensitivity toward negative CS stimuli and induced the escape reactions. All these results suggest that the whole anterior striatum encodes positive and negative information and plays a crucial role in controlling, not only involved in approach for reward, but also in active avoidances from predictable aversive events or contexts.

Disclosures: **A. Richard:** None. **Y. Saga:** None. **G. Drui:** None. **V. Sgambato-Faure:** None. **L. Tremblay:** None. **E. Hoshi:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

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Program#/Poster: 633.22/KK9

Topic: D.15. Basal Ganglia

Support: Army Research Laboratory under Cooperative Agreement Number W911NF-10-2-0022

PA Department of Health Formula Award #SAP4100062201

Title: Parcellating the internal and external globus pallidus using diffusion based clustering

Authors: *P. BEUKEMA[1,2], T. VERSTYNEN[3];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA;
[3]Carnegie Mellon Univ., Pittsburgh, PA

Abstracts: The internal and external segments of the globus pallidus have been difficult to distinguish in human neuroimaging due to poor visualization of nuclear boundaries with standard structural MRI based approaches. Segmentation of other sub-cortical nuclei has been shown using k-means clustering on the gray matter diffusion signal [Deoni et al. NeuroImage., 2007]. Similar diffusion weighted imaging (DWI) approaches may be useful for isolating the segments of the pallidal nuclei as well. Using an MNI-space orientation distribution function (ODF) reconstruction method [Yeh & Tseng, NeuroImage., 2011] on diffusion spectrum imaging data from 60 neurologically healthy adults (29 male, mean age = 26 years), we examined the diffusion structure of the internal and external segments of the globus pallidus using two metrics: quantitative anisotropy (QA) and orientation of the peak fibers in the ODF. Using euclidean distance, we segmented the pallidum into its approximate internal and external segments. For each segment, we then extracted the QA values for both the primary and secondary fibers as well as the fiber directions in the axial, coronal, and sagittal planes. We found that QA of both the primary and secondary fibers is higher in the GPe relative to the GPi. In the axial plane, orientation of the secondary fibers peaked at +/- 1.5 radians in the GPi whereas the GPe lacked any significant peaks. In the coronal and saggital planes, the distribution of the primary and secondary fibers largely overlapped. Using k-means clustering on euclidean distance, QA and peak fiber orientation, we generated probabilistic maps of the left GPi and GPe. The volume of the GPi is approximately 40% of the volume of the GPe, similar to others estimates of the relative sizes of the two nuclei in humans [Keuken M.C. et al. NeuroImage, 2014]. These results show that DWI can provide an unbiased and semi-automatic parcellation of the pallidum which will benefit future fMRI and structural studies of the basal ganglia pathways.

Disclosures: P. Beukema: None. **T. Verstynen:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.23/KK10

Topic: D.15. Basal Ganglia

Support: PA Department of Health Formula Award #SAP4100062201

Title: Differentiating serial cue prediction from motor sequence learning during long term skill training

Authors: *B. T. LYNCH[1], A. TING[1], S. WILHELMI[2], D. MARCHETTO[1], T. VERSTYNEN[1]; [1]Psychology, [2]Biol., Carnegie Mellon Univ., Pittsburgh, PA

Abstracts: Acquiring a novel sensorimotor sequence requires either learning a serial order of response cues or binding a sequence of motor actions together, or both. Using an indirect cuing version of the serial reaction time (SRT) task, we measured the independence of cue and response learning across a 5-day training period. On each trial, a centrally presented symbol (Cyrillic letters) cued subjects to press one of 4 keys on a keyboard with their right hand. On each day, the mapping from cue to key was pseudo-randomly assigned and subjects were trained to learn this new mapping. After two blocks (144 trials per block) of randomly ordered cues, subjects were trained on a hidden 12 item sequence for two blocks, followed by another random block and then a final sequence block. Subjects were randomly placed into two groups (N=15, 6 males per group). The Cue group was exposed to the same sequence of visual cues over all 5 training days. The Response group was exposed to different orders of visual cues but repeated the same sequence of key presses across days. Learning-related changes in response time showed a significant group-by-day interaction ($F(4,112)=3.72$, $p = 0.007$). Response times during sequence blocks, relative to random blocks, reached asymptote in the Cue group by Day 3, but no such asymptote was present in the Response group. Accuracy improved over time (main effect: $F(4,112)=9.06$, $p<0.001$) but both groups learned to improve their accuracy similarly across days (group-by-day interaction: $F(4,112)=2.30$, $p=0.064$). This advantage for learning consecutive cues did not appear to relate to knowledge of the sequence since both groups showed similar levels of explicit awareness, based on a post-hoc questionnaire. These results show that there is an immediate advantage to learning sequences of visual cues over sequences of actions during long term skill training.

Disclosures: **B.T. Lynch:** None. **A. Ting:** None. **S. Wilhelmi:** None. **D. Marchetto:** None. **T. Verstynen:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.24/KK11

Topic: D.15. Basal Ganglia

Support: Marie Curie International Reintegration Grant, IRG-230976

Avenir program from INSERM

Marie Curie International Incoming Fellowship, IIF253873

CONACyT, México

Title: Action monitoring by the striatum is critical for accurate execution of procedural memories

Authors: *P. E. RUEDA-OROZCO, D. ROBBE;
Inst. De Neurobiologie De La Méditerranée, Marseille, France

Abstracts: The striatum is crucial for the acquisition of procedural memories such as action sequences and motor skills but its role during the execution of already learned procedures is unclear. Here we created a task in which rats learned a running sequence with precise trajectory, running speed and acceleration timing. After training, tetrode recordings in the dorsolateral striatum (DLS) revealed robust representations of running speed, position and time but no correlates of acceleration. Speed and position representations were weak in naive rats performing the running sequence under guidance of the experimenter and increased after learning. Finally, DLS inactivation augmented variability of sequence execution. Altogether the data revealed a new moment-to-moment action monitoring function of the DLS operating through contextual and movement-related representations that is instrumental for accurate execution of learned action sequences. This finding provides a new framework to understand the contribution of the basal ganglia to motor control.

Disclosures: **P.E. Rueda-Orozco:** None. **D. Robbe:** None.

Poster

633. Systems Physiology and Behavior

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.25/KK12

Topic: D.15. Basal Ganglia

Support: PA Department of Health Formula Award #SAP4100062201

Title: The difference between stopping and deciding not to go: Behavioral, imaging and modeling evidence

Authors: *K. E. DUNOVAN[1,2], T. MOLESWORTH[3], T. VERSTYNEN[2,3];
[1]Psychology, Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; [3]Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstracts: While general inhibitory control relies on overlapping cortico-basal ganglia circuitry, the various subtypes of behavioral inhibition (e.g., stopping vs. go/no-go decisions) may reflect separable corticostriatal pathways (Swick. et al. NeuroImage 2011). We tested the behavioral and neural separability of two types of inhibitory control using a modified stop-signal task. Subjects were instructed to stop a rising bar when it intersected a target line (500ms after onset) by pressing a key. Subjects were instructed not to respond if the bar does not intersect the line. In Reactive stopping trials, the bar would stop at various intervals in its trajectory. In Proactive stopping trials, the bar would stop 50ms before intersecting the line and subjects were told to make a go/no-go decision based on a color cue indicating the probability that the bar will stop on any given trial. Behaviorally (N=61, 28 male) we found a weak correlation between stopping performance in Reactive and Proactive tasks ($r=0.24$, $p=0.03$, r-square = 0.06) suggesting that the ability plan a go/no-go decision is only weakly coupled with ability to suppress an unwanted action. In addition, performance in the Reactive task was modulated by reward contingencies, while Proactive task performance was not. Finally, event-related fMRI analysis (N=28, 7 male) showed that successful Proactive stopping differentially engaged rostral prefrontal areas in the superior frontal gyrus and anterior cingulate, while Reactive stopping engaged more caudal premotor and pre-SMA regions. From this behavioral and neural evidence, we propose a contingent two-stage decision model of behavioral inhibition. The model frames go/no-go decisions as a competitive drift-diffusion process in which the rate of evidence accumulation towards the go boundary is modulated by contextual factors (e.g.. probability or reward). In the event of a stop-signal, a second process is initiated in which a strong inhibitory signal must override the current level of evidence in order to suppress the response. This nested decision

model provides a plausible framework for how different executive processes interact during inhibitory control.

Disclosures: **K.E. Dunovan:** None. **T. Molesworth:** None. **T. Verstynen:** None.

Poster

633. Systems Physiology and Behavior

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Program#/Poster: 633.26/KK13

Topic: D.15. Basal Ganglia

Support: PA Department of Health Formula Award #SAP4100062201

Title: Highway from the Danger Zone: Interactions between uncertainty and cost in spatial estimation

Authors: *K. JARBO[1,2], R. FLEMMING[3], T. VERSTYNEN[1,2];
[1]Psychology, Carnegie Mellon Univ., Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstracts: Cost calculations in spatial sensorimotor tasks are made in a statistically optimal manner (Wu, Delgado, & Maloney, 2009); however, the accuracy and precision of this performance is affected by the saliency of the sensory cues (van Beers, Baraduc, & Wolpert, 2002). It remains unclear how cost estimation is influenced by sensory uncertainty. In two experiments with a novel paradigm, we evaluated the interaction between cost calculations and sensory reliability. Adult participants used a mouse to estimate the mean location of a target zone defined by a Gaussian distribution of white dots presented for 300ms in a random screen location. In some conditions, a Gaussian distribution of red dots that defined a distractor (i.e., danger zone) simultaneously was presented to the left or right of the target. In Experiment 1 (N=6), we examined whether spatial decisions are affected by the presence of a distractor at different levels of spatial uncertainty: low or high target zone variance versus low or high danger zone variance. We observed a significant selection bias away from the danger zone, $t(5) = -2.68$, $p = 0.022$, and decreased accuracy, $t(5) = -4.95$, $p = 0.002$, in the high variance condition, with no effect of the danger zone on target mean estimation in the low variance condition. Given that the distractor did not affect spatial selections at low target variance, in Experiment 2 we examined the effects of penalty and spatial uncertainty of the distractor. Reward gains and penalty losses decreased exponentially as a function of the spatial distance between the final selection and the mean of the target and danger zones, respectively. Participants (N=7) were tested under three conditions: low danger zone variance with no penalty, and low or high danger

zone variance with penalty. Only the high variance penalty manipulation resulted in a significant selection bias away from the danger zone, $t(6) = 3.01$, $p = 0.012$, and reduced accuracy, $t(6) = 4.13$, $p = 0.003$. Taken together, these results confirm that sensory uncertainty can bias cost calculation processes during spatial estimation, whereby people are more influenced by penalty costs in high variance contexts.

Disclosures: K. Jarbo: None. **R. Flemming:** None. **T. Verstynen:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.27/KK14

Topic: D.15. Basal Ganglia

Support: PA Department of Health Formula Award #SAP4100062201

Army Research Laboratory under Cooperative Agreement Number W911NF-10-2-0022

Title: Learning to stop or waiting to go: Targets of adaptive Bayesian updating during inhibitory control

Authors: *T. D. VERSTYNEN, L. SCHOLL, T. MOLESWORTH;
Carnegie Mellon Univ., Pittsburgh, PA

Abstracts: When planning an action, it is sometimes necessary to terminate an initiated motor program before it is fully executed. This ability to stop is adapted probabilistically based on previous experience (Shenoy & Yu, 2011). Here we evaluated whether this process reflects the acquisition of internal Bayesian priors for stopping and whether this adaptation occurs at the motor inhibition process or action selection (i.e., go) process. In a modified version of the stop-signal reaction time (SSRT) task, participants saw a rising bar approach a target line near the top of the computer screen. The bar intersected the line 500ms after trial onset and participants were told to stop the moving bar as close to the target line as possible by pressing a key (Go trials). On a subset of trials (31%), the bar stopped before hitting the target line. Participants were instructed to not press the key if the bar did not intersect the line (Stop trials). On a vast majority of Stop trials (71%) the bar stopped at a randomly selected time, sampled from one of 3 distributions: Uniform, Early gaussian (mean=250ms, std=35ms), Late gaussian (mean=350ms, std=35ms). These context trials were used to establish a temporal probability of stopping and subjects were

assigned to one context type (N=25 per group). The remaining Stop trials were used to estimate the SSRT, with preset stop times ranging from 200-400ms. Consistent with a normative Bayesian updating model, the SSRT was modulated by context trials ($F(2,74)=10.96$, $p<0.001$), with the Early group having the longest SSRT (217ms), followed by the Uniform (206ms) and Late (188ms) groups. However, contrary to learning a prior on the stop-signal itself, it appears that the contextually-mediated behavioral changes were driven by changes in the Go trial reaction time (RT) instead of the stop signal itself ($F(2,74)=10.07$, $p<0.001$): Early (RT=535ms), Uniform (RT=542ms), Late (RT=556ms). Our results indicate that adaptive priors are learned on the trigger to initiate an action, by either accelerating or delaying the go decision, rather than on the inhibitory systems themselves.

Disclosures: T.D. Verstynen: None. **L. Scholl:** None. **T. Molesworth:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 633.28/KK15

Topic: D.15. Basal Ganglia

Support: NSF SMA-0835976

Title: Simulating conditions in which striatal learning assigns behavior control to the fastest-computed reward-predictive representations of cues and contexts

Authors: *S. PATRICK[1], D. BULLOCK[2], A. GORCHETCHNIKOV[2], A. SOHAIL[2], M. VERSACE[2];
[1]Cognitive and Neural Systems, [2]Boston Univ., Boston, MA

Abstracts: Research has documented shifts during learning between the cortical representations that control behavior. A well-known case is the shift during maze learning from a place strategy, involving cognitions that depend on visibility of distal landmarks, to a habit/response strategy, involving only local cues. Whereas shifts to fast habitual behaviors occur when contingencies are stable, they need to be undone whenever contingency changes render formerly valid representations invalid as guides to rewarding behaviors. Viewed abstractly, the brain's action selection system needs to reassign behavior control to whatever representations are currently valid, and among those, to ultimately favor representations that can be computed in the shortest time, with least cost. Often the information that guides shifts is the pattern of behavior-dependent

reward occurrence and omission, which has systematic effects on dopamine signals. Research implicates different compartments of striatum in the mediation of behavior control by distinct types of representations. The research reported here used computer simulations of dopamine-dependent learning at cortico-striatal synapses to study how the direct and indirect pathways of the basal ganglia can assign and reassign behavior control among cortical representations that take different times to compute and that have predictive validities that change in accord with shifting contingencies of reinforcement. Besides representation validity and the time it takes to compute a representation, factors varied in these simulations included: distinct learning rates in different striatal compartments; distinct asymptotic values of cortico-striatal synaptic strengths in different striatal compartments; and the degree of asymmetry between effects of dopaminergic signal changes on synaptic plasticity in the direct and indirect pathways. Beyond control-switching, the model variants were also assessed for their ability to exhibit "savings" of previous learning across an interval when a contingency is temporarily inoperative or even reversed; such savings are indicated when reacquisition of behavior control by a representation is much faster than initial acquisition. Comparison of the successful model variants with unsuccessful ones illuminates factors that may be vital for the ability of the striatum, as part of an adaptive action selector, to use reinforcement learning to assign behavior control to the fastest-computed representation of cues and contexts that are currently valid guides to action selection.

Disclosures: S. Patrick: None. **D. Bullock:** None. **A. Gorchetchnikov:** None. **A. Sohail:** None. **M. Versace:** None.

Poster

633. Systems Physiology and Behavior

Location: Halls A-C

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Program#/Poster: 633.29/KK16

Topic: D.15. Basal Ganglia

Support: NHLBI/NIH T32 HL079010

NSF SMA-0835976

Title: A neural model of sleep deprivation effects on motor preparation and response: simulating adenosinergic, dopaminergic and cholinergic effects

Authors: *D. H. BULLOCK[1], M. A. ST. HILAIRE[2,3,4];
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Disorders, Brigham & Women's Hosp., Boston, MA; [4]Div. of Sleep Med., Harvard Med. Sch., Boston, MA

Abstracts: Increases in reaction times (RTs) are observed on tasks of planned and reactive movements during sleep deprivation. These sleep deprivation effects are partly mediated by accumulations of extracellular adenosine in the basal forebrain (BF), which includes arousal-related corticopetal cell types such as cholinergic cells of the nucleus basalis. To compute predicted effects of adenosine within BF and on RTs, it is necessary to model the strong GABAergic projections to BF from the striatum, as well as pathways by which these subcortical interactions can affect cortically-mediated cueing of motor responses in RT tasks. We therefore extended an existing model of cued action control by circuits spanning cortex and basal ganglia (BG, including striatum). The enhanced model explains how the laminar frontal cortex interacts with parietal cortex, BG/BF, thalamus and the superior colliculus to generate planned and cued movements. In addition to modeling mean sleep deprivation effects on two standard saccade tasks, the model can generate RT distributions comparable to empirical distributions from the psychomotor vigilance task, a test of sustained attention that requires a manual response to a visual cue with a high signal rate. Under sleep deprivation, the RT distribution shifts towards longer RTs despite a co-occurring increase in premature responses. Analysis focuses on four key additions to the prior model: (1) a preparatory process that primes activity in the BG direct pathway in expectation of the cue to act; (2) habituative (thus waning) excitation of the BG's indirect pathway during the fixation interval; (3) BG-influenced cholinergic cell populations in the BF whose cortical projections modulate attention to fixation and cue stimuli; and (4) adenosinergic interactions, primarily in the BG's direct and indirect pathways, that modulate cholinergic cortical arousal as well as cue-dependent BG signaling. Beyond simulating effects of sleep deprivation on oculomotor and manual responses, this detailed circuit model helps understand the local actions in BG/BF of two wake-promoting pharmaceutical agents: caffeine, an adenosine antagonist and modafinil, a dopaminergic agonist.

Disclosures: D.H. Bullock: None. **M.A. St. Hilaire:** None.

Poster

634. Motor Skill-Learning

Location: Halls A-C

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Program#/Poster: 634.01/KK17

Topic: D.17. Voluntary Movements

Support: NIA grant R01 AG041878

McKnight Scholar Award

Sloan Research Fellowship

NSERC Postgraduate Scholarship (Doctorate)

Title: Decay of motor memories is independent of context change detection

Authors: *A. E. BRENNAN, M. A. SMITH;
Harvard Univ., Cambridge, MA

Abstracts: Recently learned movement patterns gradually decay without an error signal. The prevailing hypothesis for this is that recently acquired motor memories are intrinsically volatile, resulting in an inevitable decay in the absence of ongoing error signals that allow performance decrements to be corrected. A recently proposed alternative posits that motor memories decay only if a change in context is detected (Vaswani & Shadmehr 2013). This hypothesis would require a novel architecture for error-based learning and memory storage, with a bank of highly stable but rapidly addressable parallel memory stores accessed by context. However, this new hypothesis has not yet undergone widespread scrutiny. Using the same experimental paradigm as Vaswani & Shadmehr, we tested the context change hypothesis by masking the transition from a training period to a retention period. This was achieved using variable error clamp (vEC) trials during the retention period to enforce movement variability that matched the variability of the training period, since the context change hypothesis maintains that movement variability is a key contextual feature and that maintaining movement variability continuity can effectively mask the context change between training and retention blocks. The vEC retention period was compared to a zero-error clamp (zEC) based retention period, where all movements were clamped to have zero error with minimal movement variability, resulting in a sudden change from the training period variability. The context change hypothesis predicts that, compared to the zEC retention period, the vEC retention period would substantially delay the onset of decay and reduce the amplitude of decay as subjects would have greater difficulty detecting the context change. Despite the vEC-based retention period resulting in dramatically improved continuity in movement variability and reward frequency following the training period, we found with 95% confidence that the population average delay was only 1 trial or less in both the zEC and vEC retention periods and that the zEC and vEC retention periods had very similar decay magnitudes (58±6% vs 62±5%, $p>0.6$). These results are in stark contrast to the 90 trial delay and the nearly complete elimination of decay previously reported for the vEC retention period. Further analysis reveals that the previous findings were likely due to biased parameter estimation and unbalanced experimental design. These results suggest context detection does not drive the observed decay in the absence of error, but instead that trial-by-trial decay is an integral part of error-based learning.

Disclosures: **A.E. Brennan:** None. **M.A. Smith:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: NIH R01 AG041878

Sloan Fellowship

McKnight Fellowship

Title: Time scales of motor memory in cerebellar ataxia

Authors: ***M. A. SMITH**, A. M. HADJIOSIF;
Sch. Engin., Harvard Univ., CAMBRIDGE, MA

Abstracts: The cerebellum is believed to be crucial for motor learning, and cerebellar damage can profoundly impair motor learning ability in humans and animals. Computational studies have demonstrated that multiple adaptive processes with different time scales contribute in parallel to motor learning; however, we do not yet understand whether the cerebellum differentially contributes to specific time scales of learning. Recent work from our laboratory has shown that the fastest time scale in motor adaptation is based on a temporally-labile motor memory that decays away rapidly with a time constant of just 15-20 seconds, whereas slower timescales display a level of temporal stability that is at least 100-fold greater. Here we performed a trial by trial reanalysis of the data from three recent studies (Smith & Shadmehr 2005, Hemminger et al 2010, Gibo et al 2013) that demonstrated impaired motor adaptation ability in participants with cerebellar damage in order to examine whether this damage differentially affects temporally-labile versus temporally-stable learning. . The main idea behind the new analyses we performed is that these studies had paradigms in which multiple movement directions were randomly interleaved and rest breaks between movements were occasionally offered during the training epoch, resulting in large movement to movement differences in the time interval between consecutive movements in the same direction. This allowed the temporal stability of the newly learned motor adaptation to be determined. Remarkably, we found significantly greater reductions in temporally-stable adaptation compared to temporally-labile motor adaptation in

individuals with cerebellar damage ($p<0.01$). Decreased temporally-stable adaptation was observed when either movements to different directions or rest breaks intervened between consecutive movements in the same direction. In contrast, temporally-labile adaptation appeared to be either entirely spared or even slightly improved in individuals with cerebellar damage. These results suggest that the different time scales of motor adaptation have distinct neural bases and that the fastest time scale does not depend on cerebellar function.

Disclosures: M.A. Smith: None. **A.M. hadjiosif:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: NIA Grant R01 AG041878

McKnight Scholar Award

Sloan Research Fellowship

Title: A modified random walk describes the low dimensional structure of motor variability in reaching trajectories

Authors: *Y. R. MIYAMOTO, M. SMITH;
Harvard Univ., Cambridge, MA

Abstracts: Ever-present movement-to-movement variations in our motions prevent us from exactly repeating our actions. This motor variability is a fundamental feature of our motor system, but little is known about how it evolves during movement. Here we investigated this evolution in an experiment in which subjects performed rapid (300-450ms) point-to-point reaching arm movements to trace a smooth 20cm path displayed on a monitor, without visual feedback about hand position. Subjects demonstrated considerable trial-to-trial variability in their hand paths; however, this variability was surprisingly low-dimensional, as over 99% of the variance in hand path shape was explained by just 3 principal components (PCs). These PCs were strikingly consistent in shape ($r > 0.96$ between each individual's PCs and the group mean) and fraction of variance accounted for (90±3%, 8±3%, & 1±0.5%) across individuals. Remarkably, we found that a simple random walk could largely account for the low dimensional

structure we uncovered, predicting (1) the time course by which variability evolved during the motion (average R2 = 0.81 across subjects), (2) the shapes of the 1st three PCs (average R2 = 0.83, 0.89, & 0.86 across subjects), and (3) the fraction of the variability in hand path shape that each accounted for (predictions of 85%, 8%, & 3%). Despite the apparent success of this model in describing the pattern of variability we observed, we noticed that variance in subjects' hand paths often evolved superlinearly with time, whereas variance increases linearly for a pure random walk. To address this discrepancy, we modified the random walk model (Model: x(n+1) = x(n) + A*(x(n) - x(n-1)) + Gaussian noise), by supplementing each step of the walk (x(n+1) - x(n)) with a single "momentum" factor (A), representing the motion conservation present in viscoelastic dynamics, that propagates the previous step (x(n) - x(n-1)). This revised model, with only a single additional parameter, accounted for the vast majority of the remaining variation in the hand path shapes unexplained by the pure random walk model. In particular, it explained (1) the shape of how variability evolves during the course of subjects' hand paths (average R2 across subjects = 0.99), (2) the shapes of the 1st three PCs (average R2 across subjects = 0.99, 0.97, & 0.99 for PC1, PC2, and PC3 respectively), and (3) the dimensionality of the variability (the 1st three PCs accounted for 93%, 5%, & 1% of the modeled motor variability). The ability to accurately model the propagation of motor variability with such a simple model may prove to be a valuable tool for further understanding both the nature of motor variability and its consequences for motor control.

Disclosures: Y.R. Miyamoto: None. **M. Smith:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: NIA grant R01 AG041878

McKnight Scholar Award

Sloan Research Fellowship

Title: The effect of visual feedback latency on the retention and internal representation of visuomotor learning

Authors: *A. M. HADJIOSIF, K. E. MALLETT, M. A. SMITH;
Harvard Univ., Cambridge, MA

Abstracts: Delays in visual feedback may have important consequences for motor learning, as this learning is often driven by the comparison between observed and expected sensory feedback. Previous studies (Kitazawa et al 1995, 2002) have suggested that delays in the presentation of endpoint-only visual feedback (VFB) reduce the rate and amount of visuomotor adaptation, even for delays as short as 50ms. This sensitivity to very small delays is especially important as LCD projectors used in some experimental setups can have latencies as large as 100ms. However, when VFB of motion is continuously provided rather than being relegated to the endpoint, the effects of visual latency are unknown. Here we investigate the effects of small VFB latencies between 25 and 300ms on the learning of a visuomotor rotation (VMR) during point-to-point reaching arm movements. Following a 240-trial baseline period, we trained 4 groups of subjects for 120 trials in a single movement direction on a ±30° VMR. Each group of 10-11 participants experienced a different visual latency (25, 50, 87 or 300ms) present in both the baseline and training periods. After training, VFB was removed and subjects were tested for retention after a 60s wait period and then tested for generalization in 19 different target directions. The smallest latency we tested corresponded to the base delay inherent in our experimental setup, which we optimized down to 25ms using the combination of a low-input lag, fast-response 120Hz LCD display and latency-optimized experimental software with asynchronous graphics output. Surprisingly, we found that VFB latency had little effect on the VMR learning curves, as neither early (trials 1-10) nor asymptotic learning (trials 100-120) were significantly affected by VFB latency ($p>0.17$ in both cases). However, we found large differences in retention of this learning following a 60s wait period with the 300ms group (57% retention) displaying smaller retention than the other groups (73-81% retention, $p<0.05$ in all three cases). VFB latency affected the generalization of motor learning even more profoundly. All groups displayed distinct broad and narrow components of the generalization across movement directions. However, the amplitude of the narrow component, and thus the fraction of the generalization it accounted for, was specifically reduced by feedback latency ($p<0.001$ vs. $p>0.5$ for the narrow and broad components, respectively), with reductions of 40 and 55% observed in the 87 and 300ms groups (both $p<0.01$). These findings indicate that visual feedback latency determines both the retention of learning and its internal representation, reflected in the pattern of generalization.

Disclosures: **A.M. Hadjiosif:** None. **K.E. Mallett:** None. **M.A. Smith:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: NSHRF Grant MED-DI 1551

Title: MEG-based functional connectivity changes with motor imagery training: Evidence for motor imagery as an acquired skill

Authors: S. N. KRAEUTNER[1], T. BARDOUILLE[3], *S. G. BOE[2];
[1]Psychology/Neuroscience, [2]Physiotherapy, Dalhousie Univ., Halifax, NS, Canada; [3]IWK Hlth. Sci. Ctr., Halifax, NS, Canada

Abstracts: Motor imagery (MI) is a form of practice in which an individual mentally rehearses a motor task, facilitating skill acquisition in the absence of physical practice (Jeannerod, 1995). MI has many clinical applications, including brain-computer interface and stroke rehabilitation. Previous research suggests that skill acquisition via MI is facilitated by repetitive activation of brain regions in the sensorimotor network similar to that observed in motor execution (ME; Hetu, 2013). This activation is influenced by differences in one's ability to perform MI (Wei, 2010), suggesting that MI ability is an acquired skill. In fact, spatial activation patterns during MI become more similar to that of ME with training. These training-related changes in MI activation have yet to be investigated from a network connectivity perspective. In showing how MI training drives network changes, the current study further demonstrates that the ability to employ MI for skill acquisition is a learned skill. Non-disabled participants (N=10; 24.7 +/- 3.8 years) performed both ME and MI of a unilateral seven-sequence button press task over three days. Magnetoencephalography (MEG) was utilized to capture neural activity. Coherence-based functional connectivity analysis was examined between eighty cortical nodes in the beta frequency band (15-30 Hz) and a partial least squares analysis conducted to compare coherence-based functional connectivity between session 1 and 3 (McIntosh, 2004). Preliminary findings indicate that functional connectivity was altered as a function of session, with network composition differing ($p < 0.05$) from session 1 to 3. Network changes included more lateralized (ie. in the contralateral hemisphere) activity during MI with more similar patterns of activity observed between MI and ME across sessions. Taken together, the current results indicate that the brain network underlying MI changes with training, further indicating that MI itself is a learned skill. Future work will utilize graph theory (Rubinov, 2010) to quantify changes in network characteristics during MI training, and examine the amount of MI training necessary to effectively facilitate skill acquisition in the absence of physical practice.

Disclosures: S.N. Kraeutner: None. **T. Bardouille:** None. **S.G. Boe:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: James S. McDonnell Foundation Grant 220020220

Title: Acquisition of skilled trajectory control in both arms after multiple days of unilateral reach training

Authors: *M. D. HARRAN[1], J. C. CORTES[1], J. W. KRAKAUER[2], T. KITAGO[1];
[1]Motor Performance Lab., Columbia Univ., New York, NY; [2]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstracts: In this study we sought to investigate how the control of planar reaching trajectories improves over multiple days of practice and whether the untrained arm also shows improvements. Most previous studies of motor learning and intermanual transfer have used either sequence or adaptation tasks. Here we used kinematics to examine changes in movement trajectories in both arms after unilateral training on a visually-guided reaching task. Seventeen healthy, right-handed subjects (mean age=23.94 yrs, 8M) participated in this study. The study was conducted over 5 consecutive days, and consisted a Pre-Test block on day 1, two Training blocks on days 1-4, and a Post-Test block on day 5. The task performed during Test and Training blocks was designed as a measure of motor control of the proximal arm: subjects were instructed to make out-and-back, gravity-supported planar reaching movements by moving a cursor from a center start circle to eight radially-arrayed targets (10 cm distance). Subjects were randomly assigned to train with either their dominant or non-dominant arm. For the Pre- and Post-Test blocks, both trained and untrained arms were tested, in random order. Each Test and Training block consisted of 24 trials per target, with targets presented in a pseudorandom order. We examined measures of endpoint and overall trajectory kinematics for the outward movement: systematic error (the distance between the mean endpoint position and the center of the target), variable error (square root of the determinant of the spatial covariance matrix at the endpoint), and curvature (area between the trajectory path and a straight line to the target, normalized for movement extent). We compared these measures in each arm under three conditions: without training, after training in that arm, and after training in the other arm. We found improvements in the trained arm for both curvature and systematic error, but not for variable error. Our preliminary analysis suggests that there is also improvement in motor skill in the untrained arm, with a marked reduction in systematic error. There was no improvement in variable error or

curvature for the untrained arm. This may due either to the fact that these measures came down less than systematic error in the trained arm or because only certain components of learned trajectory control manifest in both arms after unilateral training.

Disclosures: M.D. Harran: None. **J.C. Cortes:** None. **J.W. Krakauer:** None. **T. Kitago:** None.

Poster

634. Motor Skill-Learning

Location: Halls A-C

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Program#/Poster: 634.07/KK23

Topic: F.01. Human Cognition and Behavior

Title: Manual asymmetry in motor skill learning

Authors: R. L. MCGRATH, *S. S. KANTAK;
Moss Rehabil. Res. Inst., Elkins Park, PA

Abstracts: Motor skill learning involves a process wherein one learns to synthesize novel movement capabilities in absence of perturbation such that they are able to perform and retain the movement skill with higher accuracy, consistency, efficiency, coordination and flexibility. Performance asymmetry has been reported in motor control and adaptation to kinematic and dynamic perturbations of goal-directed actions. In this study, we investigated asymmetry in acquisition and retention of a complex motor skill that requires speed and accuracy for optimal performance. Further, we examined if skill learning asymmetry is influenced by arm dominance. 6 right-handed (RH) and 6 left-handed (LH) adults practiced 2 distinct tracks during different sessions separated by 2-4 weeks. During separate sessions, participants practiced with their dominant (DOM) or non-dominant (NDM) arm in a pseudo-randomized order. Performance changes during practice were characterized by improvements in accuracy while practicing within prescribed movement time ranges. Learning was quantified by changes in the speed-accuracy tradeoff (SAT) function measured at baseline and a day after practice ended. There were no baseline differences in the SAT function between the DOM and NDM arms. All participants improved their performance with their DOM and NDM arms with practice. With practice, the RH participants demonstrated significantly higher improvements in the SAT function for the dominant compared to the non-dominant arm. We did not find significant differences between the two arms for the LH group. With the limited practice provided for a complex motor skill, right-handed individuals demonstrate dominant arm advantage for learning. Extended practice

may be indicated for improving skill learning with the non-dominant arm in the RH individuals. In RH individuals, asymmetry in skill learning may likely be related to the hemispheric specialization effects reported for motor control.

Disclosures: R.L. McGrath: None. **S.S. Kantak:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: NIH/NINDS NS053962

Title: Stability of precision drawing skills acquired with the non-dominant hand and associated changes in functional connectivity between sensorimotor hand representations

Authors: *B. A. PHILIP[1], S. H. FREY[1,2];
[1]Dept. of Psychological Sci., [2]Brain Imaging Ctr., Univ. of Missouri, Columbia, MO

Abstracts: Patients may suffer from unilateral impairment of the dominant right hand from many causes, including neurological or peripheral injury or disease, which result in forced use of the non-dominant limb. Previous studies suggest that skilled left-hand behavior may entail a functional role of the left (ipsilateral) cerebral hemisphere, especially in dominant hand amputees. However, few studies have investigated the behavioral and neural changes involved in learning a new skill with the left non-dominant hand, and long-term retention. Here, we trained 19 healthy right-handed adults (age 27 ± 8, 13 female) to perform a precision drawing task (PDT) with their left hand. In this task, participants used a pen to draw continuous lines (45-180 mm) within provided boundaries (3, 4, or 5 mm tolerance); primary dependent measures included endpoint speed and smoothness. The training regime involved 10 days of left hand training, with a fixed number of training trials on each day (15-25 minutes). We tested participants' right hand performance before and after training. Participants returned for follow-up testing sessions 1 week, 1 month, and 6 months after the end of training. In addition, we identified training-correlated changes in functional connectivity with seed regions in bilateral hand sensorimotor cortex, via resting-state magnetic resonance imaging (fcMRI) before and after training. Seventeen participants (89%) showed significantly improved left hand performance across training. The performance difference between hands decreased significantly in the

smoothness domain. Despite discontinuation of left-hand training, performance improvements were stable over time: six months after training, 12/14 (86%) participants still showed significantly improved left hand performances. Preliminary fcMRI analyses indicate that a network of bilateral premotor-parietal areas showed a learning-correlated increase in functional connectivity with the right (trained) sensorimotor cortex. Critically, this network included the left (untrained) sensorimotor cortex. These results suggest that training-related improvements in left hand skill can be remarkably stable across time and may involve experience-dependent changes in interhemispheric connectivity. The implications of this work for rehabilitation are considered.

Disclosures: B.A. Philip: None. **S.H. Frey:** None.

Poster

634. Motor Skill-Learning

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Topic: D.17. Voluntary Movements

Support: NIH Grant K08 NS072183

University of Michigan

Title: An automated skilled reaching task to study fine motor control

Authors: *D. J. ELLENS, M. GAIDICA, S. PENG, D. K. LEVENTHAL;
Neurol., Univ. of Michigan, Ann Arbor, MI

Abstracts: Rodent skilled reaching is a well-established model for the investigation of fine motor performance and learning, with high homology to human reach-to-grasp movements. In this task, rats reach for, grasp, and eat food pellets in a sequence of stereotyped movements. Current incarnations require constant attention by the experimenter to place food pellets within reach of the animals. Furthermore, analysis of the reaching movements requires detailed evaluation of high-speed videos. Both are time consuming, limiting the number of animals that can be tested as well as the number of reaches that can be quantified per animal. An automated version of this task would allow more interventions to be tested in more animals, but must be robust and reproducible. The goal of this study was to develop such a task for use in future investigations of motor skill acquisition and performance. Our version consists of a standard skilled reaching chamber with a slot at one end through which the rat must reach to grab a pellet

off a shelf. Reaches are detected by a photobeam across the slot, which triggers acquisition of high speed (150-300 Hz) video. Individual video frames are triggered by TTL pulses that can synchronize the video with behavioral events or electrophysiological recordings. When the rats move to the back of the chamber, a photobeam break triggers a linear actuator to deliver another pellet to the shelf. Prior to test sessions, the rats' (adult male Long Evans) reaching paw is colored green with nail polish, allowing its trajectory to be tracked automatically. We found that, as reaching skill improves, the variance within multiple kinematic parameters decreases. This suggests that our automated analyses can track the refinement of motor skills over time, and will allow us to investigate the effects of multiple different interventions efficiently.

Disclosures: **D.J. Ellens:** None. **M. Gaidica:** None. **S. Peng:** None. **D.K. Leventhal:** None.

Poster

634. Motor Skill-Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.10/KK26

Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI (Grant-in-Aid for JSPS fellows, 25-4917)

JSPS KAKENHI (Grant-in-Aid for Specially promoted Research 24000012)

Title: Human left fronto-parietal cortices are associated with acquisition of skill-switching ability

Authors: *S. UEHARA[1,2], N. MIZUGUCHI[1], S. HIROSE[1], S. YAMAMOTO[3], E. NAITO[1,4]; [1]Ctr. For Information and Neural Networks (CiNet), Natl. Inst. of Information and Communications Technol., Suita City, Osaka, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Sch. of Hlth. and Sport Sciences, Osaka Univ. of Hlth. and Sport Sci., Osaka, Japan; [4]Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan

Abstracts: Learning motor skills is one thing, acquiring skill-switching ability is another. Here we provide behavioral and neuroimaging evidence to support this view. We scanned brain activity with fMRI, while thirty-two healthy right-handers practiced two different types of sequential finger movement tasks with their non-dominant left hands in randomized order. A half of the participants underwent the experiment without prior practice (non-prior-practice group), while the other half practiced the tasks in blocked manner on the day before the experiment

(prior-practice group). In the non-prior-practice group, the task performance gradually improved during the experiment. This represents the learning of motor skills themselves. Interestingly, detailed analysis showed that the performance was relatively worse when the participants should perform the different task from that in the previous trial (switched task) compared to performing the same task (non-switched task). This performance deterioration in the switched task gradually decreased through the experiment, indicating the acquisition of the skill-switching ability. Importantly, this phenomenon was also observed in the prior-practice group, although their skill learning was well promoted through the prior-practice. Compared with the non-switched task, the activity in the fronto-parietal regions increased only in the left hemisphere when the brain prepared to perform the switched task. However, the activity of these regions gradually decreased in parallel with the improvement of the switched task performance. These lines of evidence demonstrated that the ability of switching motor skills is acquired by the brain separately from the learning of motor skills per se, and that the fronto-parietal cortices in the human left hemisphere is involved in acquiring the skill-switching ability.

Disclosures: **S. Uehara:** None. **N. Mizuguchi:** None. **S. Hirose:** None. **S. Yamamoto:** None. **E. Naito:** None.

Poster

634. Motor Skill-Learning

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Program#/Poster: 634.11/KK27

Topic: D.17. Voluntary Movements

Support: Wellcome Trust Senior Research Fellowship GR066676MA to Dr V. Rema

Title: Long-term deficits in performance of skilled forelimb pellet retrieval behaviour seen in rats with unilateral motor cortex lesion

Authors: *R. CHAUDHARY, J. VENKATESH, V. REMA;
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Abstracts: Focal injuries to the brain produces loss of tissue at the injured site resulting in deficits in behavior. In addition contralateral intact cortex has been speculated to influence behavioral functions of the injured hemisphere. Here we examined whether behavioral functions of uninjured hemisphere will alter the deficits in behavioral functions of the injured hemisphere. Long Evans rats were trained on skilled forelimb pellet retrieval task. There was no forelimb

restraint during training or in their home cages. Based on the natural ability to use the forelimbs the rats were classified into two groups: (i) “Ambidextrous” animals could perform the task equally with both forelimbs and both forelimbs were trained; (ii) Unidextrous, animals could perform the task only with one forelimb and that forelimb was trained. In addition we had another group of ambidextrous animals in which only the preferred forelimb was trained, “pseudo-unidextrous”. Once the animals behavior attained plateau performance the forelimb motor cortex contralateral to the preferred forelimb was lesioned by sub-pial aspiration. Skilled forelimb pellet retrieving behaviour of the forelimb connected to the injured motor cortex was tested following the lesion. We found significant deficits in the behavioral performance. These deficit were long-lasting. Our result show that the prior training of the forelimb connected to the intact cortex, after unilateral cortical lesion does not alter the behavioral deficits exhibited by forelimb connected to the injured cortex. Funding for this study was from International Senior Research Fellowship Grant GR066676MA from The Wellcome Trust to Dr V. Rema

Disclosures: **R. Chaudhary:** A. Employment/Salary (full or part-time):; National Brain Research Centre. **J. Venkatesh:** A. Employment/Salary (full or part-time):; National Brain research Centre. **V. Rema:** A. Employment/Salary (full or part-time):; National Brain Research Centre. 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.; Welcome Trust Senior Research Fellowship.

Poster

634. Motor Skill-Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.12/KK28

Topic: F.01. Human Cognition and Behavior

Support: KTIA_NAP_13 (Neurocognitive disorders of frontostriatal sytem)

> KTIA_NAP_13 (Neurocognitive disorders of frontostriatal sytem), Bolyai Janos Research Scholarship of the Hungarian Academy of Science

Title: Differential vulnerability of different forms of skill learning in Parkinson’s disease

Authors: *A. LUKACS[1], F. KEMENY[1], G. DEMETER[1], I. VALALIK[2], M. RACSMANY[1]; [1]Dept. of Cognitive Sci., Budapest Univ. of Technol. and Econ., Budapest, Hungary; [2]Dept. of Neurosurg., St. John's Hosp., Budapest, Hungary

Abstracts: The striatal dopaminergic dysfunction in Parkinson's disease (PD) has been associated with deficits in skill learning in a number of studies, but the results are inconclusive so far. Motor sequence learning (especially sequence-specific learning) is found to be deficient in the majority of studies using the Serial Reaction Time Task (SRTT; Siegert, Taylor, Weatherall, & Abernethy, 2006; Jackson et al., 1995; Ferraro, Balota and Connor, 1993; Pascual-Leone et al., 1993, Muslimovic et al., 2007; Gobel et al., 2013; but see Kwak et al., 2012), although results are contradictory when verbal response is required instead of button presses (Westwater et al. 1998; Smith, Siegert and McDowall 2001). While problems with motor sequences seem to be prevalent, PD patients show intact performance on Artificial Grammar Learning (AGL) tasks, suggesting that the sequencing problem may be response type- or task type-dependent (Smith, Siegert and McDowall 2001; Witt, Nühsman and Deuschl, 2002) Acquisition of nonsequential probabilistic associations also seems to be vulnerable as evidenced by impaired PD performance on a probabilistic category learning task (Knowlton, Mangels et al., 1996; Shohamy, Myers, Onlaor, & Gluck, 2004). Our aim was to explore the nature of the skill learning deficit by testing different types of skill learning (sequential versus nonsequential, motor versus verbal) in the same group of Parkinson's patients. 14 patients with PD (mean age: 59.77 range: 45.5-74) were compared to age-matched typical adults using 1) a Serial Reaction Time Task (SRTT) testing the learning of motor sequences, 2) an Artificial Grammar Learning (AGL) task testing the extraction of regularities from auditory sequences and 3) a Weather prediction task (PCL-WP), testing probabilistic category learning in a non-sequential task. In motor sequence learning on the SRTT task, the two groups did not differ in accuracy; PD patients were generally slower, and analysis of z-transformed reaction times showed no evidence of sequence learning in PD. A deficit in artificial grammar learning was present only as a tendency in the PD group. The PD group showed evidence of learning on the PCL task, and their learning performance was not statistically different from that of the control group. These results partly support and also extend previous findings suggesting that motor skill learning is vulnerable in PD, while other forms of skill learning are less prone to impairment. Results are also in line with previous assumptions that mechanisms underlying artificial grammar learning and probabilistic categorization do not depend on the striatum (Reber & Squire, 1999; Skosnik et al., 2002).

Disclosures: A. Lukacs: None. **F. Kemeny:** None. **G. Demeter:** None. **I. Valalik:** None. **M. Racsmany:** None.

Poster

634. Motor Skill-Learning

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Program#/Poster: 634.13/KK29

Topic: F.01. Human Cognition and Behavior

Support: R01HD053793

T32HD007414-20

Title: Enhancement of motor skill memory through reconsolidation

Authors: *N. F. WYMBS[1,3], A. J. BASTIAN[3,2], P. CELNIK[1,2];
[1]Physical Med. and Rehabil., [2]Neurosci., Johns Hopkins Univ., Baltimore, MD; [3]Kennedy Krieger Inst., Baltimore, MD

Abstracts: Recent work on memory reconsolidation has shown that previously consolidated motor skill memory can be weakened following reactivation and subsequent exposure to destabilizing intervention. Since other forms of memory can be enhanced through reconsolidation, we wondered if motor skill memory could be strengthened following reactivation and exposure to a different type of intervention. Here, we tested if motor memory could be enhanced overnight following reactivation using a novel behavioral intervention. Participants learned a thumb-index finger pinch force task where force mapped logarithmically to lateral cursor displacement. Subjects moved the cursor sequentially to a set of 5 targets. There was 3 sessions (4 blocks of 30 trials each). Following an initial training session (0 hr), participants returned after a 6-hour delay, and the following day (24 hr). Memory for this SVIPT task has been shown to consolidate within 6 hours. Thus, participants first reactivated their consolidated memory when they returned after the 6-hour delay. Next, they either continued on the previously learned function, or given a "variable trial intervention." This involved the intermixed presentation of 6 new logarithmic functions, along with the original function. New functions were fit using cursor endpoint variance (+/- 3SD, 1SD increment) to each of the 5 targets derived from an independent dataset. These could not be distinguished from the original function. The effect of intervention was measured as offline learning: difference between the first block the following day (24hr) from the final block during the initial session (0hr). Exposure to the variable skill task after reactivation led to greater offline learning despite the lack of any performance improvement during the intervention. On the other hand, the group that trained only on the previously learned function showed little evidence of offline learning despite improved performance during the intervention session. Additional controls revealed that enhanced offline learning was specific to the reactivation of consolidated skill memory, and not simply variable practice in general. Moreover, how skillful participants responded to the intervention determined the magnitude of offline learning. In particular, those with high accuracy following large

fluctuations in trial-to-trial variability had greater offline learning. These findings provide evidence that reconsolidation of motor skill memory can be constructive. Subtle variability appears to provide a powerful means to protect and further enhance previously consolidated memory, likely through the continued engagement of plasticity-like processes.

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Poster

634. Motor Skill-Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.14/KK30

Topic: F.01. Human Cognition and Behavior

Title: Minimal observational viewing of an animated model enhances the physical performance of a motor skill

Authors: *J. J. BUCHANAN, I. PARK;
Texas A & M Univ., COLLEGE STA, TX

Abstracts: Practice may take the form of physical repetitions of an action and practice may also be induced through observation of a model. This experiment examined the contribution of physical and observational practice to the development of a novel motor memory. The task had the forearm held in a supine position as the elbow and wrist rhythmically flexed and extended in the sagittal motion plane. The novel motor pattern was a -90° relative phase pattern between the elbow and wrist in combination with joint amplitudes of elbow = 92° and wrist = 70°. The -90° offset means that the wrist lags behind the elbow throughout the cycle. There were three groups that practiced for two days: 1) physical only (30 trials per day); 2) physical-observation (6 physical, 24 observe); and 3) observation only (30 trials). The stimulus for all three groups was an animated stick figure consisting of upper-arm, forearm-elbow, and wrist-hand segments. The stick-figure was displayed on a computer monitor. The primary hypothesis was that all three types of practice would benefit the learning of the relative phase offset, yet only physical and physical-observation practice would benefit the learning of joint amplitudes. The training of the physical and physical-observation groups was supplemented with visual feedback. The physical and physical-observation groups were characterized by large improvements on the -90° relative phase offset in two retention tests, with the observers showing only a moderate improvement. The physical practice group's performance was the most stable, with no difference in stability between the physical-observation and observation groups. The physical group also matched the required amplitudes better than the other two groups. The physical-observation group did not

benefit from their limited practice with regard to scaling joint amplitudes. Physical practice produces novel motor memories in the form of stable attractors in the landscape of relative phase, and also benefits the scaling of joint amplitudes. Very little physical practice is necessary to enhance the benefit of observation supporting the idea that relative phase provides a source of information that links the visual perception of actions to the production of actions and vice versa. Even though physical and observational training may activate many of the same neural areas in the action-observation network, scaling muscle activity patterns to produce required joint amplitudes requires extensive physical practice and proprioceptive feedback.

Disclosures: J.J. Buchanan: None. **I. Park:** None.

Poster

634. Motor Skill-Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.15/KK31

Topic: D.17. Voluntary Movements

Title: Applying “unusual” action contexts to familiar tools: How tools adopt new functions

Authors: *J. C. MIZELLE, L. A. WHEATON;
Sch. Applied Physiol, Georgia Tech., ATLANTA, GA

Abstracts: As we interact with our environment, we build representations of objects and actions associated with those objects. Consider the act of stirring coffee, where we might normally associate the utensil “spoon” as the correct tool by which to best achieve the action goal of stirring. These associations are strengthened by repeated exposure, and our representations of tools develop canonical usage contexts. However, we may find ourselves in a position where the “normal” tool for a specific task is not at hand. With this work, we sought to identify the brain mechanisms by which we flexibly apply new behavioral contexts to tools with well-formed canonical usage contexts. Fifteen healthy, right-handed young adults participated in a functional MRI study in which images of correct, incorrect and "plausible" tool use were shown. Each image was preceded by a neutral text description of an action (e.g., “Stir coffee”), against which images were evaluated for contextual correctness. Participants were instructed to silently judge whether the images showed contextually correct, contextually incorrect or contextually unusual (but plausible) tool use, and made no overt motor or verbal responses. Our results suggest that left parietofrontal activations when viewing tool use in correct contexts (match with the text prompt) are augmented over both incorrect contexts (mismatch with the text prompt) and

plausible contexts (not a match, but possible). We further observed viewing tool use in plausible contexts activated right precentral and middle frontal areas over viewing tools use in both correct and incorrect contexts. No greater activations for viewing tool use in incorrect contexts were seen over correct or plausible contexts. These findings improve our understanding of the flexible nature of tool use and suggest a reliance on right-hemisphere structures related to encoding unusual tool-related actions.

Disclosures: J.C. Mizelle: None. **L.A. Wheaton:** None.

Poster

634. Motor Skill-Learning

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Topic: D.05. Visual Sensory-motor Processing

Support: Supported by grant from the German Research Fundation (DFG), RA 2183/1-1.

Title: Effects of temporal delay on implicit and explicit representations of hand position in tool use

Authors: *M. K. RAND, H. HEUER;
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Abstracts: To investigate how the CNS combines seen cursor positions and felt hand positions under a visuo-motor rotation paradigm, we previously examined implicit and explicit judgments of the proprioceptively sensed directions of hand movements performed on a digitizer while looking at a rotated visual feedback of those movements on a monitor. We found different biases in coupling seen and felt movement directions for the two types of judgment, indicating the existence of distinct explicit and implicit neural representations of hand direction. The present study further examined the effects of a short delay on the biases for each type of judgment in order to examine the memory dynamics of implicit and explicit representations. A delay of 6 s. was inserted between the end of the aiming movements and two types of judgment of the movement directions (delay condition). This condition was compared with a condition where no delay was inserted (no-delay condition). The results showed that the bias of the explicit judgments of the proprioceptively-sensed hand direction toward the visual direction of the cursor was about twice as strong as the bias of the implicit judgments. The explicit judgments of hand direction had significantly larger variability than the implicit judgments. These characteristics

were consistent with those of our previous study and unchanged by the inserted delay. Furthermore, when the individual biases within each judgment type were correlated between the delay and no-delay conditions, high correlations were found in both types of judgment. This indicates a stability of individual judgments within each judgment type. In contrast, when the individual biases were correlated between the implicit and explicit judgments, no correlation was found in both delay and no-delay conditions. Taken together, distinct characteristics of implicit and explicit representations were maintained over the inserted delay period, suggesting that both types of representations were enduring with respect to memory dynamics. The present results strengthen the notion of distinct explicit and implicit neural representations of hand direction.

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Poster

634. Motor Skill-Learning

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Program#/Poster: 634.17/LL1

Topic: D.17. Voluntary Movements

Support: USU Research Catalyst Grant A28037

National Swimming Pool Foundation

Title: Changes in electrodermal activity during motor learning: A proxy for attention?

Authors: J. E. GARDNER[1], A. C. RAIKES[1], *S. Y. SCHAEFER[1,2,3];
[1]HPER, Utah State Univ., Logan, UT; [2]Physical Therapy, [3]Ctr. on Aging, Univ. of Utah, Salt Lake City, UT

Abstracts: Motor learning theories predict that humans require high levels of attention to perform novel motor tasks, but little to no attention for learned tasks. Thus, practicing a task may decrease the amount of attention required to perform it. To test the theoretical relationship between attention and task practice, we used a physiological proxy for attention known as electrodermal activity (EDA). We hypothesized that EDA would decrease as they practiced a novel motor task. We also hypothesized that EDA would be higher overall in older adults than in younger adults when performing the same task. Because advancing age tends to limit one's ability to divide attention among multiple tasks, we expected an age-related increase in attentional requirement during motor performance. Two groups of subjects (young, n=5;

age=22.4 ± 2.1 yrs and older, n=5; age=75.8 ± 5.2 yrs) trained on a novel upper extremity task over three days. For each trial, we measured 1) EDA using commercially available wrist-worn sensors and 2) task performance, defined as movement time. EDA data were decomposed offline into tonic (underlying) and phasic (responsive) components. Results showed that in both groups, tonic EDA varied substantially between subjects and between training days, with peak EDA levels occurring mid-training. Tonic EDA levels were, however, comparable between the older and young groups. Overall, both groups demonstrated improvements on task performance and decreases in EDA levels over the course of training. This non-invasive, wireless technique may be a feasible alternative to expensive, non-portable methods for measuring attention during movement. Future studies are needed to explore the relationship between motor performance and tonic vs. phasic EDA, and to test the extent to which EDA is a valid proxy for attention.

Disclosures: J.E. Gardner: None. **S.Y. Schaefer:** None. **A.C. Raikes:** None.

Poster

634. Motor Skill-Learning

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Program#/Poster: 634.18/LL2

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG031769-01

Title: Error detection ability contributes to the compromised motor learning in older adults

Authors: *Y.-T. CHEN, M. KWON, E. A. CHRISTOU;
Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL

Abstracts: Error detection, defined as the ability to detect the difference between the desired and actual motor performance, is a crucial part of motor learning. Nonetheless, it is unclear whether age-associated impairments in motor learning are related to compromised error detection ability. The purpose of this study, therefore, was to determine whether compromised error detection contributes to impaired motor learning in older adults. Twenty young (25.1 ± 3.9 yrs, 10 men) and twenty older adults (71.5 ± 4.8 yrs, 10 men) participated in this study. Half of the subjects practiced 100 trials of a rapid goal-directed task with ankle dorsiflexion and were tested one day later with elbow flexion (ipsilateral transfer). The other half of the subjects acted as the control group and only performed the task with elbow flexion. The targeted position was 9^{o} for ankle dorsiflexion and 18^{o} for elbow flexion. The targeted time was 180 ms for both tasks. After each

trial, subjects predicted the endpoint of their performance by reporting the endpoint coordinates (position and time) of the peak displacement followed by visual feedback of the movement trajectory relative to the target. Motor learning (ipsilateral transfer) was quantified with the performance error during elbow flexion. Error detection ability was quantified as the difference between the actual and predicted performance. Only young adults were able to ipsilaterally transfer the goal-directed task from ankle dorsiflexion to elbow flexion (Δ from control group: young: -27%; older: -3%). The enhanced ipsilateral transfer in young adults relative to older adults was related to their better error detection ability during ankle practice (R^2=0.29). Furthermore, older adults who exhibited better error detection ability during practice with the ankle task exhibited enhanced ipsilateral transfer (R^2=0.51). These findings provide novel evidence that compromised error detection ability with aging contributes to motor learning impairments in older adults.

Disclosures: Y. Chen: None. **M. Kwon:** None. **E.A. Christou:** None.

Poster

634. Motor Skill-Learning

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Program#/Poster: 634.19/LL3

Topic: D.17. Voluntary Movements

Support: RO1 NS056839

RO1 NS078791

Title: Skilled motor learning increases basilar dendritic spine density on pyramidal neurons in layer II/III of mouse motor cortex

Authors: *T. CLARK[1], A. SITKO[2], T. A. JONES[1];
[1]Inst. for Neurosci., The Univ. of Texas At Austin, Austin, TX; [2]Dept. of Neurosci., Columbia Univ., New York, NY

Abstracts: Two photon (2P) imaging has enabled repeated visualization of cortical dendritic spine dynamics over time *in vivo*, but analysis is generally restricted to the most superficial layers of cortex. Such imaging in mice has revealed that motor skill learning (of a skilled reaching task) leads to a rapid formation followed by selective elimination of dendritic spines on the apical dendritic tufts of layer V pyramidal neurons in trained motor cortex, without a net

change in dendritic spine density.(Xu et al., 2009, Nature, 462: 519). In contrast, prior rat studies have found that skilled reach training increases quantities of layer V apical and layer II/III basilar dendrites and spines, as well as synapse numbers in these layers, as assessed with traditional light and electron microscopy. It has been unclear whether to attribute these conflicting results to differences in species, imaging, behavioral methods or the dendritic subpopulations examined. Here we asked whether spine densities on a deeper subpopulation of dendrites, the basilar dendrites of layer II/III pyramidal neurons, change in mice as a result of training on the same skilled reaching task as used by Xu et al (2009). Fifty-two C57/bl6 mice were trained with one forelimb on the single seed retrieval task, an adaptation of the single pellet retrieval task used in rats. Following the last day of reach training, brains were stained with Golgi-Cox. Individual spines on the basilar dendrites of forked layer II/III pyramidal neurons in motor cortex contralateral to the trained limb were visualized. Results indicate an overall increase in dendritic spine density on the terminal segments of layer II/III basilar dendrites. These results suggest that subpopulations of pyramidal neuron dendritic spines are differentially influenced by skilled motor learning.

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Poster

634. Motor Skill-Learning

Location: Halls A-C

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Program#/Poster: 634.20/LL4

Topic: F.01. Human Cognition and Behavior

Support: MSU Denver Undergraduate Research Grant

Title: Perception of hill steepness is altered by motor skill learning in winter sports athletes

Authors: *C. A. ERICKSON, A. R. ZAVILLA, C. B. WALTERS;
Psychology, Metropolitan State Univ. of Denver, Denver, CO

Abstracts: Perception is the process by which we make sense of the world, but rather than being a static filter, our perceptions of reality are dynamic and fluid. The physical state of our bodies changes our perceptions of the world. This effect is termed “Embodied Perception.” It has been postulated that embodied perception allows us to react to environmental stimuli without active thought, thus increasing efficiency in our actions (Proffitt, 2006). In a widely cited study, Proffitt et al. (1995) demonstrated that fatigue alters perception of the steepness of a hill. The current

study examined the role of motor skill expertise on perception of hill steepness. Skiers and snowboarders share the same mountain terrain, yet the skill sets for these sports are very different. Thus, we were able to examine the role of motor skill expertise on perception of hill steepness using a within-subjects design by asking experienced snowboarders with limited skiing experience to estimate the steepness of various runs at a ski resort while wearing either their own equipment (snowboard) or rented equipment (skis). Participants made three measurements (visual, verbal, and haptic) at multiple locations at a Colorado ski resort. Consistent with previous reports (Proffitt, 2006), participants overestimated the steepness of runs regardless of actual hill steepness or level of experience with equipment worn. Participants' estimations of the steepest runs were more dramatic than their estimations of the less steep runs. Visual estimates were highest when participants were wearing unfamiliar equipment (skis) and looking down from the top of the steepest run tested. For the visual estimate, there was an interaction between run difficulty and experience level using a repeated-measures ANOVA ($p<0.05$, $N=9$). This within-subjects result demonstrates that expertise in a motor skill alters perception of the visual world. The possible implications of this study are twofold. First, the results of this study are of interest to the winter sport industry. A better understanding of how students perceptions of mountain terrain changes as their skill level improves can provide ski and snowboard instructors with supplemental training tools. Second, the results expand our current understanding of embodied perception. Embodied perception provides us an instant evaluation of our ability to perform an action by adjusting our sensory experience as a function of resources available for performance of the action and potential consequences of the action. These results indicate that, in addition to temporary states, motor skill learning also alters visual perception of hill steepness.

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Poster

634. Motor Skill-Learning

Location: Halls A-C

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Program#/Poster: 634.21/LL5

Topic: F.01. Human Cognition and Behavior

Title: The curse of task specificity: Skill becomes more task specific with practice

Authors: *J. W. KRAKAUER, D. M. HUBERDEAU, P. ROY, K. MCNALLY, O. AHMAD, A. M. HAITH;
Johns Hopkins Univ., Baltimore, MD

Abstracts: Motor skills such as skiing, driving or riding a bike are characterized by long learning curves that take months to years to master. What is being learned for any given task, and how does it affect the behavior and actions in variants of that task? For instance, are expert skiers worse in an absolute or relative sense at snow boarding than novice or moderate skiers? That is to say, is increasing task-specificity the price of expertise? This can be tested by assessing generalizability at different time points during longitudinal training on a particular task. We hypothesized that prolonged training in a task would result in reduced generalizability; with more skilled participants performing worse in an absolute sense on a task variant than less skilled performers. Selective or partial generalization of acquired motor ability has been demonstrated before (Darainy, et al. Exp. Brain Res, 2006), while in some tasks, no generalization is evident, even for conditions that seemingly only differ in terms of context (Tremblay, et al. J. Neurosci. 2008). What is the difference between motor abilities that generalize to novel conditions and those that do not? There is evidence that neural representations of certain behaviors can shift over the course of training (Daw, et al. Nat. Neurosci, 2005). We predicted that in the course of learning a motor skill, the neural representation for the appropriate action will shift over the course of training and that this results in greater task-specificity (i.e. worse generalization). To test this hypothesis, we introduce a custom-built video game played on tablet computers such as the *iPad*. Our game simulates a driving scenario and can be tailored to test specific hypotheses. We set up an experiment in which participants practiced the driving game on a particular track for 30-minutes on each of 5 consecutive days. We assessed generalization by introducing a set of probe trials on either the third day of learning or the fifth day. Probe trials consisted of a track that is the mirror image of the training track. We found that participants degraded to the same performance level during the probes regardless of when the probe was introduced, even though participants had reached a higher level of performance on the training track on the fifth day. This result indicates that, in life-like motor tasks, performance improvements beyond a certain threshold level represent task-specific gains. One shortfall of this study is that with only 5-days of training, no participant reached what can fairly be called expert levels of performance at the task, which may explain why we failed to observe worse generalization on day 5 in an absolute sense.

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Poster

634. Motor Skill-Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.22/LL6

Topic: F.01. Human Cognition and Behavior

Support: NIH TL1TR000148

NIH K24HD074722

Title: Cortical connectivity at rest predicts consolidation of a novel motor skill

Authors: *J. WU[1], F. KNAPP[3], N. VARZHAPETYAN[2], R. SRINIVASAN[1], S. C. CRAMER[2]; [2]Neurol., [1]Univ. of California, Irvine, Irvine, CA; [3]Maastricht Univ., Maastricht, Netherlands

Abstracts: Individuals show significant variation in motor learning. A previous study from our group found a resting-state measure of cortical connectivity was a good predictor of motor skill acquisition. The current study extends the previous study to examine whether resting-state connectivity is a good predictor of motor training consolidation. METHODS. Twenty-eight adults (age=19.6±0.4 years, m±se) underwent 3 minutes of resting-state dEEG recording prior to 20 minutes of motor training. Subjects returned 24 hours later to test consolidation of motor training. Motor learning task. The task was a precision targeting task in which subjects made flexion and extension wrist movements to 7 potential target locations in response to a visual cue. EEG methods. Continuous raw dEEG were filtered, segmented, and cleaned of extra-brain artifacts with visual inspection and Infomax-Independent Component Analyses. Mean coherence with left M1 seed region was derived for the high beta (20-30 Hz) frequency band, which is associated with motor system function, and regressed again behavioral scores using partial least squares (PLS). RESULTS. With training, the group demonstrated consolidation of motor training, improving from 25.0±1.8% targets hit at Day 1 to 40.4± 2.1% targets hit at Day 2 (m±se, $p<0.0001$). The PLS model of resting-state beta coherence with left M1 predicting this consolidation ($R^2=0.64$) identified significant electrodes in left premotor (PM), left primary sensory (S1), and left parietotemporal (PAR) region. Specifically, individuals whose resting connectivity prior to training, was greater in left M1-left PM ($p=0.04$, $r=0.38$) and left M1-left S1 ($p=0.06$, $r=0.36$) electrodes showed better consolidation; individuals with greater connectivity in left M1-left PAR ($p=0.01$, $r=-0.46$) electrodes showed worse consolidation. CONCLUSIONS. The current results demonstrate resting-state dEEG connectivity is a strong predictor of short-term motor learning. Similar to our previous study, M1-PM and M1-PAR coherence showed a strong relationship with behavioral performance. In conjunction with the previous study, the current results demonstrate measures of resting-state functional connectivity inform both motor skill acquisition and consolidation.

Disclosures: J. Wu: None. **F. Knapp:** None. **N. Varzhapetyan:** None. **R. Srinivasan:** None. **S.C. Cramer:** None.

Poster

634. Motor Skill-Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.23/LL7

Topic: F.01. Human Cognition and Behavior

Title: Time course of plastic changes in brain structure accompanying skill acquisition

Authors: *E. WENGER[1], S. KÜHN[1], J. VERREL[1], J. MÅRTENSSON[2], U. LINDENBERGER[1], M. LÖVDÉN[3,1];
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Abstracts: Recent evidence suggests that the adult human brain structure shows potential for plastic changes in response to altered environmental demands. Many studies conducted in the field of structural plasticity follow the implicit assumption of a monotonic increase in brain volume accompanying learning. However, it has also been proposed, mostly based on work in animals, that learning-related volume increases can emerge relatively rapidly (e.g., within a few days), but then renormalize during further behavioral training and continuously stable performance. We set out to investigate the time course of plastic changes in brain structure accompanying motor skill acquisition. We trained 15 younger right-handed adults for 7 weeks to draw and write with their non-dominant hand (i.e. left) hand on a touch-sensitive tablet PC. In contrast to a standard pretest-posttest design, magnetic resonance (MR) images were acquired every other day, resulting in approximately 20 MR sessions per person over the course of the whole study, including both structural and functional imaging sequences. The data pre-processing of the structural T1-weighted images was performed using voxel-based-morphometry (VBM8 toolbox). Participants' performance level increased considerably in both writing and tracing tasks. We found that motor training of the left hand triggered changes in gray matter volume in task-relevant areas, namely left and right motor cortex as well as left and right putamen. In right motor cortex, the initial expansion was followed by a partial renormalization of structure in the presence of stable and heightened motor performance. A typical pretest-posttest design would not have uncovered these dynamics. Our findings corroborate the proposition that training-induced neural plastic changes may entail an initial increase in gray matter that is followed by partial renormalization. This is consistent with findings based on animal models, where plasticity often consists of an initial expansion of regional brain volumes, followed by a later phase of efficient reorganization and partial stabilization of new structures, combined with the elimination of pre-existing structures. Our results demonstrate the importance of including

multiple measurement occasions into MR study designs to accurately depict training-induced gray matter changes.

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Poster

634. Motor Skill-Learning

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Program#/Poster: 634.24/LL8

Topic: F.01. Human Cognition and Behavior

Support: NSERC

Title: Kinesthetic motor imagery of a newly learned dance is easier with eyes closed: Modulation of alpha power and subjective imagery ratings by eye state and expertise

Authors: *P. M. DI NOTO[1,2,3], J. M. CHARTRAND[1], G. R. LEVKOV[4,2], R.-A. ANDREW[1], M. J. WILAND[1], J. F. X. DESOUZA[1,4,2,3,5];
[1]Psychology, [2]Ctr. for Vision Res., [3]Neurosci. Grad. Diploma Program, [4]Biol., York Univ., Toronto, ON, Canada; [5]Canadian Action and Perception Network, Toronto, ON, Canada

Abstracts: Recordings of the alpha band (8-13Hz) reveal increased power (synchronization) when eyes are closed
(EC) and decreased power (desynchronization) when eyes are open (EO; Berger, 1929). Similarly,
anecdotal evidence on kinesthetic motor imagery (KMI) from an internal first-person perspective stipulates
clearer imagery when eyes are closed. This follows previous evidence for suppression of task-irrelevant
areas during motor imagery as reflected by alpha synchronization, and increased cognitive load and alpha desynchronization in task-relevant areas (Pfurtscheller et al., 1999). In order to test whether KMI clarity is affected by eye state or familiarity with the stimulus (Calvo-Merino et al., 2005), we examined alpha power among ballet dancers (n=21), dancers in other non-ballet genres (n=25), and non-dancers (n=15) during three tasks: baseline (with EC and EO), learning, and KMI (EC and EO). After each block of the learning and KMI tasks, subjects were asked to rate their learning or imagery on a scale from 1 (Perfectly accurate/clear) to 5 (No accuracy/imagery). Significant group effects were found for the learning task, with the unfamiliar non-dance group demonstrating higher alpha power compared to the highly-familiar ballet ($P<0.001$) and intermediate non-ballet ($P<0.001$) groups, indicative of decreased cognitive load in nondancers while observing and learning an unfamiliar dance sequence. Additional group effects were observed with ballet dancers providing significantly lower ratings during learning

(P<0.01) and KMI
(P<0.05) compared to non-dancers, reflecting enhanced learning and imagery in familiar experts relative to non-experts. Across all subjects, significantly more vivid imagery was reported during the EC condition
compared to EO (P<0.001) and, as expected, alpha power was significantly higher during KMI-EC
compared to KMI-EO (P<0.001) in all electrode sites except for left temporal and especially in occipital
sites. Consistent with preliminary findings in this sample (Di Noto et al., 2014), desynchronization was
observed during KMI-EC relative to baseline (P<0.001) while alpha power increased relative to baseline
during KMI-EO (P<0.001). This pattern of alpha (de)synchronization during KMI provides evidence for a
putative mechanism that facilitates clearer and more effective KMI when eyes are closed, with increased
cognitive load resulting in desynchronization during KMI-EC relative to baseline and additional suppression of visual signals resulting in alpha synchronization relative to baseline during KMI-EO.

Disclosures: **P.M. Di Noto:** None. **J.M. Chartrand:** None. **G.R. Levkov:** None. **R. Andrew:** None. **M.J. Wiland:** None. **J.F.X. DeSouza:** None.

Poster

634. Motor Skill-Learning

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 634.25/LL9

Topic: F.01. Human Cognition and Behavior

Support: Parkinson Society Canada

Title: Network recruitment during motor visualization of a dance in controls and expert dancers

Authors: ***P. DHAMI**[1], L. J. WILLIAMS[2], S. MORENO[3], R. J. BAR[4], J. F. X. DESOUZA[1]; [1]York Univ., Toronto, ON, Canada; [2]Child and Family Res. Ctr., Women and Children's Hosp., Vancouver, BC, Canada; [3]Rotman Res. Inst., Baycrest Hosp., Toronto, ON, Canada; [4]Canada's Natl. Ballet Sch., Toronto, ON, Canada

Abstracts: The objective of the current study was to investigate what networks are recruited in both controls and expert dancers when visualizing dance movements to a piece of music that neither group had any previous experience performing to. The study included 5 controls that had no dance experience, and 5 expert ballet dancers. All subjects underwent functional magnetic resonance imaging (fMRI) that included baseline and motor visualization blocks. The motor visualization blocks consisted of subjects visualizing performing dance movements to 1 minute of music in a blocked design with fixation periods of 30 seconds and 5 repeats of the same music. In order to investigate what networks were recruited due to the visualization, task partial least squares (PLS) was used, a multivariate technique that can identify groups of brain regions that change as a function of the tasks. Task PLS provided a single significant latent variable ($p < 0.05$, that accounted for 52.73 % covariance in the data) that reflected a common whole brain activity pattern that differentiated the motor visualization task from the baseline task in both groups. The baseline network included the right superior frontal gyrus, right middle temporal gyrus, left middle temporal gyrus, left middle frontal gyrus, left anterior cingulate cortex, right supra marginal gyrus and the right middle orbital gyrus. The motor visualization network that was found to be common in both groups included the left superior temporal gyrus, right rolandic operculum, right inferior frontal gyrus, left precuneus, right cerebellum, the left insula lobe and the supplementary motor area. Largest areas of activation as based on the voxel cluster size in the motor visualization network included the left superior temporal gyrus, right rolandic operculum and the supplementary motor area. The results indicate that although the two groups were in stark contrast in regards to level of expertise in dancing, when asked to visualize performing dance movements to a musical piece that they never had performed to, the expert dancers recruited a similar network to that of the controls.

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Poster

635. Reaching Learning

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 635.01/LL10

Topic: D.17. Voluntary Movements

Support: NIH R01 HD060306

Title: Limited effect of temporal constraints on feedback in learning to control movement velocity

Authors: *S. HILLENBRAND, K. OKPARA, T. TU, R. IVRY;
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Abstracts: Behavioral studies from a range of tasks, as well as computational models, suggest important temporal constraints for error based learning. Delay conditioning, visuomotor adaptation, and force field learning fall off when the interval between events relevant for learning extends beyond a few hundred milliseconds. This may reflect expiration of the eligibility for cerebellar plasticity, with reduced learning capacity as the predicted and actual feedback become temporally separated. Studies of these constraints typically introduce a delay between an action and feedback of that action. We used a range of temporal manipulations to investigate the importance of temporal regularity and ecological validity in a sensorimotor learning task. Participants performed a virtual shuffleboard task in which the terminal location of a puck was determined by hand velocity at the point of "release." Targets appeared at one of four distances from the start line, thus, ideal performance involved learning the mapping between hand velocity and distance. In a first experiment, we compared conditions in which the feedback appeared at the moment of release or after a 1 s delay. Contrary to studies of visuomotor adaptation, performance was unaffected by the delay. In a second experiment, we compared three groups. In the Random group, the feedback delay varied from trial to trial and was independent of hand velocity, ranging from 200 - 1200 ms. The same range was used for the Physics group, but the feedback time was defined by Newtonian laws (e.g., longer delay for longer throws). We also included a Reverse Physics group where we inverted the distance-time relationship (e.g., shorter delay for longer throws). Performance for the Physics group was superior to the Random group. Surprisingly, a similar improvement was observed in the Reverse Physics group. In a third experiment, the same three groups were tested, but the virtual friction was modified such that the range of feedback times was reduced to 200 - 700 ms, a range that may be more "cerebellar" and/or less subject to explicit strategies. Here we found no difference in performance between the three groups. Thus, temporal regularity can facilitate learning, but this effect is absent with shorter intervals. The cost observed with random timing in Exp. 2 may be attentional, related to the feedback occurring at unexpected times. The minimal sensitivity to temporal constraints exhibited in this task may stem from the fact that learning in this task requires modulation of hand velocity (e.g., gain) rather than hand position. Preliminary fMRI results using a variant of this task will clarify the neural contributions to movement velocity adjustments.

Disclosures: **S. Hillenbrand:** None. **R. Ivry:** None. **K. Okpara:** None. **T. Tu:** None.

Poster

635. Reaching Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 635.02/LL11

Topic: D.17. Voluntary Movements

Title: Long-Term learning in adaptation paradigms: Cognitive versus motor memory

Authors: *D. M. HUBERDEAU[1], J. W. KRAKAUER[2], A. M. HAITH[2];
[1], [2]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstracts: Adaptation paradigms are often thought to offer a model of long-term motor memory through the phenomenon of savings - faster adaptation the second time a perturbation is observed. However, recent findings have shown that behavior in adaptation paradigms is governed by both implicit motor learning and explicit, cognitive processes (Taylor et al., J Neurosci, 2014). The long-term memory exhibited through savings might therefore reflect implicit learning, explicit learning, or a combination of both. Here we attempted to determine the contribution of implicit and explicit processes to long-term learning based on differences in the amount of preparation time required by each process. We have recently shown that different components of learning can be dissociated based on their reaction time requirements (Haith et al., SfN 2013). If the RT is artificially limited - forcing a movement with less RT than would have otherwise been chosen by the participant - subjects express only a limited amount of their overall learning. Our interpretation of this finding is that the typical response time (RT) prior to initiating a reaching movement in the presence of a visuomotor perturbation includes a window of time for applying an explicit strategy. If preparation time is limited, application of the purported strategy is hampered (Fernandez-Ruiz et al., Behav Brain Res, 2011), leaving only implicit components of learning to be expressed on those trials. In the present study, we exploited this approach to determine which processes contribute to savings. To test this, we established a learn-relearn paradigm with occasional low RT probe trials interleaved among high RT trials within two adaptation learning sessions. We observed clear savings in high RT trials. However, in low RT trials, behavior was indistinguishable across days. This observation suggests that savings in adaptation tasks is due to the application of an explicit strategy and may not in fact represent a long term motor memory at all. Longer adaptation studies consisting of repeated exposures have shown that - under the appropriate conditions - the adaptation rate can increase further with subsequent exposure episodes (Gonzales-Castro et al., Curr Biol, 2014). We therefore asked if repeated exposure to the same perturbation leads to lasting changes in behavior

expressed during low RT trials. We demonstrate that under the appropriate conditions, behavior in low-RT trials can be influenced by prior experience, representing a more genuine example of long-term motor learning in adaptation paradigms.

Disclosures: D.M. Huberdeau: None. **J.W. Krakauer:** None. **A.M. Haith:** None.

Poster

635. Reaching Learning

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Program#/Poster: 635.03/LL12

Topic: D.17. Voluntary Movements

Support: NIH NS078311

Title: Rebuilding of motor memory: Increased efficiency at recall after time away from practice

Authors: *S. E. PEKNY[1], R. SHADMEHR[2];
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Abstracts: Can we minimize inefficiencies in our actions? That is, how we learn to eliminate motor commands that are effortful and yet irrelevant to success of the task? To study this puzzle, we trained human volunteers to reach in a force field. With practice, participants learned to produce forces that compensated for the field, generating task-relevant motor commands. As expected, training also resulted in generalization, the transfer of learning to other movements. In our task, any generalized forces were unnecessary and task-irrelevant. Critically, these generalized forces did not lead to kinematic errors, leaving no explicit cues or errors to indicate that this component of behavior was resulting in task-irrelevant motor commands. Could this inefficient generalization be reduced? We found that practice alone could not provide the conditions necessary to eliminate generalization, as participants continued to produce effortful and unnecessary forces after hundreds of trials. A short break of 3 or 30 minutes following initial training also did not aid in improving the efficiency of movements, as generalization force output remained constant during additional practice. However, following a 6 hour break, the production of generalization forces significantly decreased when the task was resumed, despite a maintained level of performance to the training direction. The drop in generalization force output observed following a 6 hour waiting period was identical to the decrease observed following a 24 hour break, indicating that 6 hours fell within the critical time period necessary to achieve this drop in generalization. Further experiments showed that whereas time away from practice was necessary

for this reduction, it did not result in a spontaneous drop in these forces. Rather, passage of time made the task-irrelevant commands eligible for reduction, but recall of the task-relevant commands was necessary to allow for the reduction of the task-irrelevant commands. These results illustrate a previously unknown property of motor memory: practice (and not time) improves the task-relevant component of motor memory, whereas time (and not practice) makes the task-irrelevant component eligible for reduction, a process that may depend on reconsolidation of motor memory.

Disclosures: S.E. Pekny: None. **R. Shadmehr:** None.

Poster

635. Reaching Learning

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Program#/Poster: 635.04/LL13

Topic: D.17. Voluntary Movements

Support: NSF 1137237

Title: Neural correlates of human motor control

Authors: M. S. D. KERR[1], K. KAHN[1], H.-J. PARK[3], S. THOMPSON[3], J. BULACIO[3], J. GONZALEZ-MARTINEZ[3], J. T. GALE[3], *S. V. SARMA[2];
[2]Biomed. Engin., [1]Johns Hopkins Univ., Baltimore, MD; [3]Cleveland Clin., Cleveland, OH

Abstracts: While the most common forms of neural data collected from humans are EEG and functional magnetic resonance imaging (fMRI), in our experimental setup we utilize stereo-tactic electroencephalography (SEEG). This includes the implantation of many depth electrodes, each of which can supply up to 10 independent channels of neural data. They collect a local field potential (LFP) style signal that represents the aggregate firing of many neurons in the region of the contact. A small number of epilepsy patients whose seizures do not respond to traditional treatment have this procedure done as a means to identify the seizure focus for resection. This modality gives a unique combination of temporal and spatial resolution than cannot be achieved using either fMRI or EEG. In this experiment, subjects with SEEG implants used a manipulandum to control a on-screen cursor and conduct a center-out reach task. Each trial, they were instructed to move in a specific speed range. Also, in approximately 1/5 of trials, a force field (perturbation) was applied of varying intensity and from a random direction. The perturbation started at the onset of movement and persisted until the subject held the cursor over

the target. While manipulandum based tasks are frequently done with health human subjects, they have never been done with similar neural data collection. The long-term purpose of this study is to tease out the neural correlates of human motor control and elucidate the mechanism by which the brain achieves such robust motor control in an uncertain environment. The unusual nature of the data collection method and patient pool will also provide the ability to ask a wide variety of questions. We can search for the neural correlates of movement speed and direction in structures such as the hippocampus. In addition, approximately 40% of subjects are clinically depressed and we are acquiring the relevant neurophycological test scores. Depression is associated with psychomotor retardation and this data set will provide an unprecedented way of comparing the motor processing of depressed versus non-depressed adults. While primary data analysis is ongoing, preliminary results indicate the right supracalcarine cortex modulates throughout the duration of movement. There is a substantial increase in the power in gamma and high gamma ranges (50-150 Hz) coexisting with a suppression of power in frequencies less than 30 Hz. Interestingly, this dual effect persists for the duration of the movement indicating that the area plays an ongoing role in feedback correction and not just a transient shift in attention.

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Poster

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AHA greatrivers postdoctoral fellowship, #12POST8710004

Title: Hemisphere Specific Ipesilesional deficits in Predictive and impedance control mechanisms

Authors: *V. YADAV[1], D. C. GOOD[2], R. L. SAINBURG[1,2];
[1]Kinesiology, The Pennsylvania State Univ., University Park, PA; [2]Neurol., The Pennsylvania State Univ., Hershey, PA

Abstracts: Based on our previous empirical research with stroke patients and healthy young participants, we proposed that handedness arises from differential specialization of each arm for distinct control processes: that the dominant hemisphere/arm are specialized for predictive control, while the nondominant hemisphere/arm are specialized for impedance control. We now directly test this control-based hypothesis by investigating inter limb differences in adaptation to two novel robot-applied force fields; a predictable velocity squared field that benefits adaptations through predictive mechanisms, and an unpredictable curl field that should advantage impedance control mechanisms. Previous findings in healthy participants revealed that the nondominant arm showed superior adaptation in the unpredictable field, while the dominant arm showed superior adaptation in the predictable field. We now examine the neural correlates of these control mechanisms by examining the effects of unilateral stroke to either the right or left hemisphere on ipsilesional arm adaptation. Our results reveal that following unilateral stroke to the right side of the brain, the reliance on predictive mechanisms increases as indicated by larger aftereffects following motor adaptation to either fields. Where as, following left hemisphere stroke, the subjects showed more reliance on impedance mechanisms as indicated by small to none after effects. Our preliminary findings suggest that unilateral stroke to the right side of the brain degrades impedance mechanisms, whereas stroke to the left side of the brain affects predictive mechanisms.

Disclosures: **V. Yadav:** None. **R.L. Sainburg:** None. **D.C. Good:** None.

Poster

635. Reaching Learning

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SRPBS MEXT

JSPS KAKENHI Grant number 26730073

Title: A computational model for learning reaching movement in 3-Dimensional Space and in force fields from scratch

Authors: *H. KAMBARA, H. SHIMIZU, D. SHIN, N. YOSHIMURA, Y. KOIKE;
Tokyo Inst. Technol., Yokohama, Japan

Abstracts: Human's musculoskeletal has many degrees of freedom and is actuated by dozens of muscles. The neural motor control system has to learn how to control such complex dynamics in a trial-and-error manner. In this study, we applied the motor control-learning model to reaching movements of upper limb moving in three-dimensional space. The motor control-learning model is based on the reinforcement learning algorithm that enables trial-and-error learning. We tested whether accurate reaching movements of complex musculoskeletal system can be acquired in a trial-and-error manner by reinforcement learning algorithm. The musculoskeletal of the upper limb was modeled as the two-link dynamical system composed of the shoulder joint with three degrees of freedom, the elbow joint with one degree of freedom, and twenty muscles with viscoelastic property. The feedback command signals to each muscle are learned by reinforcement learning algorithm and feedback-error-learning. The value of reward signal in the reinforcement learning is determined under the tradeoff between movement accuracy and energy consumption. As the result of simulation, we observed an increase in the amount of reward signal gained in each trial. In addition, the distance between target position and hand position at the end of each trial became smaller as the number of trial increased. We also applied the motor control-learning model to reaching adaptation to viscous-force and divergent-force fields in 2D-space. As the result of simulation, we observed several kinematic features, e.g. almost straight path and bell-shaped speed profile after adaption, huge after-effect after adapting to viscous-force fields, increase in the maximum speed after adaptation. The results of the computer simulations suggests that reaching movements of the upper limb with complex dynamics like biological musculoskeletal system could be trained in a trial-and-error manner based on the reinforcement learning algorithm.

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Poster

635. Reaching Learning

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Support: NIH Grant T32EB003383

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NIH Grant T32GM007057

Title: Changes in error-sensitivity account for sensorimotor savings

Authors: D. HERZFELD, *R. SHADMEHR;
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Abstracts: When a subject experiences a perturbation, they adapt their motor output on the next trial to reduce the error. We have suggested that the brain methodologically selects how much it is willing to learn from error. This modulation of behavior, or error-sensitivity, changes based on the history of errors that the subject experiences. Here, we asked whether changes in error-sensitivity, due to the history of experienced errors, can account for fundamental motor learning observations such as 'savings' and 'meta-learning'. Savings refers to the observation that when a subject practices a task with perturbation (A), and then the perturbation is removed, they exhibit faster re-learning of (A). We show that not only can a model which incorporates a memory of errors account for savings, but it can also account for meta-learning: prior exposure to a perturbation (B) generalizes to perturbation which has never been experienced. A prediction of our hypothesis is that we should be able to modulate savings by restricting the errors that are experienced during learning. Right-handed volunteers (n=50) participated in a visuomotor rotation experiment. The control group experienced a +30° (A) perturbation followed by washout and relearning of +30°, a protocol that should produce savings. According to our hypothesis, savings is present because the initial exposure of (A) represented a stable environment, increasing error-sensitivity to the errors, and these errors were revisited during subsequent test of (A). If so, we should be able to produce savings in a very different way: expose subjects to perturbation (B, -30°) and then present sudden washout (BNA), an example of meta-learning. When perturbation (A) is introduced gradually savings should be eliminated. That is, when the perturbation is introduced gradually (GNA), the magnitude of the experienced errors is significantly smaller than those experienced during testing of (A). Similarly, savings can be eliminated in the BNA group by reducing the magnitude of the experienced errors during washout. In the BGNA group, subjects learned (B) and then were gradually washed out (GN). During subsequent test of (A), we hypothesize that this group will not show savings. Our results confirmed our predictions: the ANA group showed significant savings (paired t-test, $p < 0.001$). In addition, the BNA group also showed savings of (A) compared to naïve ($p < 0.05$). In addition, we found no evidence of savings when the magnitude of the errors is significantly reduced: GNA ($p > 0.5$) or BGNA ($p > 0.1$). These results suggest the phenomena of savings and meta-learning may be partially explained by the history of errors that the subject experiences.

Disclosures: D. Herzfeld: None. **R. Shadmehr:** None.

Poster

635. Reaching Learning

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Program#/Poster: 635.08/LL17

Topic: D.17. Voluntary Movements

Support: CIN 2013-01

Title: Influence of the training schedule on intermanual transfer in the cart-pole balancing task

Authors: *N. LUDOLPH[1,2], M. A. GIESE[1], W. ILG[1];
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Abstracts: INTRODUCTION: A current topic in motor learning is how multiple learning and control mechanisms interact to produce overall improvement. In this study, we examined the intermanual transfer of control knowledge subsequent to different training schedules for the acquisition of the cart-pole balancing skill. METHODS: 17 right-handed subjects have been examined in the computer-simulated cart-pole balancing task. Continuous lateral forces were applied to the cart by the subjects using a joystick-like device in order to balance a pole which is attached to the cart. Subjects trained for 90 minutes using their right hand and switched afterwards for 30 minutes to the left hand. We tested two training schedules for the right-handed training: (i) gravity was gradually (g_inc=0.1) increased after every successful trial starting on g_0=1.0m/s^2 up to a maximum level of g_max=3.5m/s^2; (ii) subjects started directly on the maximum gravity level (g_max). Trials were counted as successful, if the pole angle and cart position remained within the valid ranges for 30 seconds. In addition to trial success or failure, subjects received up to 10 points per second depending on the system state and applied force. During the post-training phase gravity was constant (g_max) and performance of left-handed control was examined. RESULTS: First analysis shows that subjects in group (ii) instantaneously transfer the balancing skill to the left hand and do not further improve (N=9, $p \approx 0.57$, paired). In contrast, subjects in group (i) still improve during the left hand examination (N=8, $p<0.01$, paired). Accordingly subjects in group (i) tend to be worse at the beginning of the left hand examination. We did not find a significant performance difference at the end of the right-handed training between groups. Comparing group (i)'s left hand performance with the right-handed training of group (ii) yields that even though group (i) is better at the very beginning (first 10 minutes, $p<0.05$, unpaired) the performances are similar after 30 minutes. CONCLUSION: Previous studies suggested (Obayashi 2004, Anguera 2007) that control knowledge might be

shared between both hands. We hypothesize that the tested training schedules lead to the maintenance of different representations which allow intermanual transfer to a different extent. Brain imaging and stimulation techniques might provide further insight in which brain areas are involved and responsible for the representation in the tested conditions. REFERENCES: Obayashi S (2004), The Cerebellum 3 (4), pp. 204-211. Anguera JA et al (2007), Brain Research 1185, pp. 136-151.

Disclosures: **N. Ludolph:** None. **W. Ilg:** None. **M.A. Giese:** None.

Poster

635. Reaching Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 635.09/LL18

Topic: D.17. Voluntary Movements

Support: NIH Grant R01HD073147

Title: Anodal tDCS of dorsolateral prefrontal cortex and cerebellum enhance different aspects of motor learning in a visumotor adaptation task

Authors: ***S.-L. LIEW**[1], J. J. RAMIREZ[3], P. A. BUTCHER[4], L. G. COHEN[2], J. A. TAYLOR[4], P. A. CELNIK[3];
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Abstracts: An increasing number of studies have suggested that learning in visuomotor adaptation tasks may be the result of both implicit and explicit learning processes. In a visuomotor adaptation task, implicit learning is thought to be the result of training an internal model to predict movement errors. The cerebellum has been shown to play a critical role in this form of implicit learning. Studies have shown that anodal tDCS of the cerebellum improves adaptation in healthy individuals and that patients with cerebellar damage show deficits in adaptation. In contrast, explicit learning involves the utilization of explicit instructions or the generation of cognitive strategies to improve performance. The dorsolateral prefrontal cortex (dlPFC), which has also been associated with working memory, fluid reasoning, and problem solving, is thought to play a critical role in this form of explicit learning In the present study, we predicted that anodal tDCS over the cerebellum would enhance the error-based learning mechanism, while anodal tDCS over dlPFC would enhance the strategy-based component of a

visumotor adaptation task. To test this, we recruited 47 individuals, split into three groups: anodal cerebellar stimulation (CB, n=16), anodal dlPFC stimulation (PFC, n=16), and sham stimulation (SHAM, n=15). We used a paradigm from Taylor and colleagues (2014), in which participants directed a stylus towards a target on a circle, flanked by numbers, presented on a computer screen. Participants were asked to try to get their cursor on the target, and also to verbally report the number they were aiming at in order to get their cursor on the target. In this way, both explicit (aiming report) and implicit (internal model) components of each movement were measured. Importantly, vision of the hand was occluded. After 120 baseline trials, a 45-degree clockwise rotation was introduced for 160 trials, followed by a block of 40 trials without feedback and 40 trials with feedback. Results suggest that individuals receiving anodal dlPFC and cerebellar stimulation reduced target errors faster than the SHAM group. In addition, the dlPFC group demonstrated a greater explicit component (aiming angle) compared to CB or SHAM groups, while the CB group showed a modest increase in the internal component (internal model) over both groups. Overall, these results suggest that anodal stimulation of either cerebellum or dlPFC enhance motor learning during a visuomotor adaptation task and may exert their effects through different aspects of visuomotor adaptation.

Disclosures: S. Liew: None. **J.J. Ramirez:** None. **P.A. Butcher:** None. **L.G. Cohen:** None. **J.A. Taylor:** None. **P.A. Celnik:** None.

Poster

635. Reaching Learning

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 635.10/LL19

Topic: D.17. Voluntary Movements

Title: Learning to coordinate a redundant motor system: The role of postural comfort

Authors: *S. L. BARTON, B. R. FAJEN;
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Abstracts: During the production of a movement, the central nervous system (CNS) must organize components of the motor system around some desirable goal or outcome. A difficulty in this process is the reality that there are often more motor components than are strictly needed to satisfy the constraints of the task at hand. This excess of components creates redundancy in the movements and configuration of the motor system such that there exist multiple ways of realizing action goals. While redundancy is a source of computational complexity, it can also

provide stability and flexibility in action. Studies have shown that the CNS can exploit redundancy to reduce variability in action goals by strategically co-varying the actions of redundant motor components (Latash, Scholz, & Schöner, 2002; 2007). Such co-variation reduces error in the action goal by distributing variability across the set of redundant motor solutions that yield the same outcome. While this strategy has been demonstrated in a number of well-learned movements, this account critically assumes that redundant motor solutions are generally equitable. This contradicts findings that the comfort associated with particular configurations of the body are strong determiners of how an action is performed (Rosenbaum, van Heugten, & Caldwell, 1996). To understand how comfort might impact how the CNS handles redundancy, we asked subjects to solve a novel motor control problem involving coordinated arm movements. Using a full-body motion capture system, the angles of six arm joints were mapped onto the position of a cursor in a 2D virtual environment (Ranganathan, Adewuyi, & Mussa-Ivaldi, 2013). Subjects were required to move this cursor between four targets by adopting different arm postures. The mapping between arm posture and cursor position was both redundant and unintuitive, meaning subjects needed to learn how to resolve the redundancy problem. We found that over the course of practice, subjects improved the accuracy of their cursor movements. However, we did not find evidence of strategic co-variation between arm joints. Instead, subjects constrained their movements to a subset of solutions that were consistent with more comfortable body postures. We also found a reduction in the overall complexity of movements, and an increase in the similarity between postures adopted at the targets. Taken together, these findings suggest that reducing variability through co-variation is not the only explanation for how the CNS coordinates a redundant motor system. The comfort of particular motor solutions is important for constraining the set of redundant actions, as well as improving motor performance in a novel motor task.

Disclosures: **S.L. Barton:** None. **B.R. Fajen:** None.

Poster

635. Reaching Learning

Location: Halls A-C

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Program#/Poster: 635.11/LL20

Topic: D.17. Voluntary Movements

Support: European Research Council, ERC, 260424

Title: Motivational influence on error-based motor learning in stroke

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Abstracts: One of the main processes for learning new motor memories is through the correction of previous errors (error-based motor learning). Distinct components of this learning can be distinguished: adaptation, retention, and re-adaptation. It is largely unknown how reinforcement (punishment, reward) influences these components. Such knowledge would be important for devising more efficient rehabilitation schedules, e.g. following stroke. Adaptation and retention are thought of as distinct processes, with the former linked to the cerebellum, and the latter to the primary motor cortex (M1) (Galea et al., 2011). Given the role of the cerebellum in the processing aversive stimuli (Moulton et al., 2011), and of dopamine in both motivational processes and motor skills retention in M1 (Hosp et al., 2013), a dissociable influence of reward and punishment on error-based motor learning could be hypothesized (Galea et al., submitted). We tested here how reinforcement influences the components of error-based learning after stroke. Subjects affected by a first-ever chronic stroke, with no major cognitive deficits (MMSE >24), performed a force-field (FF) adaptation reaching task using a robotic manipulandum with their paretic arm. Patients affected by a cerebellar stroke, neglect or major psychiatric/cognitive/other neurological disorders were excluded. During baseline (day 1), patients performed 480 reaches towards eight targets. This permitted us to individually tailor the task for the subsequent days. In particular, we selected the two targets with minimal error at baseline and the FF direction (clockwise or counterclockwise) enhancing the main direction of error at baseline. On day 2 and 3, reaches to the two selected targets were performed. After 100 trials baseline, a velocity-dependent FF was applied (350 trials) on day 2 (adaptation) and 3 (re-adaptation), followed by a wash-out (150 trials). In three separate groups, comparable for age, sex and clinical impairment, patients received reward, punishment or null monetary feedback during day 2 adaptation, based on their end point angular error. Subjects were able to adapt in all groups, as shown by the progressive reduction of error and by the significant after-effects (in accordance with Patton et al., 2006). Preliminary results further reveal that patients in the reward group adapted (and re-adapted) faster and retained longer than patients in the punishment or null feedback groups. Ongoing analysis will investigate whether the response to feedback could be related to the lesion location/size. To date, these results support the use of motivational feedback to enhance error-based motor learning in stroke.

Disclosures: G. Quattrocchi: None. **S. Bestmann:** 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.; European Research Council, ERC, 260424. **J.C. Rothwell:** None. **J.M. Galea:** None. **R. Greenwood:** None.

Poster

635. Reaching Learning

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Program#/Poster: 635.12/LL21

Topic: D.17. Voluntary Movements

Support: NIH Grant R01HD040289

Title: Successful reward learning requires a balance between exploration and motor variability and outlasts error-based learning

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Abstracts: Human motor learning depends on a suite of behavioral mechanisms that includes error based adaptation and reinforcement learning. Here we asked 1) how learning a new reaching movement via these two mechanisms differs in acquisition and retention, 2) why learning via reinforcement is often unsuccessful and 3) if a different reinforcement schedule can improve learning. We studied healthy volunteers learning a reaching movement under visuomotor rotations. The reaching movements were 10 cm rapid shooting movements to a single target in a KinArm exoskeleton robotic device. In experiment 1 (E1) participants learned to counteract a 15° visuomotor rotation that was introduced gradually over 320 trials. The adaptation group could see their errors via cursor feedback representing hand position throughout each movement. In contrast, the reinforcement group did not see the cursor and instead received binary reward feedback at the end of each movement informing them if were within the 12° target. After learning, participants performed 100 trials without visual or reward feedback in order to assess retention of any learning. All participants in the adaptation group showed complete adaptation to the visuomotor rotation, but participants in the reinforcement group were divided into those able (learners) and unable (non-learners) to track the rotation. Reinforcement learners tracked the target similarly to the adaptation group during acquisition, but retained the new pattern longer once feedback was removed. To assess differences between reinforcement learners and non-learners, individual subject data were fit with a model that has two components of variability in the movement: inherent motor system noise and variability stemming from exploration behavior. When rewarded, the model updates its estimate of reach direction based on

the exploration variability used on that movement. Fits showed that non-learners exhibited high motor noise relative to exploratory variability. In experiment 2 (E2), a new group of healthy volunteers received binary reinforcement feedback that rewarded reaches lying between the mean reach angle of their preceding 10 trials and the rotated 15° target. Despite being rewarded in approximately 50% of trials some individuals remained unable to track the rotation. The total number of learners, however, was increased relative to the reinforcement group of E1. E2 learners also showed improved learning and similar retention of the rotated movement compared to the reinforcement group in E1 suggesting that learning by binary reinforcement feedback may be improved by a reinforcement schedule that is modified based on subjects' behavior.

Disclosures: A. Therrien: None. **A.J. Bastian:** None. **D.M. Wolpert:** None.

Poster

635. Reaching Learning

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Program#/Poster: 635.13/LL22

Topic: D.17. Voluntary Movements

Support: NSF Grant SES 1230933

NSF Grant CMMI 1200830

Title: Reward feedback accelerates motor adaptation

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Abstracts: Recent findings have demonstrated that reward feedback alone can drive motor adaptation. However, a number of questions remain unanswered. For example, it is not yet clear whether reward feedback alone can lead to learning when a perturbation is introduced abruptly, or how it can modulate the learning process. Here, we provide a more informative reward feedback that decays continuously with increasing error. We asked whether it is possible to learn an abrupt visuomotor rotation by reward alone, and if combining both reward and sensory feedback could modulate the learning process. We designed a novel visuomotor adaptation task during which subjects experienced an abruptly introduced rotational perturbation. Subjects (N = 46) grasped the handle of a robotic arm and made 15 cm rapid out-and-back reaching movements

to move an on-screed cursor from a home circle to a target arc. After 50 Baseline trials, subjects experienced Rotation trials, where the cursor underwent an abrupt 30 degree rotation with respect to the hand. After 450 Rotation trials, the environment returned to a 0 degree rotation for the remaining 50 trials (Washout). Subjects were assigned to groups that received either visual feedback of the cursor alone, reward feedback alone, or a combination of reward and visual feedback. A continuous reward feedback (i.e. a reward gradient) was presented to the subjects in the form of trial score. We tested subjects in a linear reward landscape, where the reward decayed linearly with distance from the target, and a cubic landscape, where the reward decayed more steeply with distance from the target. Results demonstrate that it is possible to learn from reward feedback alone and that the combination of reward and sensory feedback accelerates learning. No significant differences were observed in average error at the start or end of learning between the groups that received only reward feedback (no visual feedback) and the group that received only visual feedback (no reward feedback) ($p > 0.05$). Interestingly, we also observed that the combination of reward and visual feedback accelerated learning. We fit an exponential function to the individual subject error data early in the learning phase to determine the learning rate, c. We found that learning rates in the groups that received a combination of reward and visual feedback were significantly faster than the learning rate measure for the group that received only visual feedback ($p = 0.047$, $c = 0.457 \pm 0.063$, $c = 0.212 \pm 0.063$, respectively). These findings suggest that it would be promising to use reward feedback, either to supplement or substitute sensory feedback, for the development of improved neurorehabilitation techniques.

Disclosures: **M.K. O'Brien:** None. **A.A. Nikooyan:** None. **A.A. Ahmed:** None.

Poster

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Program#/Poster: 635.14/LL23

Topic: D.17. Voluntary Movements

Support: NSF BCS 1031899

Title: Responsibility-weighted multiple internal models explain stochastic decay in motor adaptation

Authors: ***Y. OH**[1], N. SCHWEIGHOFER[2];
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Abstracts: Following motor adaptation, performance level decays. However, the mechanism underlying this decay is unclear. In linear state-space models, performance decays passively in the absence of error as a sum of exponentials with different time constants (Smith et al., 2006; Kording et al., 2007). However, such a view may need to be re-evaluated because different unlearning protocols differently affect faster relearning or savings (Kitago et al., 2013), and because memory decay is specific to a context in which it was acquired (Ingram et al., 2013). In addition, a recent study showed that decay may not initiate immediately after the error is clamped to zero (Vaswani & Shadmehr, 2013). Instead, individuals had various lags before onset of decay, indicating that decay is a stochastic rather than a deterministic process. Moreover, subjects having experienced a loose clamp condition (errors varied around zero) exhibited longer decay lags. Because linear state-space models fail to explain both stochastic decay and savings after washout, we adopted the framework of multiple internal models (Lee & Schweighofer, 2009) with the Kalman filter banks (Hanlon & Maybeck, 1999). Each internal model makes its own prediction on sensory consequences, and a weight is assigned to each model based on its prediction error. Then, the motor output is determined from a weighted average of state estimations (Ghahramani & Wolpert, 1997; Wolpert & Kawato, 1998; Berniker & Kording, 2008, 2011). Here, we consider "the baseline model" as a natural internal model experienced from no perturbation, and rapid performance decay following adaptation is considered as a result of weight increase of the baseline model. This occurs when the prediction of the baseline model is more accurate than that of the adapted model. In error-clamped condition, such a rapid change in weight requires an accidental deviation of a motor command from the adapted one due to motor variability, thus the process is stochastic by nature. One prediction from this model is that individuals with larger motor variability are likely to experience shorter decay lags than individual with smaller variability, and our pilot data support this relationship. Similarly, savings is also explained as a recall of the adapted model: when the same perturbation is abruptly applied after washout, the prediction of the adapted model is much better than the baseline model, thus its weight increases quickly, and the motor performance changes "back" to adapted level. In our pilot data with repeated blocks of washout and relearning, subjects showed increased savings at every repetition, suggesting that savings is close to recall than relearning.

Disclosures: Y. Oh: None. **N. Schweighofer:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: C. Caro research scholarship for distinguished graduate students in Bioengineering

Title: A framework for updating optimal feedback control strategies that predicts continuous action selection in complex motor tasks

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Abstracts: The brain coordinates the continuous coupling between perception and action in the presence of uncertainty and incomplete knowledge about the world. From an engineering point of view, this mapping is enabled by control policies. However, the mechanisms that underlie learning such policies remain elusive, particularly in complex motor tasks. We propose here that in such tasks the brain makes continuous decisions for the generation of complex trajectories by learning Optimal Feedback Control (OFC) (Wolpert & Landy, 2012; Todorov & Jordan, 2002; Nagengast et al, 2009) based on the identification of unknown intrinsic and extrinsic task parameters (Sylaidi & Faisal, 2012). We present experiments and theory that can capture the temporal dynamics of motor learning on a trial-by-trial basis.Human subjects (N=15) were instructed to move a virtual tool of unknown dynamics from start to target locations in an unintuitive task that translated hand velocity into control forces on the object state. Subjects received a performance feedback after the completion of each trial in the form of a cost function capturing the end point error, a control penalty and an end state penalty. For the considered experimental setting we proposed a motor learning model, which tests the hypothesis that the brain selects actions by updating the unknown task parameters. These determine both arm and object dynamics and are updated in a locally linear system identification process, which in turn enables a decision on the form of the Optimal Feedback Control policy. The proposed framework updates learned parameters in gradient descent steps driven by the error between predicted and actual object movements in each trial. Our approach expands previous studies on mechanisms of task parameter adaptation, which considered fixed control policies throughout the examined experimental sessions (Berniker & Kording, 2008). Our model predicts accurately the gradual progression of human learning from trial to trial. Furthermore, the model's end-performance predictions outperform an ideal observer model, which assumes complete knowledge of task dynamics and can thus not capture partial learning, to which humans commonly converge in naturalistic task contexts. Our results suggest that the brain employs simple learning rules to support decisions implemented by near optimal control in complex object manipulation tasks. This psychophysically verified, abstract mechanism of motor learning can guide neurophysiological investigations of the underlying cortical foundation of action selection and motor learning principles.

Disclosures: **A. Sylaidi:** None. **A.A. Faisal:** None.

Poster

635. Reaching Learning

Location: Halls A-C

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Program#/Poster: 635.16/LL25

Topic: D.17. Voluntary Movements

Title: Altering effort costs in Parkinson's disease using non-invasive brain stimulation

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Abstracts: We considered an isometric task in which people held the handles of two robotic arms, one in each hand. The goal was to push on the left and the right robotic arms in such a way as to move a cursor toward targets distributed around a circle. The position of the cursor was the sum of the force vectors produced by each arm. For each direction of target the subjects chose how much force to produce with their right and left arms. This choice was remarkably consistent over repeated days, suggesting that a consistent cost dominated the choice of action. We hypothesized that the choice that people made was due to a cost that had two components: a cost for variability, and a cost for effort. To measure variability, in a unimanual task we measured variance of force for each arm at each direction of force. To measure effort, we measured the maximum voluntary force for each arm and each direction. Using an optimal control model, we estimated cost of effort in n=15 right-side affected PD patients and found that this cost was elevated on the affected side. Therefore, in PD effort costs are abnormally large on the affected side. All participants in this study developed asymmetric motor symptoms from disease onset. We applied anodal tDCS to M1 of the affected hemisphere and cathodal in contralateral M1 of PD patients (n=10). This increased the use of the unaffected side in the bimanual task, worsening symptoms. However, changing the polarity of stimulation in another group (n=10) produced an immediate reduction in variance on the affected side in the unimanual task. That is, in PD cathodal stimulation of affected M1 reduced the variance for control of movements on the contralateral side. Consistent with this, the patients altered the choices that they made in the bimanual task and cathodal tDCS made their choices more similar to healthy controls. We followed this with a 10-day study (n=8 patients). We observed consistent improvements in clinical motor scores, increased use of the affected side in the bimanual task, and reduction in noise on the affected side. In summary, we found that choices that PD patients made in their actions could be explained by a cost that had two components: variance, and effort. On the

affected side, variance is significantly increased with respect to controls, and the choices of PD patients are generally consistent with this increased variance. We can improve the choice of the PD patients in bimanual task by using cathodal tDCS on affected side while they are on dopaminergic medication. This improves the unimanual variance on the affected side and increases use of the affected side in a bimanual task. The repeated application of tDCS improved motor symptom of PD by decreasing clinical symptoms.

Disclosures: Y. Salimpour: None. **Z. Mari:** None. **R. Shadmehr:** None.

Poster

635. Reaching Learning

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Topic: D.17. Voluntary Movements

Support: KAKENHI 26242062

NEXT Program #LS034

Bilateral Joint Research Project (JSPS - F.R.S-FNRS)

Brains Back to Brussels (Belgium)

Title: Artificial manipulation of human motor memories using noninvasive brain stimulation

Authors: *D. NOZAKI[1], A. YOKOI[2], T. KIMURA[3], M. HIRASHIMA[1], J.-J. ORBAN DE-XIVRY[4];
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Abstracts: It is widely recognized that adapting an identical reaching movement simultaneously to conflicting dynamical environments is quite difficult due to the interference. However, several recent works have demonstrated that simultaneous adaptation is not impossible: For example, we have showed that participants can easily develop distinct motor memories for a reaching movement depending on whether the opposite arm is stationary or moving (i.e., unimanual or bimanual movement) (Nozaki et al., Nat Neurosci 2006). These results suggest that distinct neural representations of a movement depending on different behavioral contexts are associated

with different motor memories. That is, the distinct motor memories observed for unimanual and bimanual movements would stem from their partially distinct neural representations (Donchin et al., J Neurophysiol 2002). Here, to confirm this idea in a more causal way, we tried to examine if artificially induced changes in neural representations using transcranial direct current simulation (tDCS) could contribute to the formation and retrieval of distinct motor memories. Sixteen participants performed forward reaching movement (10 cm) while holding a handle of manipulandum with right hands (KINARM End-Point Lab, Bkin Technologies, Canada). Clockwise (CW) or counter-clockwise (CCW) force field was applied to the handle in a blocked manner (Each block consisted of 20 trials for each force field). tDCS was applied by electrodes placed over left and right primary motor cortices (M1) (i.e., bihemispheric tDCS). In the 6 blocks of training session, the CW or CCW force field was associated with anodal and cathodal tDCS to left M1, respectively. Following the training session, 2 blocks of test session were set up to assess whether the motor memory acquired in the training session was read-out according to the polarity of tDCS. To quantify the acquired motor memories, we adopted the error clamp method in which any force perpendicular to the force channel during movement was measured as aftereffect of the learning. Surprisingly, we observed that the aftereffect was clearly modulated with the polarity of tDCS. In contrast, tDCS in the test session was ineffective to modulate the aftereffect for the participants who trained the force fields with sham tDCS (N=6). These results indicate that tDCS could help to differentiate motor memories between the conflicting force fields and to retrieve appropriate motor memories. The present results are also consistent with our hypothesis that distinct neural representations of identical movements can contribute to develop the distinct motor memories.

Disclosures: D. Nozaki: None. **A. Yokoi:** None. **T. Kimura:** None. **M. Hirashima:** None. **J. Orban de-Xivry:** None.

Poster

635. Reaching Learning

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Program#/Poster: 635.18/LL27

Topic: D.17. Voluntary Movements

Support: National Institute of Information and Communications Technology

Title: Formation and destruction of motor policy alter the position and the velocity dependences during motor adaptation

Authors: *J. IZAWA[1], T. YOSHIOKA[2], R. OSU[2], H. GOMI[1];
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Abstracts: Motor primitives essentially characterize formation of motor memory. When the brain encounters a novel force environment, the brain initially updates gains of the position sensitive components of the limbs movements and, then, gradually updates gains of other components so that it can produce the ideal motor commands as a sum of these components in order to cancel the given force perturbation (Smith 2009). However, in an alternative view, motor adaptation is a process of re-optimization of motor commands for maximizing the task performance and for minimizing the motor costs (Izawa 2008, 2011). Here, we aimed to capture the effect of optimization on forming the gains of the motor primitives. To this end, we recruited two groups of subjects who were asked to reach in the curl force-field with holding the handle of the robotic manipulandum. One group experienced 450 trials of the force manipulation task in advance of the force-field adaptation block where they were asked to reach in the force channel pushing the wall perpendicularly to the reach direction. As a control, the other group experienced the same number of reaching trials without producing the additional force. Since both groups of subjects leaned to decrease the trajectory error in the comparable level, the prior force manipulation task did not influence the basis of the brain to achieve the reach adaptation. In contrast, the estimated two gains (i.e. position and velocity dependency) from the acquired motor commands were significantly dissociable between two subjects' groups. These learned gains were reasonable in terms of energy efficiency because the estimated motor costs were also distinguishable between two groups. When they encountered the second force field which had the opposite-sign with respect to the first force-field without any break, the optimality of the learned gain was collapsed. Updating gains of motor primitives for producing the ideal motor commands alone is not enough to explain these observed trial-to-trial profiles of the position and the velocity gains. Rather, the brain updated these gains to form optimal policy as a function of the neural encoding of limb movements. When the brain encountered the second novel environment after it had adapted for the first environment, it started to re-optimize the policy which is specific for the second environment. As a result, the optimality appeared to be collapsed after imposing the second environment. We captured this process by modulating the sensitivity to the motor cost by introducing the force manipulation task in advance of the force-field adaptation.

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Poster

635. Reaching Learning

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Program#/Poster: 635.19/LL28

Topic: D.17. Voluntary Movements

Title: Prismatic adaptation of movements toward visual and proprioceptive targets

Authors: *F. R. SARLEGNA[1], H. LEFUMAT[1], A. HETU[2], P.-M. BERNIER[2];
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Abstracts: Arm movements can be performed toward visual but also proprioceptive targets. Sober and Sabes (2005) and Sarlegna and Sainburg (2007) showed that distinct planning mechanisms underlie these two types of movements. Bernier et al. (2007) also argued for different control mechanisms by showing that prismatic adaptation of movements toward visual targets does not affect subsequent movements toward a proprioceptive target. Here we studied whether distinct mechanisms underlie the adaptive control of movements toward visual and proprioceptive targets. Two groups of 10 healthy right handed subjects reached with their seen right hand toward a visual (VT group) or a proprioceptive target (PT group). The P was the unseen left hand index. There were 3 experimental phases: pre-test (30 reaching trials), adaptation phase (100 trials with prismatic goggles deviating the visual field by 17°) and post-test (30 trials). In both groups, the target was continuously present, at the same location. A beep instructed subjects to reach as fast and as accurately as possible toward the target. Results showed that in the VT group, the hand path was altered rightward by the prisms on the first trial of the adaptation phase. As previously shown, subjects systematically adapted after a few trials, restoring baseline performance. In the PT group, we observed that the first reaching movement in the adaptation phase was unaffected by the prismatic goggles as the right hand index continued to accurately land above the left hand index as in the pre-test. Then, as trials were repeated during the adaptation phase, initial movement direction (at peak velocity) and final hand position drifted leftward of the target position. After-effects did not significantly differ between groups (mean=9°). When subjects in the PT group performed their first movement with prisms, their felt right hand was accurately located above the felt left hand while the seen right hand was rightward of the felt left hand. The fact that adaptation was observed subsequently suggests that vision dominates proprioception to determine the position of the reaching hand and that the comparison of right hand visual signals and left hand proprioceptive signals led to a sensory error signal and thus to adaptation. Another possibility is that a prediction error, derived from the comparison between the predicted visual hand position and the actual visual hand position, may have led to the adaptation of the reaching movements in both VT and PT groups. This would suggest that when we reach with our seen hand toward visual or non-visual targets, the error in predicting the visual consequences of the movement drives adaptation.

Disclosures: **F.R. Sarlegna:** None. **H. Lefumat:** None. **A. Hetu:** None. **P. Bernier:** None.

Poster

635. Reaching Learning

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GMU URSP

Title: The decay of motor adaptation to novel movement dynamics reveals hysteresis in motor primitive gain-space

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Abstracts: Studies of motor adaptation have consistently demonstrated that subjects adjust their movements when exposed to an external perturbation, such as a force-field applied to reaching movements. This adaptation is largely driven by the experienced movement error and various computational models have successfully described this process. One recent model by Sing *et al.* (2009) showed that the evolution of motor output during adaptation to different force-field perturbations was effectively explained as a combination of motion state primitives, specifically position and velocity. We investigated the interaction of these components during the decay of adaptation. In the first experiment, we tested subjects with either position (N=8) or velocity-dependent force-fields (N=9) and following training, immediately exposed them to a series of error-clamp trials to directly measure the decay of the learning. Similar to Sing *et al.* (2009), we found that motor output in early adaptation to either perturbation was a combination of both relevant (the motion state of the force-field) and irrelevant motion components. Additionally, in late adaption, the contribution of the task-relevant component increased to account for the majority of the adaptive response (91% for velocity-dependent learning, 85% for position). During the decay period, motor output gradually reverted to baseline levels, but throughout this period the task-relevant component was significantly greater ($\geq$ 82%) than the irrelevant component ($\leq$ 18%). Interestingly, when the respective gains were depicted in state space there was substantial hysteresis—the trajectory during learning was further from the task-relevant axis than during the decay period. In a second experiment we trained subjects (N=5) in the position-dependent force-field over two days to determine how additional training influenced the interaction of the task-relevant and irrelevant components, limb position and velocity

respectively. Training was the same on both days, but decay was only assessed on the second. We found clear savings on the second day of training (a faster learning rate) which resulted in a difference in the orientation of the respective gain space learning trajectories: the initial trajectory for learning on the second day was significantly greater in the task-relevant position component than on the first ($p = 0.012$). In summary, we find that the decay of adaptation to novel dynamics travels through a different gain-space trajectory than initial learning and the resulting hysteresis reveals the stability of the task-relevant learning. In addition, we show this hysteresis is diminished with multiple trainings.

Disclosures: K.P. Nguyen: None. **E.A. Hosseini:** None. **W.M. Joiner:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: NSERC Grant

Title: Dual adaptation is facilitated by intrinsic contextual cues and saturates upon extended training

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Abstracts: When reaching towards objects, the human central nervous system (CNS) can actively compensate and adapt to two different perturbations simultaneously (dual adaptation), though this does not simply occur upon presentation. In fact, the CNS requires distinctive contextual cues to differentiate between adaptive states. Furthermore, not all contextual cues are effective in facilitating dual adaptation. In two experiments we investigated the efficacy of contextual cues which are intrinsic to the CNS including hand and body posture, as well as the role of extended training in adapting to two opposing visuomotor rotations concurrently. Using a virtual reality paradigm, participants manipulated a projected hand-cursor using a digitizing tablet in a semi-dark room with an opaque board occluding the view of the arm. Cursor rotations of 30° clockwise and counter-clockwise were each associated with 2 distinct hand postures (i.e. precision and power grips) respectively in the first experiment and 2 distinct body rotations (i.e. leftward and rightward turn of the seat, while fixating straight) respectively in the second

experiment. Participants completed pre-training where they were instructed to reach towards visual targets with an aligned cursor, training, where they reached with misaligned cursors, and post-training where they reached without visual feedback of the hand in order to capture any aftereffects following adaptation. In addition, because recent work from our lab and others have shown that the learning rate in dual adaptation is not as steep as that of single adaptation, we implemented an extended training set in the first experiment to examine the effect of greater practice. We found that how people held the tool or oriented their body while reaching is sufficient for recalling an adaptive state (or previous visuomotor mapping) such that over time, reach errors significantly decrease despite being presented both perturbations in a randomized, concurrent manner. This adaptation was further reflected in the presence of significant aftereffects following training. No significant reduction in reaching errors nor increases in percent improvement in reach adaptation were found following extended practice suggesting that dual adaptation training reaches a saturation point. Our results suggest that intrinsic cues which produce distinct muscle synergies are effective at facilitating dual adaptation while extended training provides no additional benefits.

Disclosures: **M.N. Ayala:** A. Employment/Salary (full or part-time):; York University. **D.Y.P. Henriques:** A. Employment/Salary (full or part-time):; York 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.; Natural sciences and engineering research council of Canada.

Poster

635. Reaching Learning

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Topic: D.17. Voluntary Movements

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UTAS REGS Grant C0021896

Title: Investigating the mechanisms of repetitive trans-cranial magnetic stimulation using motor learning paradigms and *in vivo* 2 photon imaging

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Abstracts: **Introduction/Objective** Brain function is underpinned by electrical activity inducing chemically mediated communication between cells, both of which are crucial in establishing normal brain structure and function during development and maintaining them throughout life. Modulation of electrical activity by repetitive transcranial magnetic stimulation (TMS) is becoming widely recognised as a clinically applicable non-invasive intervention to improve and repair neural function. However, the mechanisms whereby such neuromodulation occurs, and how long they last, are largely unexplored. Rodent models can be useful in establishing structural and molecular mechanisms associated with neuromodulation. To this end, we have combined TMS with either a motor learning behavioural paradigm or live *in vivo* 2 photon imaging of motor cortex circuitry to investigate neuroplasticity resulting fromTMS. **Methods** We use a rodent specific TMS 8mm outer diameter circular coil to deliver intermittent theta burst stimulation (iTBS) over the motor cortex of awake adult male mice (*Mus musculus*). Thy1-GFPM mice undergo cranial window insertion overlying the right motor cortex to enable visualisation of excitatory cortical neurons in the upper layers of the motor cortex. Images of cortical circuitry are collected at regular intervals before and after iTBS and analysed for alterations in connectivity resulting from daily stimulation (600 pulses, 190 sec). For motor learning paradigms, adult C57Bl6/J mice are maintained at 90% body weight, habituated to experimental conditions and learning assessed by pellet reaching through a narrow vertical slit in a Perspex box over a period of 10 days. Mice receive either daily iTBS (600 pulses, 190 sec) or sham stimulation immediately prior to being placed in the box. All experiments are approved by University of Tasmania Animal Ethics Committee. **Results** We have established a baseline trajectory for skilled reaching task comparable to published data. Preliminary analysis on day 4 of training indicates no change in motor learning with the application of iTBS. Analysis of imaging data is ongoing. **Conclusions** The use of rodent models to address the mechanism of action of TBS, will undoubtedly pave the way forward to more appropriate uses and therapeutic applications of non-invasive brain stimulation in health and disease.

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Poster

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Topic: D.17. Voluntary Movements

Support: NSF BCS 1031899

NIH R01 HD065438

Title: Joint kinematics and synergies during motor learning with a redundant arm exoskeleton in individuals with sub-acute stroke

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Abstracts: The improvement in performance during recovery and motor training post-stroke has been thoroughly investigated via changes in hand kinematics (e.g. Rohrer et al. 2002, Van Dokkum et al. 2014). However, the longitudinal changes in the redundant arm joint kinematics space, and how these changes relate to performance improvement in hand space, are less well understood. Here, we investigated changes in hand kinematics, joint kinematics, and joint synergies in 7 post-stroke individuals with mild to moderate impairments in the sub-acute phase. Participants received motor training with the redundant ARMEO Spring device arm exoskeleton (Hocoma) twice a day over 4 consecutive weeks, 5 days per week. Each training session consisted of series of serious games in which the patients controlled a cursor via 3D displacement of the hand and forward kinematics transformation of the 6 joints (3 at the shoulder, 1 at the elbow, pronation-supination and wrist flexion-extension). We tested performance with a vertical pointing test repeated at the beginning and end of each session. We analyzed hand and joint kinematics as well as joint coordination via principal component analysis (PCA). We compared the performance measured by hand kinematics with healthy subjects (Schweighofer et al. 2013, SFN), and discussed how the improvement of performance in hand space may be influenced by joint kinematics and joint synergies.

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Poster

635. Reaching Learning

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Topic: D.17. Voluntary Movements

Support: Canadian Institutes of Health Research (CIHR)

Fonds de la recherche Québec Santé (FRQS)

Title: Suppression of visual feedback during force-field adaptation impairs motor acquisition without affecting next-day retention

Authors: *C. S. BATCHO[1,2], M. GAGNÉ[2], L. J. BOUYER[1,2], J.-S. ROY[1,2], C. MERCIER[1,2]; [1]Univ. Laval, Quebec, QC, Canada; [2]Ctr. interdisciplinaire de recherche en réadaptation et intégration sociale (CIRRIS), Quebec, QC, Canada

Abstracts: Motor rehabilitation is important to optimize recovery of function after neurological impairments. Previous studies have suggested that visual feedback may help enhancing motor performance during learning. The aim of this study was to investigate the role of visual feedback in the acquisition and retention stages of motor learning associated with training in a reaching task. Twenty-eight healthy subjects made ballistic reaching movements with their dominant arm towards two targets (Far and Near), on 2 consecutive days using a robotized exoskeleton. They were randomly assigned to a group with (VFb) or without (noVFb) visual feedback of index position during movement. On Day 1, the task was performed without (baseline) and with a force field (adaptation). To assess retention, participants repeated the task with the force field on Day 2. Motor learning was characterized with: (1) the initial angle (iANG) between the trajectory and a straight line to the target ("motor planning") and (2) the final error (fERR) ("movement accuracy"). Two-way ANOVAs (Time X Group) were performed for each condition (Baseline and Adaptation), variable (fERR and iANG) and target (Near and Far). Results showed no significant differences between groups at baseline. During force field adaptation, a significant Time x Group interaction ($p \leq 0.033$) was found for both targets. Contrast analysis revealed that the VFb-group decreased their fERR faster than the NoVFb-group on Day 1. However the fERR was similar between groups on Day 2. iANG showed a change in the feedforward control of movement during training on Day 1, and this effect was maintained on Day 2. However, little difference was found between groups. Indeed, a significant Time x Group interaction was found only for the Far target, and the contrast analysis showed a significant difference between groups only at the end of Day 1. These results suggest that despite a slower motor acquisition on Day 1, the NoVFb-group showed a similar retention on Day 2. This finding is consistent with previous studies showing that larger gains during training are not always predictive of better long-term retention. Therefore, assessing retention in the actual task is critical to determine optimal feedback conditions during motor training in rehabilitation.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH (NINDS) R01NS073952

Title: Neural basis for motor learning: Sensorimotor cortical ensembles multiplex spatial, temporal and reward-related information

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Abstracts: Motor learning is the hallmark of brain plasticity. Although motor learning has been studied extensively, the neural mechanisms that drive such motor plasticity are not well understood. A recent study suggests that reward pathways project to human M1 (Kapogiannis et al., 2008). However, how reward and reward anticipation might modulate sensorimotor cortical ensembles remains a question. We designed a task that dissociated spatial characteristics of movements from reward anticipation. Monkeys performed a center-out reaching task that incorporated a delay period between target acquisition and the reward. The duration of this period (1s vs. 3s) was signaled by the target color (red vs. blue). Monkeys were involved in the task in three different ways in different sessions: they passively observed a computer-controlled avatar arm performing the task, manually moved the joystick to control avatar arm movements, or moved the avatar arm with their brain activity. Large-scale recordings from several hundred neurons from sensorimotor cortex and posterior parietal cortex revealed robust reward anticipatory activity that preceded the reward. While some neurons (~36%) showed a buildup or sustained firing rate until reward long after movement ended, some others (~10%) fired just around reward delivery. A KNN-classifier applied to the neuronal ensemble revealed that the neuronal tuning underwent a transformation: the ensemble encoded the target location during the reach movement, and then transformed to encode reward anticipation. Furthermore, with long-

term training, the ensemble represented the anticipation earlier in the trial and sustained this activity longer during the hold time. These results show that spatial, temporal and reward-related signals are represented in a highly distributed way by cortical networks. We suggest that adaptations of these representations underlie motor learning and understanding the process of adaptation would allow optimization of reward-based learning in neuroprosthetic control.

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Poster

635. Reaching Learning

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Topic: D.17. Voluntary Movements

Support: STW Grant 12668

Title: Scaling errors down improves retention of motor adaptation

Authors: *R. J. VAN BEERS, E. BRENNER, J. B. J. SMEETS, K. VAN DER KOOIJ;
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Abstracts: Many studies on motor adaptation have focused on the factors that determine how fast subjects adapt to a perturbation. A more recent development is that motor adaptation is also studied to determine the factors that determine how well adaptation is retained. Here we examined how scaling the sensory feedback about motor errors affects retention of motor adaptation. Subjects reached to visual targets in eight directions from a fixed start location. They adapted to a visuomotor rotation, such that they had to move in a direction that differed 30 deg from the actual target direction in order to reach the target. This rotation was introduced gradually over 48 movements. Moreover, the error in the movement direction with respect to the required direction was multiplied by a gain factor to determine the displayed hand position. We tested three groups of subjects with gains of 0.5, 1 and 2. An analysis of the initial movement directions after adaptation showed that subjects in all groups had adapted to about two-thirds of the perturbation. After the adaptation phase, subjects made 200 error-clamp movements, in which they always received visual feedback that suggested that they moved in the correct direction. During this error-clamp block, the level of adaptation decreased in all groups. However, the extent of the decrease differed strongly between groups. Whereas the gain-2 group

retained only 52% of what they had learned, the gain-1 and gain-0.5 groups retained 63% and 90%, respectively. Our results show that scaling the sensory feedback about movement errors has a strong effect on retention of motor adaptation. Whereas scaling errors up may lead to faster learning, it has a negative effect on retaining what has been learned. Scaling errors down, in contrast, may lead to somewhat slower learning, but what is learned is retained very well. Downscaling of errors may therefore be a useful technique for training when it is important that what has been learned is not quickly forgotten, such as in movement rehabilitation following brain injury or stroke.

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Poster

636. Rehabilitation

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Title: Recovery in upper body mobility through practice with body machine interface

Authors: *C. PIERELLA[1,3,4], F. ABDOLLAHI[1], A. FARSHCHIANSADEGH[1,5], J. PEDERSEN[2], D. CHEN[2], E. THORP[5,1], I. SEÁÑEZ-GONZÁLEZ[5,1], F. A. MUSSA-IVALDI[1,5,4], M. CASADIO[3];
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Abstracts: People with high-level spinal cord injury (SCI) face two related problems: recovering motor skills and regaining functional independence. Assistive technologies are instrumental for them to interact with the environment and partially replace the lost functionalities, but their use is often challenging because the users need to reorganize their residual ability in unintuitive ways, e.g. sip-and-puff or head arrays. Moreover, most SCI survivors receive intense rehabilitation treatments that promote motor recovery only shortly after injury, when they are hospitalized. However, they do not continue with the same frequency after being released from the hospital. Body-machine interfaces (BoMIs) can be a valid solution to these problems. They empower people with severe motor disabilities with the possibility to control external devices, and they concurrently offer the opportunity to focus on achieving rehabilitative goals. In this study we developed a portable and low-cost body machine interface that addresses both problems. The proposed BoMI remaps the user's residual upper body mobility to the two coordinates of a cursor on a computer screen. This mapping is obtained via standard techniques for dimensionality reduction, such as principal component analysis (PCA). We hypothesize that the BoMI can be specifically programmed to engage the users in functional exercises aimed at partial recovery of motor skills, while simultaneously controlling the cursor and carrying out functional tasks, e.g. playing games. Specifically, PCA allows us to select not only the subspace that is most comfortable for the user to act upon, but also the degrees of freedom and coordination patterns that the user has more difficulty engaging. In this case study, we attempted to change the body-motion symmetry of a single SCI participant with an incomplete lesion at the C4 level. At the beginning of the experiment, the participant demonstrated a marked reduction in the mobility of the right side of the upper body compared to the left side. Thus, we changed the parameters of the BoMI so as to increase the role of the right side and tested the effect of this alteration after 2 weeks of practice. Results showed that this approach restored a higher level of symmetric mobility. The present study is the first proof of concept of the use of the BoMI in the rehabilitation field. Engaging the user in functional and entertaining tasks while practicing the interface and changing the map in the proposed ways is a novel emerging approach to home-based rehabilitation treatments provided by portable and low-cost technologies.

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Poster

636. Rehabilitation

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con il contributo del Ministero degli Affari Esteri, Direzione Generale per la Promozione del Sistema Paese

Title: Robot-assisted training of the non-paretic arm contributes to recovery for chronic stroke survivors

Authors: *M. CASADIO[1], A. DE LUCA[2], H. VERNETTI[2], C. LENTINO[3], G. CHECCHIA[3], P. GIANNONI[4];
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Abstracts: To quickly regain independence in daily life activities, stroke survivors develop and strengthen compensatory strategies that involve trunk movements and posture, as well as the unimpaired side of the body. Sixteen chronic stroke survivors (7 male, 9 female, age 63.06 ± 8.84 years, 11 right and 5 left hemiplegia, 5.96 ± 4.28 years from the acute event) participated on 20 therapy sessions based on active movements of the unaffected arm supported by exoskeleton Armeo Spring (T-Wrex). A physiotherapist helped subjects maintaining a correct sitting posture. During the treatment, unaffected limb performance was evaluated with the training device in a reaching test performed at the beginning and at the end of each session. With training, all subjects improved the not paretic arm performance (duration -0.79±SE 0.09 seconds, $p<0.0001$, accuracy $p=0.01$, smoothness $p=0.008$). The paretic arm performance of seven subjects was tested at the beginning and the end of the treatment with the Armeo evaluation test. We found a significant improvement in the task execution time (-0.72±0.22 SE seconds, $p=0.01$) and in the number of targets reached with the un-treated impaired arm. A clinician evaluated subjects before and after treatment. We found a significant improvement in the scores of the Trunk Impairment scale (+2.06±0.6 SE $p=0.005$) and in the impaired arm function: +3.87±0.99 SE points of the Fugl-Meyer Scale upper arm section ($p=0.001$). 12/15 subjects improved also the Wolf test score. The amount of improvement in the FM score is comparable to the results obtained with rehabilitation robotic treatments focused directly on the impaired arm. We plan to further investigate these findings. However, as it stands, this result highlights the importance of taking into account in the rehabilitation robotic program all body schema, instead of focusing exclusively on the most impaired side of the body. This study also suggests that the recovery we observed in chronic stroke survivors after robot-assisted rehabilitation could be mainly due to the control of compensatory strategies and the empowerment of weak or silent skills, rather than to the re-acquisition of new or lost abilities on the affected side.

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Poster

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Topic: D.17. Voluntary Movements

Support: NICHHD 1R01HD072080

NIIDRR H133E120010

Title: Age related effects on motor learning when using a novel body-machine interface

Authors: ***M.-H. LEE**[1], A. FARSHCHIANSADEGH[2];
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Abstracts: Do children and adults learn differently? This issue has important implications both from a theoretical motor development standpoint - e.g., whether particular brain regions are critical for motor learning - and from a practical/translational perspective - e.g.. whether rehabilitation protocols for children with disabilities should be similar to those used for adults. In typical motor tasks, comparisons between children and adults are often difficult since any changes in learning strategies are also confounded by changes in task familiarity and anthropometric changes such as muscle strength or body size. In order to minimize these confounds, we investigated how children and adults acquire a motor skill in a novel virtual task using a body-machine interface (BoMI). Both children and adults who were completely naïve to the task learned to use their shoulder movements to control a computer cursor in a center-out reaching task to 8 different targets. Participants wore a customized vest that had 4 inertial measurement units (IMU) attached to the participants' upper body. These 8 signals (roll and pitch from the 4 IMUs) were mapped on to the 2-D position of a cursor. The map was established using a calibration procedure to ensure that both children and adults could accomplish the task using their existing movement abilities. Both children and adults practiced for a total of 160 trials toward 4 targets. Three generalization tests during learning (pre, during and post-practice), where participants reached toward all 8 targets, were used to examine learning and generalization. Preliminary results show that initially in practice, children had longer movement times (~50%) compared to adults. This difference in movement time was also

associated with a change in the movement strategy. The analysis of the task and null space variance showed that children tended to use greater exploration (i.e. greater task space and null space variance) when compared to adults initially in learning. However, by the end of practice, the performance and exploration for the two groups were similar. These results suggest that children tend to use greater exploration initially in learning. Future studies will investigate how this difference in learning strategy may be exploited to facilitate motor learning in children.

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Poster

636. Rehabilitation

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Title: Remapping upper-body movements of individuals with spinal cord injuries to control a power wheelchair

Authors: *E. THORP[1,3], F. ABDOLLAHI[1], D. CHEN[2], A. FARSHCHIANSADEGH[1,3], M.-H. LEE[5], J. PEDERSEN[2], C. PIERELLA[1,6,4], E. J. ROTH[2,4], I. SEÁÑEZ-GONZÁLEZ[1,3], F. A. MUSSA-IVALDI[1,4,3];
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Abstracts: Traditional power wheelchair control interfaces are often inadequate for individuals with high-level spinal cord injuries who may have impaired upper-limb function. Additionally, alternative wheelchair controllers such as a head array, sip-and-puff or most EEG controllers offer only a limited vocabulary of commands and do not take advantage of what residual movement may remain. Here, we present a body-machine interface (BoMI) that uses shoulder movements to drive a power wheelchair. Specifically, shoulder movements are used to continuously control the speed and direction of a power wheelchair to achieve proportional

control. Our approach involves dimensionality reduction, where we map high dimensional shoulder kinematics as measured by inertial measurement units to a low dimensional control signal. To test the efficacy of such a control interface, after training in a simulated virtual environment, subjects performed a set of maneuvers driving a real power wheelchair using first a joystick and then our BMI. A preliminary study of skilled power wheelchair users whom had suffered injuries to the cervical level of the spinal cord, revealed that after only a few driving sessions, mastery of the interface was achieved. Performance was quantified by the path length and time needed to complete each maneuver as well as the smoothness of the maneuver. During the first session driving the wheelchair, even though performance was lower for the BoMI compared to a traditional joystick, all participants were able to complete all maneuvers using the BoMI. After only seven training sessions, all performance measures increased across participants. In the final session, performance using the BoMI was comparable to performance using the joystick. These results suggest that training in a virtual environment can translate to using the interface to control a real power wheelchair. The results also suggest that despite sufficient virtual training, there is a learning process that occurs when controlling the real wheelchair for the first time, but that high performance can be achieved over relatively few training sessions. Overall this work presents a proof of concept for a BoMI controlled power wheelchair that affords maximum flexibility while promoting continued engagement of residual motor function.

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Poster

636. Rehabilitation

Location: Halls A-C

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Program#/Poster: 636.05/MM12

Topic: D.17. Voluntary Movements

Support: NIH Grant HD069806

Title: Reorganization of finger movements to improve hand dexterity in stroke

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Abstracts: The loss of hand dexterity after stroke results significantly impacts the ability to perform activities of daily living. Hence there is a need to discover new techniques to improve hand dexterity. Here, we examine the use of a body-machine interface to induce reorganization and increase the repertoire of finger movements in stroke. The goal of the participants was to learn to control a computer cursor using finger movements. 8 individuals with chronic stroke (average 6 years post-stroke) wore a Cyberglove and the signals corresponding to flexion/extension of the MCP joint of the four digits were mapped linearly on to the position of a cursor. The task was to move the cursor back and forth between two targets positioned at the left and right of the screen. In order to encourage participants to reorganize their movements, we gradually changed the weightings in the map between the finger movements and the movement of the cursor. For example, the weights of the index, middle, ring, and little fingers (I,M,R,L) were initially set to (1,1,1,1) - which requires a power-grasp pattern. This weights would be gradually changed during the course of the trial to (1,1,-1-1) - which requires individuation between the IM and RL fingers. Each trial consisted of between 50 and 100 targets, and participants practiced the task for 4 sessions spread over two weeks. We measured the repertoire of finger movements generated in these trials using a principal component (PCA) analysis. Results showed that stroke survivors were able to reorganize their finger coordination patterns to adapt to the changing weights and reach the targets. Moreover, in 6 out of 8 subjects, the variance accounted for by the first PC (which indicated the dominant coordination pattern) decreased with practice (from 82 to 77%), indicating that participants were able to expand their repertoire of finger movements. These results suggest that the use of a body-machine interface facilitated the reorganization of finger coordination patterns in stroke survivors. There was exploration of novel coordination patterns, which may provide a way of breaking maladaptive synergies. This technique may provide a potentially powerful paradigm in rehabilitation to alter existing coordination patterns.

Disclosures: R. Ranganathan: None.

Poster

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Title: Kalman-based control of a virtual wheelchair using shoulder movements

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Abstracts: We describe a novel body-machine interface for the continuous control of a 2D cursor and a virtual wheelchair using four inertial measurement units (IMUs) placed on the user's upper-body. A calibration paradigm where human subjects follow a cursor with their body as if they were controlling it with their shoulders generated a map between shoulder motions and cursor kinematics. This map was used in a Kalman filter to estimate the desired cursor coordinates from upper-body motions. The cursor's position was then mapped into the coordinates of the virtual joystick controlling the virtual wheelchair. Subjects performed a center-out reaching task with blind and generalization trials. After practice, subjects were instructed to navigate through a virtual map and perform a variety of maneuvers commonly used to asses wheelchair control. All subjects improved their performance with practice on the center-out reaching task and were able to successfully complete the virtual navigation task. Our work demonstrates the potential of non-invasive IMU-based body-machine interface systems as an alternative or complement to brain-machine interfaces for accomplishing wheelchair control in 2D space. The engagement of users of assistive devices in control actions performed by the available mobility has the potential to connect the performance of functional tasks with the practice of rehabilitation-oriented physical exercises. The present study may serve as a platform for people with high-tetraplegia to control assistive devices such as powered wheelchairs using a joystick.

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Poster

636. Rehabilitation

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Program#/Poster: 636.07/MM14

Topic: D.17. Voluntary Movements

Support: NIH Grant R01NS053606

Title: Time required to predict movement distributions

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Abstracts: While human motor ability is often described in terms of the average performance in goal directed actions, such an approach ignores the natural variation of movement in daily life. Allowing human subjects to perform self-directed motor exploration in an experimental setting could reveal information about their unique movement tendencies. Before such an experimental tool can provide meaningful insights, methods are needed to determine the best practices for interpreting variable movement. One important feature is how much information (data) is required to construct a probability distribution that reliably predicts an individual's movement patterns. In this study, we analyzed the movement of stroke survivors (n=10 from a previous study) and healthy individuals (n=5) derived from self-directed motor exploration to investigate whether simple kinematic variables differed in terms of the amount of data required. We found that the amount of data required for such characterizations decreased with order (i.e., 13.7 ± 1.7 minutes for position (mean ± SE), 9.6 ± 1.9 minutes for velocity, and 6.4 ± 2.4 minutes for acceleration for healthy individuals). Data requirements for stroke survivors followed a similar trend, but required less time (i.e., 44% less for position, 35% less for velocity and 61% less for acceleration, but only significant for position information; $p < 0.05$). These results from self-directed movement identify the characterization time as an important feature for tracking movement differences using statistical distributions. Such tools can complement more standard clinical and engineering assessments, help track recovery, and can guide the design of novel neurorehabilitation training.

Disclosures: Z. Wright: None. **M. Fisher:** None. **F. Huang:** None. **J. Patton:** None.

Poster

636. Rehabilitation

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Program#/Poster: 636.08/MM15

Topic: D.17. Voluntary Movements

Support: Undergraduate Research Opportunity Program at the University of Michigan

Title: Inter-limb transfer effects following leg motor skill learning

Authors: *C. KRISHNAN[1], R. RANGANATHAN[4], M. TETARBE[2], L. L. LETHERWOOD[3]; [1]Physical Med. & Rehabil., [2]Physical Med. & Rehabilitation; Biomed. Engin., [3]Physcial Med. & Rehabilitation; Mechanical Engin., Univ. of Michigan, Ann Arbor, MI; [4]Kinesiology, Michigan State Univ., East Lansing, MI

Abstracts: Several lines of research indicate that motor learning is a key component of recovery after neurological injuries such as, stroke or spinal cord injury. There is also evidence to suggest that learning in one limb transfers to the other limb, which is termed as inter-limb transfer. We have recently developed a novel functional motor learning paradigm for gait rehabilitation; however, less is known on the retention and inter-limb transfer effect of our paradigm. Therefore, the purpose of this is study is to evaluate the consolidation and inter-limb transfer effects of a leg motor skill learning task in a group of neurologically intact adults. A secondary purpose was to evaluate whether the inter-limb transfer effects are side-specific. Twenty-two young adults (11 dominant group, 11 non-dominant group) were tested on two consecutive days. Participants performed a foot target-tracking task that necessitated modifications in their hip and knee flexion while walking on a treadmill. On Day 1, the dominant group performed testing with their dominant leg (i.e., preferred leg for kicking). On Day 2, they were tested for their dominant leg retention and non-dominant leg transfer effects using the same paradigm. The non-dominant group performed the same sequence beginning with their non-dominant leg instead. The changes in tracking error were computed to study the learning effects. The results indicate that repeated practice of the leg motor learning task resulted in significant reduction in target-tracking error in both the groups ($P < 0.05$). For the right group, the error in target-tracking during the first block of Day 2 was similar to those observed on block 10 of Day 1, indicating that subjects retained their performance improvements. In contrast, the error in target-tracking during the first block of Day 2 was slightly lower to those observed on block 10 of Day 1 in the left group ($P < 0.05$), indicating that there was some offline learning. Similarly, inter-limb transfer effects were better for the left group in comparison to the right group, although both groups showed transfer effects ($P < 0.05$). These results indicate that inter-limb transfer effects are present for our leg motor skill learning task and appear to be side-specific. The inter-limb transfer effects of learning observed in our target-tracking paradigm provide an opportunity to facilitate recovery of the impaired limb via training of the less impaired limb. The results have meaningful implications for gait rehabilitation in individuals with stroke or other neurological disorders.

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Poster

636. Rehabilitation

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Program#/Poster: 636.09/MM16

Topic: D.17. Voluntary Movements

Support: R01NS053606

Title: Parameterization of error in time versus space for goal-directed movements

Authors: ***M. FISHER**[1,2], F. HUANG[1,3], Z. WRIGHT[1,2], J. PATTON[1,2];
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Abstracts: Manipulation of error feedback has been of great interest to recent studies in motor control and rehabilitation. Typically, researchers examine motor adaptation in terms of scalar metrics, for example, the initial direction error or maximum deviation for each trial. However, yet such an approach overlooks details about how error evolves through the movement. We believe that statistical distributions of movement error through the extent of the trajectory can reveal unique patterns of adaption and possibly reveal clues to how the motor system processes information about error. Using multiple data sets where subjects experience intermittent distortions to sensorimotor conditions, this study describes different possible ordinate domains, focusing on representations in time and state-space, used to quantify reaching errors. We hypothesized that error described in various ordinate domains would differ in their predictive power. We calculated the coefficient of determination (R2) between the probabilistic error model and the experimental data. We found that the R2 values for error during intermittent exposure differed between ordinate domains (ANOVA with repeated measures, p=0.0042), with larger values for time (mean: 0.3390, CI: 0.254, 0.425) compared to path length (mean: 0.0965, CI: 0.0675, 0.1255) and distance along target path (mean: 0.2435, CI: 0.1884, 0.2985). These results show that errors represented in a time domain exhibit the least variance and allow for the highest predictive model of reaching errors. The development of such predictive models will give rise to specialized methods of robotic feedback for training of motor skills, improving upon previous techniques of error augmentation.

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Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: a MHLW grant (BMI)

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KAKENHI (#23300151)

KAKENHI (#24800087)

Title: A BMI-based robotic exoskeleton for neurorehabilitation and daily actions: Elbow and wrist movements controlled by EEG and EMG signals

Authors: ***T. KAWASE**, Y. SATO, K. KANSAKU;
Sys Neurosci Sect, Dept of Rehab for Brain Funct, Res. Inst. of Natl. Rehabil. Ctr., Saitama, Japan

Abstracts: The brain-machine interface (BMI) or brain-computer interface (BCI) is an interface technology that utilizes neurophysiological signals from the brain to control external machines or computers, and we have developed a BMI-based occupational therapy-assist suit (BOTAS) for paralyzed upper extremities. Sensorimotor rhythm, P300 (Komatsu et al., 2010), steady-state visual evoked potential (SSVEP) (Sakurada et al., 2013) and electromyography (EMG) signals (Kawase et al., 2012, 2013) were used to drive the in-house assist suit for reaching and grasping movements. We developed a wearable BMI-based exoskeleton for neurorehabilitation and daily actions (BRENDA) for reaching and grasping movements with an elbow and fingers (Kawase et al., 2014). In this study, we developed a BRENDA (type II) for reaching movements with an elbow and a wrist. The BRENDA had a two motors to assist reaching movements with flexion/extension of an elbow and a wrist. The motors were controlled based on signals extracted from EEG and EMG. A LED panel for eliciting SSVEP with 38Hz green/blue flickering stimuli was attached to a forearm portion of the BRENDA. Electrodes for EMG measurement were attached to an arm sleeve beneath the BRENDA. An amplifier of the biological signals, batteries and a PC for controlling the whole system were stored in pouches, and the user wore the pouches

at the waist. In an experiment, the BRENDA worn by an able-bodied subject (a 29-year-old male) realized reaching movements with flexion of the elbow, which were triggered by EMG signals, and with flexion of the wrist, which were triggered by SSVEP elicited by 38Hz green/blue flickering stimuli. The new exoskeleton may be useful for practical rehabilitation and support of daily actions based on the users' intention.

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Poster

636. Rehabilitation

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Program#/Poster: 636.11/MM18

Topic: D.18. Brain-Machine Interface

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National Institute of Biomedical Imaging and Bioengineering Grant 5T32EB003383-08

Title: Development of virtual integration environment sensing capabilities for the modular prosthetic limb

Authors: ***B. A. WESTER**[1], K. FISCHER[1], T. GION[1], G. HOTSON[2], J. DOWNEY[5], M. FIFER[3], F. TENORE[1], J. BEATY[1], A. RAVITZ[1], M. MCLOUGHLIN[1], R. GAUNT[5], J. COLLINGER[5], N. CRONE[4], S. SWETZ[1];
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Abstracts: The Virtual Integration Environment (VIE), a software emulation of the Modular Prosthetic Limb (MPL) with shared communication interfaces, was designed as an integration and training tool for clinical research in the fields of upper extremity prosthetics and rehabilitation. The VIE provides both a 3D graphical visualization and physical simulation of the MPL including the 26 articulating joints and 17 independently controllable virtual motors operating and interacting within a virtual world. The VIE simulates physical object interactions with the virtual MPL (vMPL), including contact, grasping and fingertip force, and allows

grasping, transporting, and repositioning of objects. Limb data and virtual sensors percepts are generated through control of the vMPL, from object interactions, and event handling within the environment. Via defined communication interfaces, these data and percepts are available to the clinical operator. In addition, the data and percepts mimic those reported by the physical MPL. The VIE has been developed as a framework allowing creation of multiple virtual scenarios that facilitate clinical training and real-time operation of the vMPL, as well as offline data analysis. Because of its versatility and portability, the VIE is well suited to support development of closed-loop experimental systems for both motor control and sensory feedback, and for eventual clinical use of a physical MPL system. Here we describe the architecture and capabilities of the VIE for closed-loop control, as well as its implementation in clinical work within the scope of the DARPA's Revolutionizing Prosthetics and other programs.

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Poster

636. Rehabilitation

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Program#/Poster: 636.12/MM19

Topic: D.17. Voluntary Movements

Title: Neural mechanisms of mirror feedback: An EEG study based on virtual reality

Authors: *G. GARIPELLI, V. LIAKONI, D. PEREZ-MARCOS, T. TADI;
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Abstracts: Recent research in neurorehabilitation has found mirror therapy to be more effective than conventional treatment for improving motor function. However, the neural mechanisms underlying mirror therapy remain unclear. On the other side, virtual reality (VR) based cognitive therapy has been shown to be helpful in inducing cortical plasticity and promoting functional recovery of arm/hand movements. We have combined both approaches into a novel VR platform (MindPlayPRO, MindMaze SA, Switzerland) for effective neurorehabilitation. In the current study, we explored the neural markers associated with the execution and observation of goal-directed movements under mirrored visual feedback in a virtual environment. Understanding these neural mechanisms will guide customization of neurorehabilitation procedures using VR systems. We recorded EEG data (64 electrodes) of nine healthy participants (22-35 years) while

they performed a reaching task in VR under three visual feedback modes: a) direct mapping, with the right arm mapped onto the virtual right arm; b) mirror mapping, with the right arm mapped onto the virtual left arm; and c) passive video control, with action observation of pre-recorded movements of the virtual left arm. Mirror mapping led to higher negative slow cortical potentials (SCPs) (0.1-1.5 Hz) compared to direct mapping in central electrodes (maximum at Cz; paired t-test, $p<0.001$, Bonferroni corrected). Interestingly, the hemispheric laterality (difference between C3 and C4) was significantly lower in the mirror mapping ($p<0.001$). Additionally, we conducted single-trial analysis for elucidating the neural signatures of mirrored feedback using linear discriminant analysis (LDA) between direct and mirror mapping. The performance of LDA revealed a source of discriminability on the ipsilateral electrodes (maximal at C2 and C4 electrode), with ROC area under curve of 67+/-4%. The mere action observation in passive video control led to significantly weaker activity compared to direct and mirror mapping in the central electrodes (C3, Cz and C4, $p<0.001$). The analysis of SCPs suggests that the proposed mirror visual feedback in a virtual environment can increase the cortical excitability of the hemisphere ipsilateral to the movement. This finding is in line with observations of previous neurophysiological studies on mirror therapy and may have important implications in the design of effective rehabilitation procedures using virtual reality. Further studies with hemiparetic patients may reveal how the current evidence is translated into clinical settings.

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Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: DARPA Grant N66001-10-C-4056

Title: Psychophysical, neural, and learning curve metrics toward optimizing training with a brain-computer interface

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Abstracts: Training is an oft mentioned yet infrequently analyzed component of a brain-computer interface (BCI) paradigm. Numerous studies using multiple recording modalities have

documented evidence of neural adaptation that occurs during BCI learning with the help of biofeedback. However, a standardized training regimen to optimize the rate of this adaptation through task difficulty has not been well developed to date. This concept of an "optimal difficulty" is not trivial. Making a task too difficult may cause the subject to lose interest and stop trying, effectively halting further learning or even regression. Conversely, making a task too easy may provide insufficient feedback (i.e. errors) for further learning and refinement of motor control. Thus, finding a difficulty "sweet-spot" that balances motivation with error feedback would be paramount to optimizing the learning curve for a novice BCI user. With this in mind, the objective of this project was to more closely examine markers of BCI skill acquisition and propose a framework for accelerating this process. In this study, we examined the time course of learning in a non-human primate utilizing single-unit recordings for control of a robotic arm in a 3D BCI task. We first established a baseline difficulty metric using software simulations as well as robot simulations with randomized neural signal playback. By systematically adjusting the difficulty of the task and parameterizing the subject's learning curve based on his history of trial successes and failures, we then estimated the subject's level of skill acquisition both on a trial-by-trial basis as well as across sessions and adjusted task difficulty accordingly. In addition to using performance metrics derived from binary successes and failures to evaluate skill level, we also examined continuous-valued psychophysical metrics of the BCI reach path in order to define skill level in terms of traits corresponding to natural reaching movements. Finally, we correlated neural activity metrics (R^2, depth of modulation, network dynamics, etc.) against learning curve trends in order to develop complementary markers of BCI skill acquisition. Using this training approach, our test subject showed consistent improvement in the 3D task over a period of weeks, as well as examples of within session learning and skill acquisition even as motivation appeared to decline. In addition, our method of characterizing difficulty may be generalized to various methods of altering task difficulty or assistance. Overall, the results presented here may have implications not only for improving BCI user training, but also for optimizing physical rehabilitation regimens.

Disclosures: **J.J. Williams:** None. **R.N. Tien:** None. **A.B. Schwartz:** None.

Poster

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Topic: D.18. Brain-Machine Interface

Support: FINEP Grant 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: The Walk Again Project: Analysis of brain activity of spinal cord injury patients during training with a BMI

Authors: *R. C. MOIOLI[1], F. L. BRASIL[1], S. SHOKUR[1], A. L. LIN[1], K. FAST[1], N. PERETTI[1], A. TAKIGAMI[1], D. SCHWARZ[2,3], E. MORYA[1], M. A. L. NICOLELIS[1,4,2,5,3]; [1]Edmond and Lily Safra Intl. Inst. of Neurosci. of Natal (ELS-IINN, Natal, Brazil; [2]Biomed. Engin., [3]Ctr. for Neuroengineering, [4]Neurobio., [5]Psychology and Neurosci., Duke Univ., Durham, NC

Abstracts: Brain-machine interfaces (BMI) rely on electrophysiological signals to operate. These can be recorded using invasive and non-invasive methods: the first provides deeper insights into neuronal information processing whilst the latter overcomes current operational and technical obstacles, especially in studies involving human subjects. As such, spinal cord injury (SCI) patients can greatly benefit from non-invasive BMIs to alleviate locomotion impairment. In this work, we characterize the neuronal activity represented by EEG recordings from 8 paraplegic patients involved in a novel, hybrid EEG/EMG based control training paradigm, towards learning how to control an exoskeleton. More specifically, at each of the 4 phases of training - motor imagery, virtual actuator, commercially available gait orthosis (Lokomat, Hocoma) control, and 15 dof lower limb lower limb exoskeleton control - we recorded and analysed the data from 32 active EEG electrodes (BrainAmp, BrainProducts) carefully distributed above specific motor areas of the patients' scalp surface. The analyses include the motor imagery performance as well as the spatial and temporal patterns of neuronal activity. Independent component analysis (ICA) was employed to identify independent cortical sources and highlight any evidence of learning and neuronal plasticity over the training process. Through this data analysis paradigm we intend to determine the impact of diverse training approaches to brain dynamics, but also to devise novel algorithms that can enhance the clinical relevance of BMIs. Acknowledgments: The authors thank Alberto Santos Dumont Association for Research Support (AASDAP) and the 156 people involved in this project.

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Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: FINEP Grant 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: The walk again project: Brain-controlled exoskeleton locomotion

Authors: *A. LIN[1], D. SCHWARZ[2,3], R. SELLAOUTI[6], S. SHOKUR[1], R. C. MOIOLI[1], F. L. BRASIL[1], K. R. FAST[1], N. A. PERETTI[1], A. TAKIGAMI[7,8], S. GALLO[9], K. LYONS[10], P. MITTENDORFER[12], M. LEBEDEV[2,3], S. JOSHI[11], G. CHENG[12,13], E. MORYA[1], A. RUDOLPH[14], M. NICOLELIS[1,2,3,4,5];
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Abstracts: As part of the Walk Again Project, a state-of-the-art robotic exoskeleton was designed to enable patients with spinal cord injury (SCI) to perform lower-limb locomotion via brain activity. Hydraulic generators were used to control 15 degrees of freedom (DOF) of the exoskeleton. Real-time trajectory corrections were calculated from data gathered by gyroscopes, strain gauges, force-torque, and pressure sensors positioned along the exoskeleton's limbs, giving it the ability to pivot laterally to stabilize its center of mass. At the interface of the subject's body and exoskeleton, multi-modal sensors were placed to monitor interaction with the exoskeleton. To enable brain-controlled locomotion, movements were discretized into higher level control

states. A small computing unit was utilized to 1) receive biosignals from subjects, 2) interpret them as state transitions for the exoskeleton, 3) provide visual and tactile feedback, and 4) monitor user interaction with the exoskeleton. The control system and exoskeleton functioned without the need of an external operator. Sixteen EEG and two EMG channels served as the inputs for discrete state control. EEG during motor imagery tasks was classified by linear discriminant analysis (LDA) using features extracted by common spatial pattern (CSP). The resulting classifier was visualized by the subject via a custom display. Muscle contractions detected by EMG were used to confirm the classifier and initialize state transitions. The transition was sent to the exoskeleton controller, which performed the indicated mechanical trajectory with aforementioned online corrections. Participants received tactile feedback along their inner forearms synchronized with the exoskeleton walk phase. The subject's interaction with the exoskeleton was compared for both static and dynamic walking. A total of eight SCI patients were trained using this control strategy. Results indicate that BMI-based control of an exoskeleton can become a feasible rehabilitation tool for severely paralyzed patients.
Acknowledgments: The authors thank Alberto Santos Dumont Association for Research Support (AASDAP) and the 156 people involved in this project.

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Poster

636. Rehabilitation

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 636.16/MM23

Topic: D.18. Brain-Machine Interface

Support: FINEP 01.12.0514.00

AASDAP

AACD

Itau Bank

NCCR Robotics

Title: The walk again project (wap): Sensory feedback for brain controlled exoskeleton

Authors: *S. SHOKUR[1], S. GALLO[2], J. OLIVIER[2], N. PERETTI[1], A. TAKIGAMI[3], A. L. LIN[1], K. FAST[1], R. MOIOLI[1], F. BRASIL[1], E. MORYA[1], G. CHENG[4,5], H. BLEULER[2], M. A. L. NICOLELIS[1,6,7,8,9];
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Abstracts: As part of the WAP project, we developed a state-of-the-art, 15 degree-of-freedom (DOF) autonomous exoskeleton (EXO) with hydraulic actuators for restoration of locomotion for patients with complete spinal cord injuries (SCI), who neither have the ability to control their lower limbs, nor to experience any tactile or proprioceptive sensation from the level of the lesion down. To address these issues, we proposed an innovative solution for a bidirectional communication between the patients and the exoskeleton. While the EXO is controlled using hybrid biosignals (EEG + EMG), an array of multimodal sensors covering the legs of the EXO records relevant locomotion information that is then transmitted to the patients through a vibrotactile interface placed on their upper body and arms (thus closing the loop patient-exo). After an extensive 3 month testing period with this system, starting in a virtual reality environment, subjetcs (8 SCI patients) could experience tactile/proprioceptive sensations related both to foot contact on the floor and ankle or knee position. Additional experiments investigated body representation plasticity after long term use of the EXO's tactile feedback system. Our results showed the importance of providing this type of artificial tactile/proprioceptive feedback for the subject's incorporation of a virtual (avatar) or prosthetic lower limbs (exoskeleton). As such, we propose that in order to become clinically relevant, exoskeletons will have to provide rich sensory feedback to their operators. Acknowledgments: The authors thank Alberto Santos Dumont Association for Research Support (AASDAP)and the 156 people involved in this project.

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Poster

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Program#/Poster: 636.17/MM24

Topic: D.18. Brain-Machine Interface

Support: FINEP 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: The Walk Again Project: An EEG/EMG training paradigm to control locomotion

Authors: *F. L. BRASIL[1], R. C. MOIOLI[1], S. SHOKUR[1], K. FAST[1], A. L. LIN[1], N. A. PERETTI[1], A. TAKIGAMI[2,3], K. LYONS[4], D. J. ZIELINSKI[5], L. SAWAKI[9], S. JOSHI[4], E. MORYA[1], M. A. L. NICOLELIS[2,6,7,8,10];
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Abstracts: A brain-machine interface (BMI) can facilitate direct communication from the brain to actuators in spinal cord injury (SCI) patients. Here, we propose to employ this novel approach to restore lower limb mobility by using cortical neuronal signals to control an external neuroprosthetic device known as an exoskeleton. The main challenge we faced was devising a system that was capable of reliably recording and processing electrophysiological data while remaining portable and resistant to noise and external perturbations. To address these issues, we developed a hybrid BMI (EEG + EMG) training protocol designed to operate an exoskeleton using a shared control approach. For our study, eight paraplegic patients learned a structured protocol that included: 1) executing a motor imagery task using EEG, 2) learning to use EEG to move a virtual reality based actuator, 3) using the EEG+EMG signals to control the movements of a commercially available gait orthosis (Lokomat, Hocoma), and finally 4) controlling the movements of a lower limb exoskeleton. During this training, patients were subjected to visual and auditory distractors to simulate a crowded and noisy open-air environment. A head-mounted, portable peripheral display, attached to a custom designed helmet, allowed the patients to reliably control this non-invasive BMI in a real life situation. Flowing LEDs in the helmet display provided cues and feedback to patients without need of any other external devices. All

eight patients learned to use their EEG and EMG signals to operate this BMI at comparable levels of performance obtained in a silent and controlled environment. This further supports our claim that BMIs can become clinically relevant tools for sensorimotor rehabilitation.
Acknowledgments: The authors thank Alberto Santos Dumont Association for Research Support (AASDAP) and the 156 people involved in this project.

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Poster

636. Rehabilitation

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Program#/Poster: 636.18/MM25

Topic: D.18. Brain-Machine Interface

Title: The Walk Again Project: Using a Brain-Machine Interface for establishing a bi-directional Interaction between paraplegic subjects and a lower limb exoskeleton

Authors: *M. A. L. NICOLELIS[1], S. SHOKUR[2], A. LIN[2], R. C. MOIOLI[2], F. L. BRASIL[2], N. PERETTI[2], K. FAST[2], A. TAKIGAMI[3], E. MORYA[2], G. CHENG[4], L. SAWAKI[5], R. KOPPER[6], D. SCHWARZ[1], S. GALLO[7], M. LEBEDEV[1], S. JOSHI[8], H. BLEULER[7], A. RUDOLPH[9];
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Abstracts: Worldwide, millions of people suffer from walking impairments. Brain-machine interfaces (BMI) offer a promising alternative for restoring motor behavior in patients affected by lower limb paralysis. Here, we present a new paradigm for patients affected by a complete lesion of the lower spinal cord to learn how to operate, in a safe and reliable manner, a non-invasive BMI for controlling a 15 degree-of-freedom exoskeleton, capable of generating autonomous walking. During a 6-month training period, eight patients learned to use EEG signals as part of a BMI designed to restore bipedal locomotion. This training paradigm was divided into 4 sequential steps: 1) brain-controlled virtual avatar presented on a common 2D monitor; 2) brain-controlled virtual avatar shown from a first person perspective using a head

mounted 3D display; 3) brain-control of a commercially available gait orthosis (Lokomat, Hocoma); and 4) brain-control of a custom designed 15 degree-of-freedom lower limb exoskeleton. All 8 patients were able to complete the training procedure. Specifically, they all learned how to employ EEG features to generate leg movements in all four stages. In addition, all eight patients utilized haptic feedback provided in each condition, including the exoskeleton walking, to establish a bidirectional interaction with different versions of artificial actuators (virtual and real). These preliminary results suggest that a bidirectional interaction between paraplegic patients and an exoskeleton, mediated by a brain-machine interface may serve as both a new rehabilitation tool and a new prosthetic alternative for severely paralyzed patients. Supported by: ELS-IINN, Alberto Santos Dumont Association for Research Support (AASDAP) / Association for Assistance to Disabled Children (AACD) laboratory in Sao Paulo, Brazil, which validated the proposed method. Acknowledgments: The authors thank Alberto Santos Dumont Association for Research Support (AASDAP) and the 156 people involved in this project.

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Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: NIH NCATS Grant 1ULTR001067

DARPA MTO SPAWAR Pacific Grant/Contract No. N66001-12-C-4042

Title: Restoration of sensory and motor hand function via two Utah Slanted Electrode Arrays (USEAs) in residual arm nerves after prior hand amputation

Authors: *D. M. PAGE[1], S. WENDELKEN[1], H. A. C. WARK[1], T. DAVIS[1], R. A. NORMANN[1], D. J. WARREN[1], B. GREGER[2], D. T. HUTCHINSON[1], G. A. CLARK[1]; [1]Univ. of Utah, Salt Lake City, UT; [2]Arizona State Univ., Tempe, AZ

Abstracts: Peripheral nerve interfaces can selectively stimulate and record from individual nerve fibers, potentially helping to restore sensation and movement after hand amputation. We implanted two 100-electrode Utah Slanted Electrode Arrays (USEAs) in the residual ulnar and median arm nerves of one subject (one per nerve) 21 years after hand amputation. During the 4-week implant period, experiments included stimulation, recording, and simultaneous stimulation and recording via separate USEAs. 1) Stimulation via individual electrodes evoked 106 location-distinct percepts on a single day, with subjective qualities including "tingle," "vibration," "pressure," "sting," and "movement." In blind trials, the subject discriminated between 5 unique percept locations, evoked by individual and combined stimulation of 4 electrodes (35 of 35 trials, $p < 0.0001$). The subject also discriminated between two qualities (tingle and vibration) with the same location (tip of ring finger), evoked by two different electrodes (30 of 30 trials, $p < 0.0001$). Most percepts evoked by individual electrodes changed quality or location weekly. Of the 43 ulnar and 17 median nerve electrodes that evoked percepts over the implant duration, an across-week mean of 91% and 78% changed location or quality each week, respectively. For the 43 ulnar electrodes, 72%, 63%, and 67% changed percept quality on weeks 2, 3, and 4, respectively; and 70%, 79%, and 58% changed percept location (12 regions: front/back of each digit and the hand). For the 17 median electrodes, 71%, 59%, and 47% changed percept quality, whereas 53%, 53%, and 53% (*sic*) changed percept location. Longer-duration studies may reveal improved stability over time. 2) Recording via USEAs revealed electrodes with neural firing rates that correlated with fictive movements of the phantom hand, allowing 2-DOF real-time, neurally-driven decodes of little- or middle-finger extension. In most sessions, electromyographic (EMG) signals dominated recordings, limiting on-line decodes to 1 DOF. 3) Simultaneous stimulation and recording via separate USEAs allowed the subject to modulate 1-DOF (EMG-driven) virtual-hand movement via sensorimotor feedback. The subject was instructed to flex all fingers (thus moving the virtual hand), and to indicate at which of two randomly presented finger positions (close or far) contact was perceived, as evoked by stimulation via a single electrode. Without any visual feedback of the virtual hand, the subject identified the correct contact position for 41 of 47 trials ($p < 0.0001$). These results indicate that USEAs can help to restore sensation, movement, and closed-loop sensory feedback after hand loss.

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Poster

636. Rehabilitation

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Program#/Poster: 636.20/MM27

Topic: D.18. Brain-Machine Interface

Support: DARPA Contract N66001-10-C-4056

Title: Sensing capabilities of the modular prosthetic limb

Authors: M. JOHANNES[1], K. KATYAL[1], R. ARMIGER[1], J. HELDER[1], M. PARA[1], J. BEATY[1], A. RAVITZ[1], M. MCLOUGHLIN[1], O. LASOWSKY[2], E. SCHLUTER[2], S. BENSMAIA[2], *F. TENORE[1];
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Abstracts: The Modular Prosthetic Limb (MPL) is an upper extremity neuroprosthesis designed to closely match the characteristics of the human arm it seeks to replace. It consists of 26 articulating joints driven by 17 independently controllable motors, thus allowing for anthropomorphic limb motion from the shoulder down to individual fingers. In addition, the MPL is equipped with over one hundred sensors of different types, which can provide a user with tactile and proprioceptive feedback. With these capabilities, the MPL is an ideal prosthesis with which to implement closed loop control, where a user's intent is decoded into appropriate arm and hand motions and interactions of the prosthesis with its environment are relayed back to the user in real time. Here, we review two different approaches to sensorizing the MPL's fingertips and describe sensor data from both systems that relate to contact location, contact pressure, and motion. These results will demonstrate that the depth and breadth of sensory data that can be acquired from the prosthetic fingertip meets or exceeds the sensory encoding capabilities of the neural interface in upcoming closed-loop control experiments within the scope of DARPA's Revolutionizing Prosthetics and other future programs.

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Poster

636. Rehabilitation

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Topic: D.17. Voluntary Movements

Support: The European Commission's Seventh Framework Programme (CP-IP 258654)

NCCR Robotics (51NF40-125773)

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International Paraplegic Foundation

Title: Motor cortex population dynamics in freely walking rats

Authors: *J. DIGIOVANNA[1], N. DOMINICI[2], J. RIGOSA[3], S. DUIS[2], L. FRIEDLI[2], I. VOLLENWEIDER[2], J. KREIDER[2], R. VAN DEN BRAND[2], A. PANARESE[3], S. MICERA[1,3], G. COURTINE[2];
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Abstracts: Supraspinal centers have the capacity to tune the activity of spinal circuitries throughout gait to implement volitional changes in locomotor states. We hypothesized that neuronal ensemble modulation from the motor cortex should reflect supraspinal control of leg movements during locomotion across tasks requiring perceptually-guided adjustments of gait. Rats were trained to walk on a treadmill, overground along straight and curved runways, onto a staircase, and over the irregularly spaced rungs of a ladder. After completion of training, the rats were chronically implanted with a microwire array that spanned the left hindlimb motor cortex, and with EMG electrodes into a pair of flexor and extensor muscles for each joint of the right hindlimb. Ensemble cortical modulations and motoneuron outputs were recorded simultaneously with whole-body kinematics and kinetics during all the trained tasks. We found reproducible modulation of motor cortex neurons over the gait cycle of the right hindlimb. Population dynamics could be approximated with a sinusoidal firing pattern whereby the firing rate was minimal during mid-stance, rose before the stance-swing transition, and peaked within the swing phase. These results are consistent with previously documented firing patterns in cats and rodents during locomotion. In addition, we detected task-dependent recruitment of neuron sub-populations. This recruitment, defined as neurons modulating significantly differently than at rest, increased with task complexity. Changes in population dynamics reflected task-dependent adjustments of muscle synergies and leg kinematics. These results suggest that the rodent motor cortex continuously supervises leg movements, and becomes increasingly active when the performed task requires high-level control signals to fine-tune gait patterns based on contextual information.

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Poster

636. Rehabilitation

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Topic: D.17. Voluntary Movements

Support: European Project "NEUWalk"

ERC 261247 "Walk Again"

7th Generation framework Marie Curie IFF 587504 "e-WALK"

Title: Single neuron activity and population dynamics in the leg area of the motor cortex in the freely moving rhesus macaque

Authors: ***J. LAURENS**[1], D. BORTON[1], E. BEZARD[2], G. COURTINE[1];
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Abstracts: We leveraged a recently designed multimodal neuromotor analysis platform to record population dynamics in the leg area of primary motor cortex (MI) in conjunction with contralateral hindlimb electromyography and whole-body kinematics in 4 freely moving, unconstrained, and untethered rhesus monkeys. The animals were trained to walk quadrupedally on a treadmill, across a flat runway, and over horizontal ladders of varying difficulties. Since the experiments were conducted within untethered environments, we additionally could capture spontaneous behaviors, such as bipedal walking and even backward locomotion. We exploited this rich database to conduct the first high-resolution analysis of neuronal activity in the primary motor cortex of non-human primate during locomotion. We found that the majority of neurons showed vigorous and highly reproducible modulation during locomotion. Furthermore, many neurons exhibit strikingly diverse response profiles between the locomotor tasks. Evaluation of population dynamics uncovered a strong similarity between responses during walking along a corridor or onto a treadmill regardless of whether the animals adopted a bipedal or quadrupedal gait. In contrast, locomotion along a horizontal ladder requiring precise paw placement was

associated with distinct and intrinsically more complex neuronal activity. We then built a Maximum Likelihood decoder based on neuronal population dynamics. The decoder distinguished the type of performed locomotor task with accuracy (80-95%), and could even detect the position of the leg within the gait cycle with high precision ($r2 > 0.95$). These results demonstrate that the primary motor cortex of non-human primates contains rich gait-related information encoding key kinematic parameters during the execution of natural locomotor tasks.
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Poster

636. Rehabilitation

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Program#/Poster: 636.23/MM30

Topic: D.18. Brain-Machine Interface

Title: A little elastic for a better performance: Kinesiotaping of the motor effector modulates neural mechanisms for rhythmic movements

Authors: *R. BRAVI, E. COHEN, E. QUARTA, D. MINCIACCHI;
Univ. of Florence, Florence, Italy

Abstracts: A rhythmic motor performance is brought about by an integration of timing information with movements. We have recently demonstrated that the precision of an isochronous performance, defined as performance of repeated movements having a uniform duration, was insensible to auditory stimuli of various characteristics (Bravi et al., 2014, Exp. Brain Res.). Such finding has led us to further investigate where do the determining factors of precision reside. For this purpose we used manipulation of cutaneous afferents by kinesiotaping (KT), an approach that was previously shown to improve some isokinetic performances. Subjects, tested without KT and with KT, have participated in sessions in which sets of repeated isochronous wrist's flexion-extensions (IWFEs) were performed under various auditory conditions and during their recall. Kinematics was recorded and temporal parameters were extracted and analyzed. Various degrees of improvement in the isochronous performances were evident for the KT recordings especially in terms of temporal precision. Our results indicate that, in the precision of repetitive rhythmic movements, the manipulation of cutaneous afferents plays a significant role. Whether this increase in precision is achieved by augmentation of the

efficiency in central or local neural mechanisms is to be determined, but what remains certain is that when it comes to precision, a little elastic makes the difference.

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Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: DARPA (N66001-11-C-4190)

Plastic Surgery Foundation

Frederick A. Coller Surgical Society

Title: Partial muscle regenerative peripheral nerve interfaces for prosthetic control

Authors: S. L. WOO, M. G. URBANCHEK, M. K. LEACH, J. D. MOON, P. S. CEDERNA, *N. B. LANGHALS;
Plastic Surgery, Univ. of Michigan, Ann Arbor, MI

Abstracts: Objective: Nonvascularized partial skeletal muscle grafts are notorious for their limited force-generating capacity and tendency to degenerate in the absence of reinnervation. Accompanied by peripheral nerve implantation, however, partial muscle grafts can survive and transmit detectable electromyographic (EMG) signals capable of prosthetic control. Our study investigated partial muscle graft survival in the construction of regenerative peripheral nerve interfaces (RPNIs) and further characterized their electrophysiological properties across various muscle donor sites. Methods: Twenty F344 rats were assigned to 1 of 5 groups based on muscle graft type used for RPNI construction: 1) control-whole extensor digitorum longus; 2) partial biceps femoris; 3) partial rectus femoris; 4) partial lateral gastrocnemius; and 5) partial vastus medialis. Each graft (approximately 140-mg at initial harvest) was suture-anchored to the femur, wrapped in small intestinal submucosa for tissue isolation, and implanted with the transected common peroneal nerve. After 4 months of recovery, *in situ* EMG [figure] and force testing were performed. Results: All control RPNIs (n=4) transmitted detectable EMG signals, compared to 75% of partial muscle RPNIs (n=12 of 16). Significant differences between control and partial muscle RPNIs included average mass [118±42 mg vs. 66±25 mg], EMG peak-to-peak amplitude [6.7±2.3 mV vs. 1.16±1.5 mV], and maximum tetanic force [500±615 mN vs. 137±152 mN].

Amongst partial muscle RPNIs, donor muscle was not a significant predictor of EMG amplitude after adjusting for final mass and length. Conclusions: Partial muscle graft RPNIs transmit detectable EMG signals with a 75% success rate at 4 months. This proof of concept underscores the potential to develop and refine partial muscle graft-based interfaces to harness peripheral nerve signals for high-fidelity prosthetic control. While signal size remains favorable (i.e. 10-100 times larger than signals recorded directly from peripheral nerves), further studies are warranted for optimization of partial muscle graft regeneration and signal acquisition.

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Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: NSF DGE-0718124

NSF DGE-1256082

NSF EEC-1028725

Title: Neural correlates of task dynamics in motor cortex

Authors: *A. HADDOCK[1,2,3], C. MATLACK[2,3], H. CHIZECK[2,3];
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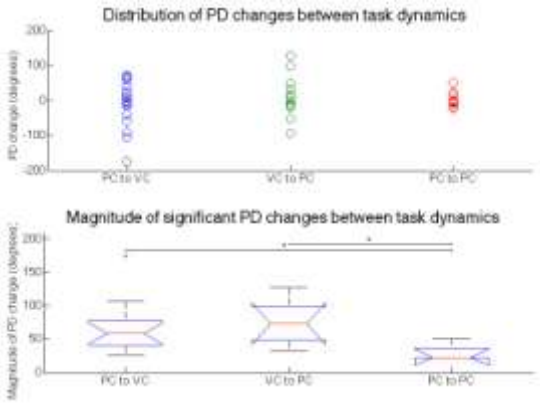
Abstracts: Learning to use a brain-machine interface (BMI) requires adaptation to a novel dynamical system mapping neural signals to output kinematics. Current BMI decoding algorithms derive velocity signals from neural populations in motor cortex to control prosthetic motion. This study seeks to clarify the cortical mechanisms accompanying the change to velocity-controlled task dynamics. We studied the tuning properties of neurons recorded in the right arm area of primary motor cortex. A macaque was trained to apply isometric wrist force to a manipulandum to control computer cursor position in a 2D pinball task. We tested two task dynamics conditions: in the first, force mapped directly to cursor position (position control, or PC); in the second, force was integrated to increment the cursor position at each time step (velocity control, or VC). Thus, there were no changes in posture or directional mapping of force

between each task, as has been studied previously. Instead, each task condition required a different temporal force profile. We analyzed the activity of 50 directionally tuned neurons ($p < 0.05$, Rayleigh test) as the macaque performed blocks of trials first in PC, then in VC, then in PC again. In this analysis we characterized each neuron by its preferred direction (PD). We found that 8 of 50 cells showed significant changes in PD ($p < 0.05$, z-test) as task dynamics shifted from PC to VC; 7 of 50 cells showed changes as dynamics shifted back from VC to PC; and, finally, 6 of 50 cells showed changes when comparing PC conditions. The top plot shows PD changes for each change in task dynamics, and the bottom plot shows that the magnitude of significant PD changes are greater when dynamics change versus the control condition ($p < 0.05$, Wilcoxon rank sum test). These results show that although most motor cortical neurons studied have fixed PD across task dynamics conditions, some neurons incur large PD changes in response to VC dynamics, suggesting a possible role in corrective movements. Further analysis will investigate the temporal characteristics of motor cortical neurons during changes in task dynamics.



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Poster

636. Rehabilitation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 636.26/MM33

Topic: D.18. Brain-Machine Interface

Support: Wallace H. Coulter Foundation

Plastic Surgery Foundation

Frederick A. Coller Surgical Society

Title: Demonstration of a regenerative peripheral nerve interface and custom recording device in a rhesus macaque

Authors: *Z. T. IRWIN[1], K. E. SCHROEDER[1], D. E. THOMPSON[1,3], S. L. WOO[2], N. B. LANGHALS[2,1], M. G. URBANCHEK[2], P. S. CEDERNA[2,1], C. A. CHESTEK[1];
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Abstracts: Peripheral nerves are a promising source for neuroprosthetic control signals, as the information carried downstream of cortex is increasingly functionally selective and thus easier to interpret. However, the clinical viability of current approaches is still hampered by low signal amplitude, interface instability, and low functional resolution within the nerve. Here, we address these issues by demonstrating the successful implantation of a Regenerative Peripheral Nerve Interface (RPNI) in a rhesus macaque, as well as the design of a neural recording system capable of low-power, wireless operation. The electronics platform can also be used with other neural signal modalities such as intracortical spiking data. The RPNI is constructed by selecting individual nerves at any level (e.g. individual fascicles) and suturing them into a small graft of unvascularized, denervated donor muscle. The graft then revascularizes, regenerates, and is reinnervated by the transplanted nerve, acting as a bioamplifier for the efferent action potentials. We transplanted three terminal branches of the median nerve, two of which acted to flex the fingers (FDS and FDP) and the third to flex the thumb (FPL), into three separate 1x3cm muscle grafts taken from the same arm. Six months after implantation, we could record a 700-1000uVp-p EMG signal from the FDS-RPNI via percutaneous fine-wire electrodes. Correct function of the RPNI was verified by correlation of EMG with finger flexion and non-correlation with finger extension and wrist flexion during a four-finger flexion/extension task. The location of the RPNI in the forearm made accidental recording of intact finger flexor muscles unlikely. Using EMG power within 100-500Hz we were able to detect flexion with 98.7% accuracy within 50ms. To complete the interface, we designed a low-power neural recording system consisting of all off-the-shelf components: an Intan bioamplifier and an Atmel microcontroller and wireless transceiver. The system is designed expressly to generate prosthetic control signals instead of higher bandwidth research-oriented data. We reduced the required amplifier power by lowering the cutoff and sampling frequencies to 1kHz and 2kSps, and the wireless data rate by only transmitting average signal power every 64ms. For 8 channels of (previously recorded) neural data, the system draws 2.3mA at 3.3V (7.6mW). For 16 channels, the system draws 9.1mW, an increase of only .19mW/channel. Together, the RPNI and low-power recording system represent a path towards clinical viability for generating long-lasting, functionally specific prosthetic control signals from peripheral nerves.

Disclosures: **Z.T. Irwin:** None. **K.E. Schroeder:** None. **D.E. Thompson:** None. **S.L. Woo:** None. **N.B. Langhals:** None. **M.G. Urbanchek:** None. **P.S. Cederna:** None. **C.A. Chestek:** None.

Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: This work was supported by NIH U44 NS067784.

Title: Wireless implantable multichannel myoelectric system validation

Authors: *D. R. MERRILL, S. HIATT, K. S. GUILLORY, C. SMITH, D. MCDONNALL; Ripple, Salt Lake City, UT

Abstracts: Modern hand and arm prostheses provide multiple degrees of freedom (DOF) of motion, yet their use is severely limited by the biological control interface. Current prostheses are controlled by EMG signals recorded from surface electrodes on the residual limb. These recordings are a composite of signals from several muscles and typically do not provide enough independent sources for simultaneous multi-DOF control. Additionally the surface recording sites are unreliable. These impediments limit transradial prostheses to two DOF with sequential control. Our objective is to provide simultaneous multi-degree of freedom prosthesis control, ultimately providing an intuitive control experience. We have developed an implantable device to detect EMG from multiple residual muscles and send the signals wirelessly to a prosthesis. This approach supports a high number of independent control signals and provides access to EMG from deep muscles that cannot be accessed with surface electrodes. The system consists of a hermetic implanted module from which six EMG leads emerge. Each lead comprises four electrode sites for 24 total recording channels. The implant receives power inductively from an external transceiver and sends digitized EMG data to the external transceiver by reflected impedance modulation. By using a single subcutaneous module for telemetry from which several leads emerge, power coupling efficiency remains high. The implant consists of established biocompatible materials including alumina and zirconia ceramics, titanium, gold, silicone, and stainless steel. We have completed initial safety and performance testing. Electrical safety and EMC were verified according to IEC 60601-1 and -1-2. Biocompatibility was confirmed per ISO 10993-1. Benchtop implant performance demonstrated the amplifier to have an input-referred noise of < 2 μVRMS, common mode rejection ratio greater than 55 dB, and neighboring channel isolation averaging 66 dB. The system was validated in a six-dog study. Devices were implanted bilaterally with electrodes implanted in deltoideous and lateral head of triceps. One week after

implantation EMG was recorded as the dogs walked freely. EMG displayed very low noise and clearly indicated swing/stance phases of gait. These efforts demonstrate the ability to amplify and transmit muscle signals and confirm safety and performance requirements. This approach has the potential to provide simultaneous multi-degree of freedom prosthesis control, especially if used with advanced hand and arm prostheses, targeted muscle reinnervation patients, and recently developed pattern recognition algorithms.

Disclosures: **D.R. Merrill:** A. Employment/Salary (full or part-time):; Ripple. **S. Hiatt:** A. Employment/Salary (full or part-time):; Ripple. **K.S. Guillory:** A. Employment/Salary (full or part-time):; Ripple. **C. Smith:** A. Employment/Salary (full or part-time):; Ripple. **D. McDonnall:** A. Employment/Salary (full or part-time):; Ripple.

Poster

636. Rehabilitation

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Topic: D.18. Brain-Machine Interface

Support: DARPA N66001-10-C-4056

Title: Improving performance of a neuroprosthetic robotic arm during object manipulation

Authors: ***J. E. DOWNEY**[1], M. L. BONINGER[1,2,5], E. C. TYLER-KABARA[1,3], A. B. SCHWARTZ[1,4], J. L. COLLINGER[1,2,5];
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Abstracts: Although we have shown how prosthetic arms controlled by motor cortex activity can aid people with tetraplegia in their day-to-day lives, we have found that standard brain-computer interface (BCI) decoders can induce undesired neuroprosthetic behavior, such as early grasping, during object manipulation (Wodlinger et. al., In Review). Some of these effects can be mitigated by training the decoder in both the presence and absence of objects to capture differences in neural activity. Training on multiple contexts can be time consuming and possibly ineffective, so a more ideal method would be to account for changes in neural firing during online neuroprosthetic use. Data for this study are from BCI control sessions of a subject with tetraplegia implanted with two 96-electrode Utah Arrays using an optimal linear estimator decoder. Initial analysis failed to reveal a consistent significant difference in single unit firing

rates when the robotic hand was interacting with objects versus when it was not. However, the summed firing rates of all recorded units had higher firing rates when the hand was interacting with objects (K-S Test, $p<0.001$). This object-interaction related increase in global activity violates the decoder's assumption of a baseline firing rate that is only modulated by desired movement kinematics. Here we show results from an online normalization procedure which corrects for the global firing rate changes. During online BCI control, the observed firing rate is normalized by a ratio of the recent (1 second) summed firing rate and the "baseline" summed firing rate observed during decoder training. Performance was evaluated using various tasks. The reach-to-grasp task required the subject to move the robotic hand to a cylindrical target and grasp it. During three sessions of testing, the grasping was successful 65% of the time without normalization, and 80% of the time with normalization. The reach-to-touch task required the same movement, except the hand had to stay open at the target, to test whether object interaction was causing undesired grasping. Success rates for this task were 65% and 85% without and with normalization, respectively. The object-transfer task required the subject to move a weighted cylinder from one side of the table to the other as many times as possible in 2 minutes. Without normalization the subject completed 0.79 transfers/min, but with normalization she completed 1.79 transfers/min. These tests show that renormalizing unit firing rates based on changes in the global firing rate improves decoder performance for object interaction tasks.

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Poster

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Topic: D.18. Brain-Machine Interface

Support: NIH Grant 5R01NS063372

Title: Neural ensemble response to learning challenges in brain-machine interfaces

Authors: *M. ARMENTA SALAS[1], S. I. HELMS TILLERY[2];
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Abstracts: The underlying neural mechanism of motor learning is a process that involves several brain structures such as motor and premotor cortices, cerebellum and the basal ganglia

(Georopoulos et al, 1982; Jueptner et al, 1997; Hikosaka et al, 2002). Detecting changes in these structures associated with learning, however, is challenged by the main variables dictating neural firing, which are kinematic and kinetic variables. Thus, we are interested in an approach which helps test the limits of neural adaptation without being constrained by such variables. We achieve this by using brain-machine interface technology, which allows us to directly relate changes in brain activity to an output experiment variable, i.e. movement of a computer cursor (Carmena et al, 2003; Jarosiewickz et al, 2008; Taylor et al, 2002). We have trained two non-human primates to perform a 3D center-out task, using microwire arrays implanted in motor and premotor cortices to drive the movement of a computer cursor using the population vector algorithm (Georgopoulos et al, 1986). After the task was well learned (accuracy $\geq$ 65%), we introduced two types of perturbations. First, a visuomotor rotation (VMR) where we perturbed the movement by 30° about the anteroposterior axis. An ideal solution to this perturbation would be to rotate the preferred directions (PD) of the contributing cells by an equivalent amount. Second, a decorrelation paradigm, where we rotated by 90° the PDs of a small subset of cells (ca. 20%), selected from the highest correlated cell pairs. An ideal solution to this task would be to identify the perturbed cells and selectively rotate their PDs. We hypothesized that the monkeys would be able to adapt and bring performance back to baseline for both perturbations. However, we expected the learning curves to be different for each perturbation. Our interest then was the neural solution found for each task. We report that the monkeys adapted to the VMR task faster than to the decorrelation one, shown when comparing performance accuracy and movement to target angles. Moreover, we observed different trends in the activity of the cells used for brain control in each task: cells' PDs changed as expected for the VMR but not for the decorrelation task, and the estimated target that the monkeys were redirecting the movement to (Chase et al, 2010) differed in each case. These discrepancies in behavior and neural activity suggest that the brain engages different mechanisms and solutions when challenged with diverse tasks, even if they are still within a motor context. We propose that the solution found may also be dependent on task difficulty, specifically on the underlying characteristics of the task perturbations

Disclosures: **M. Armenta Salas:** None. **S.I. Helms Tillery:** None.

Poster

636. Rehabilitation

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The Cleveland Clinic Lerner Research Institute

Title: Cortical control of nonlinear, musculoskeletal systems with a brain-machine interface

Authors: F. R. WILLETT[1,2,3], H. KALODIMOS[2,1,3], J. COX[2,3], *D. M. TAYLOR[2,1,3];
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Abstracts: The majority of brain-machine interfaces to date have focused on decoding a kinematic command signal (e.g., hand position or velocity) from the brain and rerouting that signal to a computer cursor, robotic limb, or a functional electrical stimulation (FES) system that reanimates paralyzed muscles. When controlling a computer cursor, the relationship between the cortical command signal and the cursor movement is linear and straightforward. In the case of a robotic or musculoskeletal limb, a control algorithm typically solves the nonlinear control problem for the user, transforming the user's kinematic commands into the joint torques or muscle stimulations needed to make the desired movement. Here, we take a different approach and explore the feasibility of a nonlinear command interface that puts the user in direct control of muscle stimulators implanted in a dynamic musculoskeletal model of the arm in a virtual world. While a direct brain-to-muscle command interface might be more difficult to control than a kinematic one (i.e., requires the cortex to approximate more complex, nonlinear functions of the movement goal and limb state), it would also give the user more freedom. Specifically, a direct brain-to-muscle command interface would give the user control over limb stiffness (through co-contraction) and over the forces exerted by the limb on the environment (useful for pushing, pulling, or picking up objects of different weights). In our experiments, we investigated the ability of motor cortical areas to learn to directly control musculoskeletal systems through two nonlinear command interfaces. In interface one, firing rates were linearly mapped to two independent muscle synergies in a dynamic simulation of a paralyzed human arm with six muscle stimulators confined to make 2D movements in a horizontal plane. In interface two, firing rates were linearly mapped to opposing muscles (brachialis and triceps) at the elbow joint alone with the shoulder joint locked. We characterized the control strategies employed by three Rhesus macaques when controlling these systems and report on the extent and limitations of their neural adaptation.

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Poster

637. Craniofacial Functions

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Program#/Poster: 637.01/NN2

Topic: D.17. Voluntary Movements

Support: NIH Grant R01DC007603

NIH Grant P30DC004661

Title: Sensorimotor control of self-timed vs. externally-timed nonspeech movements in adults who stutter

Authors: A. DALIRI[1], J. R. FLANAGAN[2], *L. MAX[1,3];
[1]Univ. Washington, SEATTLE, WA; [2]Queen's Univ., Kingston, ON, Canada; [3]Haskins Labs., New Haven, CT

Abstracts: Stuttering is a disorder that disrupts speech fluency. Speaking in certain conditions (e.g., in synchrony with a metronome, single words) enhances fluency for many affected individuals. Over the past decades, studies have demonstrated that stuttering is associated with atypical sensorimotor processing for both speech and nonspeech movements. Thus, the disorder may involve instabilities in fundamental sensorimotor mechanisms that underlie the neural control of voluntary movements in general. Here, we aimed to investigate the trial-to-trial stability of two main components involved in the planning of natural nonspeech movements. Using a task that involved grasping and moving an object, we compared stuttering and nonstuttering adults with regard to (a) the control component involved in generating appropriate motor commands to move the arm, and (b) the prediction component involved in anticipating the appropriate amount of grip force for each phase of the movement. We examined the stability of these components in conditions with self-timed vs. externally-timed movements and with cyclical vs. discrete movements. The subjects were 8 male stuttering adults and 8 age-, sex-, and handedness-matched nonstuttering adults. Subjects held between their thumb and index finger a test object instrumented with a force/torque transducer, and moved it (targeted displacement ~30 cm) without visual feedback in a sagittal plane to the side of the body. Auditory cues were used for the external timing condition. Continuous up-and-down movements were performed in the cyclical condition whereas single up or down movements were performed in the discrete condition. Variability of movement extent was used as a measure of the stability of the control component. Variability of the strength of coupling between grip force and movement

acceleration was used a measure of the stability of the prediction component. The latter measure is based on the fact that preventing slip of the object requires the central nervous system to accurately predict changes in movement acceleration, and to appropriately adjust -- in an anticipatory manner -- the grip force applied to hold the object. Movement extent variability was significantly larger for the stuttering group than for the control group. This variability decreased significantly for externally-timed vs. self-timed movements in the stuttering group but not the nonstuttering group. There was no between-group difference in the coupling between grip force and movement acceleration. Findings suggest that stuttering is associated with deficits in generating movement control signals rather than predicting the consequences of those movements.

Disclosures: A. Daliri: None. **J.R. Flanagan:** None. **L. Max:** None.

Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: Japan Society for the Promotion of Science No. 25750247

Title: Neural activation of reading words aloud in adults who stutter

Authors: *S. CHU, J. OGURA, M. WADA, K. MORI, K. OCHI;
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Abstracts: The purposes of this investigation were 1) to trace the distributed neural network of overt speech and 2) to characterize brain activation specific to reading aloud Japanese words of different lexical properties in adults who stutter and neurotypical controls. Word form stimuli of four Japanese lexical properties (familiar words, unfamiliar words, pseudowords, elongated vowels), and of Cyrillic letters as non-reading visual control were presented and compared between stuttering and nonstuttering speakers using a 1.5 Tesla MR-scanner. A clustered image acquisition technique was used to minimize speech-related movement artifacts. The stuttering group showed higher activation than the control group in the right premotor areas (BA6) and right supramarginal gyrus (BA40) for familiar, unfamiliar and pseudo- words, and vowels subtracted by the Cyrillic letter condition. Higher activation of the right insula was observed in the stuttering group for the pseudowords than the Cyrillic letter condition. The fact that increases

activity in the right insula during pseudowords condition suggests that insula may be involved in rather complicated word production and recruitment of this area for articulatory movement ensures fluent production among stuttering speakers. These preliminary results suggest that stuttering and nonstuttering speakers produce different patterns of brain activation during word production.

Disclosures: S. Chu: None. **J. Ogura:** None. **M. Wada:** None. **K. Mori:** None. **K. Ochi:** None.

Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01DC011277

Title: White matter differences in young children who stutter

Authors: *S.-E. CHANG[1], A. CHOO[1], M. K. ANGSTADT[1], D. C. ZHU[2];
[1]Psychiatry, Univ. of Michigan, Ann Arbor, MI; [2]Radiology, Michigan State Univ., East Lansing, MI

Abstracts: Stuttering affects 5% of preschool-age children, with typical symptom onset at 2-4 years of age. Despite the fact that onset and eventual recovery versus persistence of stuttering mostly occur during childhood, few studies have yet examined the neural bases of childhood stuttering. Here we present a diffusion tensor imaging (DTI) study that examined measures of white matter integrity (fractional anisotropy; FA), in a relatively large group of children who stutter between 3-10 years of age. We asked whether previously reported anomalous white matter (WM) measures in adults and older children who stutter were also present in younger children who stutter, and whether stuttering severity is correlated with FA. Specifically, we hypothesized that children who stutter would exhibit decreased WM integrity in major WM tracts (i.e., SLF) interconnecting frontal motor and auditory areas, particularly in the left hemisphere. We also hypothesized that stuttering severity may be negatively correlated with FA in the left SLF. A total of 89 monolingual English-speaking children participated in this study as part of an on-going longitudinal investigation of developmental stuttering. All children exhibited normal speech, language, IQ, and hearing development as assessed through a battery of

assessments. Stuttering and control groups were matched in chronological age and socioeconomic status. DTI data were acquired with a dual spin-echo echo-planar imaging (EPI) sequence (48 contiguous 2.4-mm axial slices in an interleaved order, FOV = 22 cm × 22 cm, matrix size = 128 × 128, number of excitations (NEX) = 2, TE = 77.5 ms, TR = 13.7 s, 25 diffusion-weighted volumes with b = 1000 s/mm2). A total of 77 volumes (40 controls, 37 stuttering) were analyzed (after excluding 12 due to excessive movement) using FSL's TBSS processing stream for whole-brain based comparison of FA between the two groups. 10,000 permutation tests were performed using the randomise program in FSL and statistical inference was based on an uncorrected threshold of $p<0.001$ with an extent threshold of minimum 10 voxels. Stuttering children exhibited significantly reduced FA relative to controls in the bilateral inferior frontal (IFG; BA44), motor and auditory regions and left cortical spinal tract, but increased FA in the right IFG (BA45). Stuttering severity was negatively correlated with FA in the BA44, motor, and parietal areas along the left SLF. These results provide first glimpses into the neural bases of childhood stuttering, which includes bilateral attenuation of WM integrity underlying sensorimotor cortical areas, and greater stuttering severity associated with left hemisphere decreases in WM integrity.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: NIH Grant DC009589

NIH Grant DC004290

NIH Grant NS075044

Title: Focal intraoperative cooling modifies speech production in a location-specific manner

Authors: ***M. A. LONG**[1], K. A. KATLOWITZ[1], R. C. CLARY[1], T. J. F. DI CASTRI[1], M. A. SVIRSKY[1], D. R. HANSEN[2], M. A. HOWARD, III[2], J. D. W. GREENLEE[2];
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Abstracts: Humans are capable of learning and executing a wide range of motor sequences, but the means by which premotor brain regions coordinate their activity to produce these behaviors is still poorly understood. Perhaps the most closely studied complex human behavior is the production of spoken language. The neural dynamics correlated with speech production have been explored using electrophysiology (Sahin et al., 2009; Bohland et al.,2010; Tankus et al., 2012; Bouchard et al., 2013; Cogan et al., 2014) or functional magnetic resonance imaging (Price, 2012). To uncover the specific roles of individual brain regions in speech production, we developed a method to quickly, reversibly, and focally lower the temperature of the cortical surface in order to slow down local circuit activity (Pires and Hoy, 1992; Yamaguchi et al., 2008; Long and Fee, 2008; Tang et al., 2010). We built a custom cooling device to affect 1 to 6 regions (mean: 2.6) in series in 15 awake patient volunteers undergoing craniotomy while they recited word lists. We measured two primary effects of cooling on speech production. First, cooling in some regions, such as the pars opercularis, resulted in a slowing of the timing of produced speech, as measured using custom-built software to identify repeatable spectrotemporal landmarks within each word. Second, cooling in other regions, including the premotor cortex, resulted in speech degradation, as measured through a range of subjective and objective measures. Independent alterations in timing or degradation of speech sounds could sometimes be elicited as a function of cooling probe location, implying segregated roles for speech production circuitry. These results have implications for the mechanisms of speech sequence generation, and they support focal cooling as an alternative option to standard speech region mapping procedures, such as electrical stimulation.

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Poster

637. Craniofacial Functions

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R00DC009629 to KS

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Title: Dopamine drives left-hemispheric lateralization of brain activity and functional connectivity during speech production: An fMRI and neural modeling study

Authors: *K. SIMONYAN[1], J. C. ZINN[1], B. HORWITZ[2], S. FUERTINGER[1];
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Abstracts: Hemispheric dominance of human speech and language is known since the time of Broca but the explanation for the physiological basis of left-lateralized brain activity and functional networks is still elusive. Our recent PET/fMRI findings suggest that left-lateralized striatal dopamine release during speech production contributes to the lateralization of functional brain networks. However, it remains unclear whether dopamine release drives or follows the lateralization of functional speech network. To address this question, we developed a physiologically informed stochastic neural population model to simulate brain activity coupled with dopaminergic neurotransmission during speech production. Twenty individual whole-brain simulations of neural activity were carried out for 70 cortical and 6 subcortical small-scale models, which were composed of excitatory and inhibitory neurons and coupled based on our experimental functional connectivity data. Speech-related dopamine release was simulated based on neural firing in substantia nigra pars compacta (SNc), and its modulatory effects were measured in the laryngeal motor cortex (LMC), which represents a final common motor cortical speech pathway. The resting-state activity without dopamine or firing rate modifications was simulated as control. Simulated functional networks were assessed using psychophysiological interaction analysis. Dopamine-induced functional networks were examined in relation to left-lateralized and bilaterally distributed structural connectivity between the SNc and LMC. Lateralization of simulated neural activity and networks was calculated using a laterality index. Simulated dopamine release from the SNc to LMC showed left-lateralization of brain activity and functional network. Compared to the bilateral release, left-SNc induced dopamine release showed more pronounced network lateralization, which was similar to that of the experimental data. Simulated resting-state activity and functional network did not show dopamine release-dependent lateralization. Artificial increases in simulated structural connectivity between left SNc and LMC did not amplify the observed dopamine-modulated lateralization of functional activity and networks. Comparisons with simulated structural connectivity suggest that left-lateralization of the functional network is not dependent on left lateralization of structural network. Rather, dopamine directly modulates the LMC activity and by this induces pronounced lateralization of simulated whole-brain activity and functional network during speech production.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Title: A multi-modal imaging system for simultaneous measurement of speech articulator kinematics compatible with human electrophysiology

Authors: *D. CONANT[1], K. BOUCHARD[1,2], G. ANUMANCHIPALLI[1], B. DICHTER[1], E. CHANG[1];
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Abstracts: Speech articulation involves the rapid, coordinated movement of speech articulators (e.g. lips, jaw, tongue, and larynx). Most neuroscience investigations of speech have relied upon static, binary features instead of dynamic articulator kinematics. However, a complete neurobiological understanding of speech motor control requires determining the relationship between simultaneously recorded neural activity and the kinematics of all articulators. Many speech articulators are internal to the vocal tract, and so simultaneously tracking the kinematics of all articulators is difficult, especially in the context of human electrophysiology recordings. Here, we describe a noninvasive, multi-modal imaging system for simultaneously tracking the movement of the lips, jaw, tongue and larynx for articulator tracking in human neuroscience at the bedside. We combined three non-invasive methods previously used separately: videography to track the lips and jaw, electroglottography to monitor the larynx, and ultrasonography to track the tongue. To characterize this system, we recorded articulator positions and acoustics from six speakers during multiple productions of nine American English vowels. We first describe processing methods for the robust extraction of kinematic parameters from the raw signals and to alignment/scaling methods to account for artifactual variability across recording conditions. To understand the relationship between kinematics and acoustics, we used regularized linear regression between the vocal tract kinematics and speech acoustics to identify which, and how many, kinematic features are required to explain both across vowel and within vowel acoustics. These results generally confirm the relationship between tongue height and tongue frontness, and the first and second formants, but also identified contributions from other articulators. We used unsupervised matrix factorization techniques to extract ‘basis sets’ of vocal tract ‘shapes’ associated with different vowels. This data-driven approach may preserve information about vocal tract ‘shape’ better than traditional point extraction methods. Finally, we developed a statistical speech synthesizer to convert measurements of the vocal tract to audible speech and were able to reconstruct perceptible speech from measured articulatory features. These results demonstrate a multi-modal experimental system to non-invasively monitor articulator kinematics during speech articulation, and describe novel analytic methods for relating kinematic data to speech acoustics.

Disclosures: **D. Conant:** None. **K. Bouchard:** None. **G. Anumanchipalli:** None. **B. Dichter:** None. **E. Chang:** None.

Poster

637. Craniofacial Functions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 637.07/NN8

Topic: D.17. Voluntary Movements

Title: Dynamics of variability and information encoding in electrocorticography recordings during production and perception of syllables

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Abstracts: Human speech production requires precise spatio-temporal patterns of neural activity in motor areas for articulation of phonemes, and in auditory areas for encoding the resulting auditory feedback. However, because of the inherent variability of neural processing, the representation of motor commands and sensory feedback vary from trial-to-trial. Understanding how the human brain manages neural variability during speech will give insight into neural processes underlying these functions. Here, we investigated the structure and dynamics of neural variability, and its relationship to information encoding, during human speech production of a large set of consonant-vowel syllables. Our goal was to understand across-trial variability of neural activity over the time course of articulation in the ventral sensory-motor cortex (vSMC, an area critical for speech motor control), and in the superior temporal gyrus (STG, an area critical for perception of speech sounds), during the ensuing auditory feedback. To this end, we recorded neural activity directly from the surface of vSMC and STG using high-density electrocorticography (ECoG) in two neurosurgical patients while they listened to and repeated consonant-vowel (CV) syllables multiple times. We extracted the analytic amplitude from the High-gamma band [75-150 Hz] (HG), which correlates well with multi-unit spiking activity, and has recently been shown to reveal important features of speech production and perception. We observed that, as with single-unit recordings from many species, the variability of HG was positively correlated with its mean. To isolate changes in across trial variability from changes in mean activity, we modeled variance as a function of mean activity and time for each stimulus on

each electrode. To better understand the nature of HG variability, we also calculated network variance by using factor analysis to distinguish between shared variability across the network and noise that is private to individual electrodes. We found that, similar to firing rate responses in primates, HG variability decreased upon stimulus presentation for individual electrodes and on a network level. We determined the time evolution of encoding each component of the CV syllable by computing information across trial-time about consonant identity, vowel identity, and CV identity. The time-course of information closely matched that of variance reduction, with maximum cross-correlations between information and variability occurring within 100ms for both brain areas. This analysis motivates studies into the neural mechanisms of variability reduction and performance gains it may provide.

Disclosures: **B. Dichter:** None. **K.E. Bouchard:** None. **E.F. Chang:** None.

Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: NSF BCS Grant 1262297

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Affective Science Training Grant

Title: Beyond lateralization: Inter-hemispheric communication coordinates vocal feedback control

Authors: ***N. KORT**[1], P. CUESTA[4], J. F. HOUDE[2], S. S. NAGARAJAN[3];
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Abstracts: While speaking, modulation of the timing and frequency vocal pitch are key features of speech that convey important semantic and affective information. While it is known that auditory feedback is used to monitor and maintain proper pitch production, the neural control of

this important aspect of speech production remains poorly understood. In this study, we examined the neural control of pitch production using time-resolved whole-brain functional imaging with magnetoencephalography. Subjects respond to an unexpected pitch shift by changing their pitch production to oppose the change, and we found bilateral increases in high gamma power (HGP) early in response to the pitch shift change followed by predominantly right hemisphere HGP enhancement by 200ms. Speaking-specific induced HGP increases, obtained by subtracting out the cortical responses during passive listening, showed right parietal and premotor cortex (peak activity by 175ms) and left posterior temporal cortex activations (peak activity at 225ms) in driving the motor response to the shift. Neurobehavioral correlations demonstrated the involvement of right frontal regions and left posterior temporal cortex lobe in the amplitude of the behavioral response while bilateral auditory and right frontal regions correlated with individual subjects' response variations to pitch shifts. Finally, connectivity analysis revealed large scale increases in inter-hemispheric communication that coordinates vocal feedback control of pitch.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: Emmy Noether Grant of the German Research Foundation to C. Kell

Title: Neural temporal information coding in sentence repetition - an ECoG study

Authors: ***J. GEHRIG**[1], M.-T. FORSTER[2], J. LEI[1], H. LAUFS[1], C. SENFT[2], V. SEIFERT[2], S. HANSLMAYR[3], C. KELL[1];
[1]Dept. of Neurol., [2]Dept. of Neurosurg., Goethe Univ., Frankfurt Main, Germany; [3]Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstracts: *Introduction* Little is known about how information is coded in the brain. Speech constitutes a hierarchically organized and temporally highly structured behavior in which different parts of the physical signal carry different information. We hypothesized that temporal information coding in speech directly relates to temporal coding in neural networks involved in speech production and perception. We thus investigated electrocorticography (ECoG) recordings

from six patients undergoing awake brain tumor surgery of the left hemisphere and focused on analyses of temporal similarity in neural signals associated with listening to, remembering and repeating the same versus different sentences. *Methods* Patients listened to prerecorded three word sentences (~1s, listening phase) and waited for 1.5s (maintenance phase) until visual presentation of a go cue for sentence repetition (speaking phase). A specific sentence was used in 30 trials (Same trials), while 70 further trials were based on 70 different sentences (Different trials). ECoG data were recorded with cortical grids (5 mm spacing, 5kHz sampling) and were analyzed using fieldtrip. Individual time-frequency spectra (2-120 Hz) were created by analyzing dpss multitapers. To identify temporal information coding in the different trials phases, we tested whether the difference in correlations between 1s sliding windows (100 ms timesteps) of Same and Different trials was significantly different from the mean difference in all trials. These temporal pattern similarity analyses were carried out both on raw timecourses and on time frequency data. *Results and Conclusions* The broadband gamma response in auditory cortex was stronger for listening to the patient's prerecorded voice compared to speech production of the same sentences while this effect was inversed in the motor cortex. Low frequency suppression during processing was most obvious in sensorimotor cortices and relatively absent in Broca's area. Here, activations were confined to the high beta/low gamma band during verbal working memory encoding and maintenance. Temporal pattern similarity was found in the raw time courses in auditory and motor cortices during listening and speaking. Spectral analyses revealed that these effects were driven by temporal information coding in delta, theta and alpha rhythms. Importantly, temporal information coding was observed in frontotemporal cortices during working memory encoding and maintenance in the beta band. Our results indicate that temporal coding in the brain is not only restricted to tracking rhythmic regularities of sensory stimuli but constitutes a more general mechanism of information coding.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (#24390431)

Title: Possible neuroplasticity of swallow-related neural network

Authors: *M. INOUE;
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Abstracts: We examined effects of continuous pharyngeal electrical or liquid stimulation on swallowing behaviors in healthy humans. Effect on voluntary swallow was elucidated by repetitive saliva swallowing test (RSST), in which subjects were instructed to swallow their own saliva as quickly as possible for 30 sec and the number of swallows was counted. Involuntary swallow was elucidated by swallowing response time (SRT), in which 0.1-ml water was injected into the pharynx and the latency of initiation of first swallow was measured. In the first experiment, 10-min pharyngeal surface electrical stimulation (5 Hz, 1 ms pulse duration) was applied and effect of stimulation on voluntary and involuntary swallows were evaluated. RSST and SRT were recorded before stimulation as control and every 10 min after 10-min stimulation for 60 minutes. While SRT was less affected by 10-min pharyngeal stimulation, the number of swallows during RSST gradually increased in 60 min. In the second experiment, 10-min liquid stimulation using distilled water or carbonated water was applied. In this experiment, the subjects were instructed to swallow 5 ml of either liquid every 10 sec over 10 min. Following the liquid stimulation, voluntary and involuntary swallows were evaluated. While RSST was less affected throughout the recording period in both cases, SRT significantly decreased in 60 min in case of carbonated water. Possible neuronal mechanisms for the modulation of swallowing initiation were discussed.

Disclosures: M. Inoue: None.

Poster

637. Craniofacial Functions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 637.11/NN12

Topic: D.17. Voluntary Movements

Support: NIH R03DC010895

Title: Adapting human videofluoroscopic swallow study (VFSS) methods to characterize dysphagia in animal models of human diseases

Authors: *M. J. ALLEN[1], T. E. LEVER[2];
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Abstracts: Objective: A logical first step in establishing animal disease models of dysphagia for translational research is the identification of objective measures (biomarkers) of swallow function/dysfunction that can be readily compared across species, including humans. However, published methods for evaluating dysphagia in animals are markedly different between species as well as between diseases, rendering it impossible to derive meaningful comparisons and predictions from these preliminary research efforts. In contrast, human dysphagia is predominantly studied using an x-ray procedure called a videofluoroscopic swallow study (VFSS). This test entails sitting or standing within the c-arm of fluoroscopy machine while voluntarily ingesting food and liquid consistencies mixed with an oral contrast agent. The goal of this study was to adapt the voluntary human VFSS methods for use with major model organisms (mice, rats, dogs, cats, and pigs) to facilitate comparative and translational investigations of dysphagia. Methods: Approximately 60 mice and 40 dogs of both sexes participated in protocol development. Mice were from our C57 and SOD1-G93A (model of amyotrophic lateral sclerosis or ALS) colonies. Healthy dogs and those with neurological diseases (e.g., ALS and Batten disease) were from existing colonies or the local community. Results: We have successfully designed species-specific methods for VFSS that include three key components: 1) recipes for flavoring oral contrast agents, 2) test chambers that permit self-feeding, and 3) a step-by-step test protocol that permits quantification of swallow physiology. Conclusions: We have developed novel methods for evaluating dysphagia that permit direct comparisons of swallow function/dysfunction between species and diseases. These methods are currently being used to establish several animal disease models of dysphagia that are suitable for translational research with humans and companion animals. Thus, this line of research has the potential to benefit human and veterinary patients with dysphagia.

Disclosures: M.J. Allen: None. **T.E. Lever:** None.

Poster

637. Craniofacial Functions

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Program#/Poster: 637.12/NN13

Topic: D.17. Voluntary Movements

Support: NIDCD; 1F31DC013230

NIDCD; R01DC008149

Title: The cross-training and detraining effects of tongue exercise in the cranial sensorimotor system

Authors: *A. J. SCHASER, M. R. CIUCCI, N. P. CONNOR;
Univ. of Wisconsin-Madison, Madison, WI

Abstracts: Purpose: Exercise-based therapies are currently used to treat voice and swallowing disorders without a clear understanding of the mechanisms that alter the cranial neuromuscular system. For instance, it is not known if tongue exercise paired with a water swallow is associated with changes limited to the tongue alone or if cross-training effects are found in other swallow-related structures, such as the larynx. It is also unknown if behavioral changes endure after therapy is discontinued, and if changes are related to neurotrophic regulation. It is hypothesized that: 1) tongue exercise will result in improved behavioral and neurotrophic outcomes in the cranial sensorimotor system across the lifespan with greater effects in lingual versus laryngeal structures, and 2) that exercise effects will diminish when the exercise program is discontinued (detraining). Methods: Forty young adult (9m) and 40 old (32m) male rats were used. The rats were randomly assigned to either a tongue exercise group (n=20), 2-week detraining group (n=20), 4-week detraining group (n=20) or control group (n=20). Baseline tongue forces and ultrasonic vocalizations were recorded for all animals. Exercise and detraining animals performed a tongue press task paired with a water swallow for 8 weeks. Detraining animals then underwent 2 or 4 weeks of detraining, respectively. Behavioral outcomes of tongue force and ultrasonic vocalizations were recorded at all time-points. Following training, laryngeal and lingual muscles and brainstem areas were removed for analysis of neurotrophic factors. Results: Preliminary results showed a decrease in tongue force and number of vocalizations with age. Following exercise in both age groups, maximum voluntary tongue force increased, with a subtle detraining effect found at both 2 and 4 weeks. Changes in vocalizations did not appear dependent on tongue exercise, based on number of vocalizations alone. Additional analyses of vocalizations and protein and mRNA quantification of BDNF, NT4/5, and TrkB in muscles and brainstem nuclei will be presented to build on our previous immunohistochemical work which has shown a reduction in TrkB in the hypoglossal nucleus with age and an increase in BDNF with exercise in young adult animals. Conclusions: Tongue exercise is associated with increased tongue forces at all ages and gains are maintained after detraining up to 4 weeks. However, tongue exercise does not appear to have an impact on number of vocalizations. Ongoing analyses examining changes in neurotrophic regulation in the cranial sensorimotor system will be presented to determine the underlying mechanisms responsible for the behavioral changes described.

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Poster

637. Craniofacial Functions

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Program#/Poster: 637.13/NN14

Topic: D.17. Voluntary Movements

Support: NIH R03DC010895

Title: Characterizing the laryngeal adductor reflex across multiple species: Implications for human and veterinary medicine

Authors: *T. E. LEVER;
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Abstracts: Objectives: A recently proposed risk factor for dysphagia and aspiration in neurologic disorders is an impaired laryngeal adductor reflex (LAR), which entails brief closure of the vocal folds in response to contact of foreign material at the laryngeal entrance. The LAR is readily triggered in healthy, awake humans by delivering a puff of air to the laryngeal entrance via an endoscope passed through the nose into the throat. LAR impairment has been studied (but not well-characterized) in conditions of health and various neurological disorders that cause dysphagia, such as amyotrophic lateral sclerosis (ALS) and stroke. The goal of this study was to develop a protocol to evaluate the LAR in mice. Methods: We designed and constructed an elaborate prototype air pulse delivery system that interfaces with an endoscope that is small enough for oral insertion to the laryngeal entrance of mice. The endoscope contains a working channel through which calibrated puffs of air can be delivered at specific phases of the respiratory cycle. The system is currently being scaled up in size for use with larger animals (e.g., dogs, pigs and horses). Results: We have successfully used this prototype system to evoke and video record LAR responses in healthy mice and mouse models of ALS under light anesthesia, thus demonstrating proof of concept. Furthermore, we have objectively quantified several novel LAR parameters that have not been previously reported for human LAR, including total LAR duration, duration of adduction versus abduction phases, and velocity of dorsal angle adduction and abduction. Conclusions: This study provides new evidence that mice have an LAR similar to humans, which can be reproducibly induced and recorded in conditions of health and disease. We propose that LAR parameters may serve as novel outcome measures to objectively quantify the effect of treatment interventions for ALS and other medical conditions that cause dysphagia.

Disclosures: **T.E. Lever:** A. Employment/Salary (full or part-time):; University of Missouri. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH.

Poster

637. Craniofacial Functions

Location: Halls A-C

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Topic: D.17. Voluntary Movements

Support: NIH Grant K23 DC011056

NIH GrantR01DC005375

Title: Dysphagia after stroke: Use of hyper-acute anatomical MRI for the study of brain networks and dysphagia

Authors: *M. GONZALEZ-FERNANDEZ[1], C. ANDERSON[1], A. E. HILLIS[2];
[1]Physical Med. and Rehabil., [2]Neurol., Johns Hopkins University, Sch. of Med., Baltimore, MD

Abstracts: Introduction: Lesion analysis is a classic technique used to identify brain-deficit correlations. The earliest location-function associations were possible through post-mortem lesion analysis of brains from individuals with known deficits. The brain-function associations that could be made using this approach were limited by changes occurring in the brain after the event as a result of healing. Early MRI imaging can allow us to make lesion site- deficit associations before changes occur as a result of brain reorganization or healing. We hypothesized that using diffusion-weighted (DWI) and perfusion-weighted (DWI) MRI in the hyper-acute stage of stoke would allow us to make these associations. Methods: DWI and PWI MRI-based lesion analysis was performed concurrent to instrumental evaluation of swallowing in subjects who sustained a supratentorial ischemic stroke. MRI images were obtained within 48 hours of stroke with instrumental examination of swallowing (videofluoroscopy) within the same timeframe to allow for the identification of brain areas that, if affected, result in specific physiologic deficits that clinically result in dysphagia. Results: Obtaining timely MRI and videofluoroscopic swallowing images was feasible. Analysis of the MRI and vidoefluoroscopic images is possible and preliminary data have allowed for the identification of possible associations in several brain locations such as the premotor cortex and inferior frontal cortex. Conclusions: Lesion analysis using DWI and PWI MRI is a feasible approach to study the brain

networks regulating swallowing. Future studies including diffusion tensor imaging (DTI) can potentially add to the relationships identified using the current approach.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: Michael J Fox Foundation

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Title: Preclinical oromotor dysfunction and brain pathology in a PINK1 knock-out model of Parkinson Disease

Authors: ***M. R. CIUCCI**, C. A. KELM-NELSON, L. M. GRANT, K. P. CULLEN, E. PAUL; Surgery-Otolaryngolgy, Univ. Wisconsin, MADISON, WI

Abstracts: Parkinson disease (PD) is devastating to oropharyngeal swallowing and the onset, progression and neural correlates of PD-related dysphagia are poorly understood. To address this, we used a genetic rat model of PD and hypothesized that oromotor and swallowing deficits would manifest early in the disease process and be related to pathologies in brain structures associated with cranial sensorimotor function. Data for vocalization and tongue function were reported previously (SfN 2013). This presentation includes new data on biting and oropharyngeal

swallowing, as well as brain pathology.. Rats with homozygous knock-out (KO) of PINK1, and WT controls (n=32) were studied at 4 and 8 months of age. Bite force and timing characteristics were measured during consumption of a 7cm piece of pasta. Videofluoroscopy was used to evaluate swallow function for mastication rate and bolus velocity. Brain tissue was analyzed for insoluble aggregated alpha-synuclein (aSyn), nigrostriatal dopamine content, and serotonergic receptor density in the hypoglossal nucleus. A repeated measures ANOVA and Fishers Least Significant Difference tests were used for the biting data. Planned comparisons between genotypes at ages 4 and 8 months were performed with unpaired t-tests for videofluoroscopy and neurochemistry data (alpha<0.05). For biting/chewing, PINK1 KO rats demonstrated a larger inter-bite interval at 8 months of age [$F(2, 74)=10.12$, $p<0.001$]. For videofluoroscopy, PINK1 KO rats showed a significantly slower mastication rate $t(15)=1.97$, $p=0.034$, and bolus velocity $t(15)=1.84$, $p=0.043$ than WT rats at 8 months.. For the PINK1 KO rats at 8 months of age: 1) aggregated aSyn was found in the substantia nigra, periaqueductal grey and nucleus ambiguus at 8 months 2) decreased pixel area for immunoreactivity for 5HT-1A receptors was found at 8 months of age. There were no significant differences in tyrosine hydroxylase immunoreactivity between WT and PINK1 KO in the substantia nigra or striatum. Overall, results indicate that rats with homozygous KO of PINK1 demonstrate early oromotor and swallowing dysfunction, prior to nigrostriatal dopamine loss. Aggregated aSyn was found in the substantia nigra as well as regions that control or modulate swallowing (periaqueductal grey, nucleus ambiguus), and reduced number of 5HT-1A receptors were found in the hypoglossal nucleus. Findings suggest oromotor/swallowing deficits occur early in the disease process and are related to pathologies outside of the nigrostriatal dopamine depletion framework.

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Poster

637. Craniofacial Functions

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Program#/Poster: 637.16/NN17

Topic: D.17. Voluntary Movements

Title: A fast and reliable paradigm for swallowing-related functional magnetic resonance imaging (fMRI)

Authors: *P. SOROS;
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Abstracts: Purpose: Swallowing-related fMRI is challenging, in particular, in patients with dysphagia. Main obstacles include task-related head motion, multiple attempts to swallow, and fatigue, which limits the time for data acquisition. The objective of this study was to develop a fast and reliable paradigm to evaluate swallowing-related brain function using fMRI. Methods: We studied swallowing-related brain activity in 22 healthy young women, who were cued to swallow their saliva every 44 s. Event-related fMRI was performed on a Siemens Trio Tim scanner at 3T. For this study, we analyzed fMRI data obtained during the first 6 swallows (5 min) of a longer experiment. Data analysis was performed with independent component analysis (ICA) using MELODIC. Results: Swallowing-related brain activity was identified in the bilateral sensorimotor cortex (lateral pre- and postcentral gyrus) and in the bilateral cingulate motor area (anterior cingulate gyrus) in all participants. Conclusions: This study demonstrates that as few as 6 acts of saliva swallowing performed within 5 min are associated with a reliable activation of key areas of the cortical swallowing network in individual participants, when analyzed with a model-free analysis approach (ICA). This analysis does not require monitoring of swallowing events and successfully separates changes in brain activity from head-motion induced artifacts. This approach needs to be validated in healthy seniors and in dysphagic patients and may be particularly useful for the study of patients with severe swallowing deficits, e.g. due to ischemic brain lesions.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: NIH R01 NS045853

Brain Research Foundation

Title: Uncoupled dynamics of magnitude and phase of beta oscillations in MIo during feeding behavior

Authors: *K. TAKAHASHI[1], Y. NAKAMURA[2], N. G. HATSOPOULOS[1], C. F. ROSS[1]; [1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Grad. Sch. of Med. and Dent. Sci., Niigata Univ., Niigata, Japan

Abstracts: Previously we have shown that the β frequency range of local field potentials (LFPs) recorded from the orofacial part of the primary motor cortex (MIo) increased its power prior to the transition from rhythmic chews to swallows. However any dynamics of beta oscillations, particularly during swallow, has never been shown. In order to investigate beta phase dynamics, we used two female were trained to feed with their right hand while restrained. We used a motion capture system to record 3D jaw kinematics and 2D videofluoroscopy to record tongue marker kinematics. Jaw movement cycles were defined by two consecutive maximum gapes. We recorded 96 channels of LFPs from a chronically implanted Utah array in MIo. We then identified the peak of the β oscillation frequency, bandpass filtered each channel of LFP over β peak ± 3 Hz, then computed the amplitude and phase of the Hilbert transform of the filtered LFPs. Then we analyzed swallowing movements and identified 5 commonly used kinematic events to assess swallowing performances: Closing - Start of thyroid cartilage movement, Elevation - Start of thyroid cartilage elevation, Top - Thyroid cartilage reaching the most up-forward position, Descent - Start of thyroid cartilage descent, Return - Return to Closing position. We observed that beta power increased more than one chew cycle prior to the onset of each event of swallowing and attenuated at Closing, Elevation, Top, and Descent. Furthermore, we found that an entrainment of phase locking of beta oscillations took place around 120 ms prior to Top timing and peak phase locking of a few cycle showed consistent frequency with the identified beta oscillation frequency. Our results indicate that the magnitude of β oscillations recorded from MIo can be used to predict the occurrence of transition from a rhythmic chew to a swallow at least one chewing cycle prior to the start of the swallow cycle. Furthermore there is a particular phase alignment prior to Top independent of magnitude of β oscillations.

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Poster

637. Craniofacial Functions

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Topic: D.17. Voluntary Movements

Support: NIH Grant R03HD07315

NIH Grant K01NS062116

Title: Functional connectivity of swallowing network areas in children with acquired or congenital hemiplegia: A pilot study

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Abstracts: Purpose: To examine the functional connectivity of swallowing network areas in five children with acquired or congenital hemiplegia with and without dysphagia. Methods: Five children with hemiplegia (age range: 6;1-11;1; 3 males) participated in a standardized clinical swallowing assessment and a resting-state fMRI experiment. The swallowing assessment was performed using the Dysphagia Disorder Survey during functional eating and a 4-point Dysphagia Severity Scale. A high-resolution T1-weighted image was acquired for registration of functional images and to obtain lesion information. Additionally, two resting-state fcMRI scans were acquired (TR=2000 ms, TE=35ms, acquisition matrix 64 x 64, FA=75 degrees, slice thickness=4, slices=36, volumes=167) and seed-based connectivity analyses were performed with 5mm radius seed regions in swallowing network areas (primary motor and sensory cortices, insula, and frontal operculum). Results: Dysphagia severity scale scores revealed that one child exhibited no dysphagia, three children exhibited mild dysphagia and one moderate/severe dysphagia. Resting fcMRI analysis showed that for the child without dysphagia all swallowing network areas showed significant and high intensity positive temporal correlations with other swallowing network areas bilaterally (e.g., primary motor and sensory cortices, the anterior cingulate gyrus and the insula) ($t=4.650$, $p<0.001$, uncorrected). Anti-correlations were seen primarily with areas of the prefrontal and visual cortex and the cerebellum bilaterally ($t=4.650$, $p<0.001$, uncorrected). For the three children with mild dysphagia swallowing network areas showed similar patterns of positive correlations but primarily on the unaffected hemisphere (and limited on the affected hemisphere) ($t=4.650$, $p<0.001$, uncorrected); and for the child with moderate/severe dysphagia the analogous positive correlations were seen only ipsilaterally (unaffected side) ($t=4.650$, $p<0.001$, uncorrected). Anticorrelations appeared weaker in the four children with different degrees of dysphagia compared to the child with no dysphagia. Conclusion: These results show preliminary evidence that the functional connectivity of important swallowing areas is altered in children with hemiplegia and dysphagia. Resting-state fcMRI appears to be a useful means for non-invasively investigating swallowing network connectivity and potential neuroplasticity in pediatric patients with dysphagia.

Disclosures: **G. Malandraki:** A. Employment/Salary (full or part-time):; Teachers College, Columbia University. **K. Friel:** A. Employment/Salary (full or part-time):; Weill Cornel Medical College. 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.; K award and R03 Grant. **J.J. Sheppard:** A. Employment/Salary (full or part-time):; Teachers College, Columbia University. **A. Gordon:** A. Employment/Salary (full or part-time):; Teachers College, Columbia 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.; Grant.

Poster

637. Craniofacial Functions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 637.19/NN20

Topic: D.17. Voluntary Movements

Support: NIH Grant 1R01DC006243

Title: Distinct roles of induced and evoked cortical oscillations for speech motor control

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Abstracts: During speaking, the brain has to continuously monitor feedback information in order to correct for unwanted production errors and update the current state of sensory-motor networks for future speech production purposes. This task requires the involvement of short-term and long-term neural processes that facilitate communication between functionally-related areas. In the present work, we studied the electrophysiological correlates of the underlying neural mechanisms of speech motor control in an auditory feedback perturbation paradigm. Electroencephalographic (EEG) responses were recorded from 3 groups of subjects (11 absolute-pitch (AP) musicians, 12 relative-pitch (RP) musicians and 11 non-musicians (NM)) while they maintained steady vocalizations of the vowel sound “a” and received brief (200 ms) pitch shifts (+/- 100 cents) in their auditory feedback. Time-frequency analysis of the EEG signals was performed on a trial-by-trial basis using a complex Morlet wavelet transform and phase-synchronized (evoked) and non-phase-synchronized (induced) neural responses to pitch shifts were extracted for delta (<4 Hz), theta (4-8 Hz), alpha (8-15 Hz), beta (15-30 Hz) and gamma (30-60 Hz) frequency bands. This analysis revealed that the onset of pitch shifts elicited an increase in the power of the evoked responses at frequencies below 10 Hz that peaked at 200 ms

and was significantly larger in AP and RP musicians compared with the NM group. In addition, an increase in the power of the induced neural response components was identified within delta and theta frequency bands that started at latencies around 1000 ms with peaks at 2000 ms following the pitch shift onset. Contrary to the evoked responses, power increases in the induced neural activity was significantly larger in the NM group compared with AP and RP musicians. These findings suggest that the brain utilizes distinct functional mechanisms to process and integrate auditory feedback information during speech motor control. We suggest that the short-latency evoked activities are involved in gating the sensory feedback information to drive compensatory vocal motor behavior. However, long-latency induced oscillations are important for long-range integration and coupling of distinct but functionally-related local neural circuits that are involved in monitoring behavioral performance and updating the state of the sensory-motor networks to accomplish future motor control goals during speaking.

Disclosures: R. Behroozmand: None. **N. Ibrahim:** None. **O. Korzyukov:** None. **C. Larson:** None.

Poster

637. Craniofacial Functions

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 637.20/NN21

Topic: D.17. Voluntary Movements

Support: Grant-in-Aid for JSPS Fellows (25-5251)

Title: Effect of transcranial direct current stimulation (tDCS) over M1 pharyngeal area on swallowing phase in post-stroke dysphagia

Authors: S. KOGANEMARU[1], F. OSHIMA[2], Y. OHASHI[2], H. FUKUYAMA[3], *T. MIMA[3]; [1]Brain Integrative Sci., Kyoto universtiy, Kyoto, Japan; [2]Japanese red cross kyoto daiichi hospital, Kyoto, Japan; [3]Human Brain Res. Cntr, Kyoto, Japan

Abstracts: Non-invasive brain stimulation has been reported to enhance functional recovery by inducing brain plasticity in post-stroke patients. Recently, some studies revealed that transcranial direct current stimulation (tDCS) given over pharyngeal area of the primary motor cortex (M1) has promoted recovery of swallowing dysfunction in acute and subacute post-stroke patients. However, its detailed mechanism of recovery is still unknown. We investigated which phase of swallowing the tDCS affects in post-stroke dysphagia by using video-fluoroscopy (VF). We had

one chronic post-stroke patient showing intractable dysphagia with nutrition intake through a gastrostomy tube due to the right subcortical infarction and the left subcortical hemorrhage occurred about 20 years ago. He received the bilateral tDCS and sham stimulation each on different day. The anodal tDCS was given over the left M1 pharyngeal area and the cathodal tDCS the right M1 pharyngeal area. Other parameters of tDCS were following; duration of 10 minutes, electrical current of 2mA and the size of the electrodes of 3 cm * 3 cm. During tDCS and sham stimulation, he also received direct swallowing training for 10 minutes by a speech therapist. After the tDCS intervention, VF revealed a shortening of the oral transit time in feeding fluid in the oral phase, of the swallowing reaction time and of pharyngeal transit time in feeding jelly and fluid in the early part of pharyngeal phase, compared to the pre-intervention. There was no apparent change in the late part of pharyngeal phase. After the sham stimulation, there was no change in both of the oral and pharyngeal phase. It suggests that tDCS over the M1 pharyngeal area combining swallowing training might be effective for improvement of coordinated movement of oral and pharyngeal organs in post-stroke dysphagia.

Disclosures: S. Koganemaru: None. **F. Oshima:** None. **Y. Ohashi:** None. **H. Fukuyama:** None. **T. Mima:** None.

Poster

637. Craniofacial Functions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 637.21/NN22

Topic: D.09. Tactile/Somatosensory

Title: Central processing of masticatory muscle sensation

Authors: T. FUJIO[1], F. SATO[1], A. TOMITA[1], *M. MORITANI[2], A. YOSHIDA[1];
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Abstracts: Sensations from the masticatory muscles and periodontal ligaments are generally recognized to be transported by the trigeminal mesencephalic nucleus (Vmes) neurons, and to be directly involved in the monosynaptic jaw-closing reflexes. It has been reported in rats that responses to stimulation of the nerves innervating the masticatory muscles can be recorded in the primary somatosensory cortex (S1) and primary somatomotor cortex (M1). Our previous finding that the Vmes neurons receive direct projections from the prefrontal cortex indicated a possibility that sensations from the masticatory muscles or periodontal ligaments are conveyed to the

prefrontal cortex. However, little is known about the central processing of these sensations. To reveal this issue, we examined the central pathways of these sensations to the cerebral cortex in the rat by using neuronal tract tracing and electrophysiological recording techniques. After application of biotinylated dextranamine (BDA) to the masseter nerve, we found BDA-labeled axon terminals mainly in the trigeminal motor nucleus (Vmo) and supratrigeminal nucleus (Vsup). After application of the BDA to the inferior alveolar nerve and the superior alveolar nerve, however, no labeling was found in the Vmes, Vmo and Vsup. Responses to stimulation of the masseter nerve could be recorded in the Vsup. Subsequently, to reveal the thalamic projections of the Vsup neurons, we injected BDA into the Vsup. BDA-labeled axon terminals were found in the medial part of the ventral posteromedial nucleus (VPM) and paracentral nucleus (PC). Responses to stimulation of the masseter nerve could be recorded in the VPM and PC which have been indicated to respectively project to the somatosensory cortex and the prefrontal cortex. Therefore, the present findings suggest that sensation from the masticatory muscles is possibly involved not only in the discriminative aspect but also the autonomic or limbic aspect of the sensory processing.

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Poster

637. Craniofacial Functions

Location: Halls A-C

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Program#/Poster: 637.22/NN23

Topic: D.09. Tactile/Somatosensory

Support: Grant No. 26293412 from the Ministry of Education, Science and Culture in Japan

Title: Neural excitation with positoron emission tomography after tooth mechanical stimulation

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Abstracts: Dentists often cannot find objective abnormal findings in patients who complain of discomfort or abnormal sensation in their dental occlusion. We hypothesized that abnormal neural transmission from the tooth is related to this occlusal discomfort sensation. Chronic tooth

contact habit may induce neural excitation from the tooth to the central nervous system and it may aggravate the discomfort sensation. However, the details about neural transmission from tooth to the central nervous system are not yet clear. In this study, we stimulated a rat premolar mechanically and observed activated brain sites using positron emission tomography (PET) and ^{18}F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG). Rats, 5-7 weeks old, were anesthetized using isoflurane inhalation anesthesia and we stimulated the upper right premolar mechanically with an electric von Frey system (Model 1601C, IITC Instruments) by measuring mechanical pressure. Before the tooth mechanical stimulation, we injected ^{18}F-FDG through the rat's caudal vein and used a stimulation intensity of 0g, 100g, 200g or 300g. We recorded ^{18}F-FDG accumulation with PET ten minutes after ^{18}F-FDG administration. At first, the PET brain images were separated into four parts (upper right, upper left, lower right and lower left) for the analysis and the peak value of striatal uptake (SUV) in each part was analyzed. The PET image showed that accumulated ^{18}F-FDG in the lower right part of the brain was higher with 300g tooth stimulation than 100g or 200g. The data showed that tooth stimulation side in the lower right part of the brain was activated with the tooth stimulation by comparing it with the other parts. Since we wanted to know the excitation point in the brain in detail, we measured SUV in the right and left sensory area, motor area, hippocampus, trigeminal ganglia and spinal cord. Unfortunately, we could not find a specific ^{18}F-FDG accumulated point with tooth stimulation. There is a possibility that unknown points in the brain might have been activated after the tooth's mechanical stimulation. Also, we found that spatial resolution of the PET was not enough and we were not able to find the specific activated point. Moreover, ^{18}F-FDG may not be good enough to trace neural activation clearly.

Disclosures: **K. Omoto:** None. **K. Maruhama:** None. **Y. Matsuka:** None. **T. Sugimoto:** None.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 638.01/NN24

Topic: D.18. Brain-Machine Interface

Title: Simplified technique for localizing speech motor cortex implantation targets

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Abstracts: Localization of implantation targets using functional MRI was first utilized in our research in 1995 to target implantation of hand motor area in locked-in subjects. Functional MRI utilizing BOLD technique indicated a large and non-specific target area over the left motor / pre-motor cortex. Since that time, identification of discrete targets by Chang and his colleagues (Bouchard et al, Nature 2013) has provided clear demarcation of the somatotopy of lips (medial), jaw, tongue and larynx (most lateral and adjacent to the Sylvian Fissure [SF]). These workers took advantage of electrocorticogram (ECOG) recordings during surgery in seizure patients. Their measurements coincide precisely with functional MRI data taken from one intact human subject in this confirmatory study who used discrete movements of lips, jaw and tongue. To apply this information to surgical implantation of electrodes, standard neurosurgical fiducial data were consulted: These data indicate that a line drawn from the tragus to the vertex passes over or slightly anterior to the central sulcus. CT scan imaging in the same subject confirmed that fiducials placed along the line from tragus to vertex lie over or close to the central sulcus. Future surgical exposure of this motor cortical area should confirm the localization as extending along this line from the SF to 30 mm above the SF (Bouchard et al. 2013). These fiducials markings should simplify targeting of implantable electrodes in individual subjects within the motor speech cortex without the need to perform functional MRI.

Disclosures: A.J. Cervantes: None. **P.R. Kennedy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 98% of Neural Signals Inc. **H. Mao:** None.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 638.02/NN25

Topic: D.18. Brain-Machine Interface

Support: BMBF KMU-innovativ

Title: Double sealed hermetic implant package for the next generation of a wireless brain-computer interface

Authors: F. KOHLER[1,3], J. ORDONEZ[1,3], T. STIEGLITZ[1,3], *S. CARDOSO DE OLIVEIRA[2,4], J. RICKERT[3,4,5], M. SCHUETTLER[2,3,4,5];
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Freiburg, Germany; [4]Brain Links-BrainTools Excellence Cluster, Freiburg, Germany; [5]CorTec GmbH, Freiburg, Germany

Abstracts: Hermeticity is a crucial technical requirement to avoid failure of moisture sensitive electronics utilized in active implantable medical devices (AIMDs). Our development of a wireless brain-computer interface (BCI) which is intended to stay functional within the human body for decades poses manifold challenges to the technical development. But not only moisture ingress has to be avoided once the device is implanted, process related hazards have to be identified and avoided as well. To allow wireless infrared data communication through our implant packages wall, we decided for a ceramic housing to host the electronics. Ceramic provides excellent hermeticity characteristics and concurrently allows a satisfying amount of infrared light to pass. We developed a solder-sealing concept for the ceramic packages which is reproducible and highly reliable. However, hazardous fumes related to the solder procedure must not enter the package since they could initiate corrosive processes. For that purpose we utilize a two-step sealing concept where we first seal our package from the inside with an epoxy resin followed by the final solder-seal. To achieve a reliable polymeric seal, we utilize a sealing tool, which allows us to align the individual components of our package as well as a heating function to melt the epoxy to form an internal seal and solder fume barrier, respectively. The tool can be utilized for various designs and geometries. It allows variations of the processing temperature as well as the mating force when individual components are joined to form the hermetic enclosure. The process itself works reliably and is easy to handle. Packages with hermeticity values ranging from 10^-10 mbar l s^-1 to 10^-12 mbar l s^-1, depending on the overall size of the package and the length of the sealed joint could be fabricated. The measured hermeticity values predict theoretical lifetimes for the individual packages ranging from 100 to more than 10000 years.

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Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 638.03/NN26

Topic: D.18. Brain-Machine Interface

Support: Internal funds of Florida Hospital for Children

Title: Estimation of intracranial P300 speller sites for brain-computer interfacing with magnetoencephalography (MEG)

Authors: *M. KOROSTENSKAJA[1,2,3], C. KAPELLER[4], R. PRUECKL[4], R. ORTNER[4], P.-C. CHEN[1,3], K. LEE[3], T. KLEINESCHAY[2,3], C. GUGER[4], J. BAUMGARTNER[3], E. M. CASTILLO[2];
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Abstracts: Rationale: Brain-Computer Interface (BCI) technology is a powerful tool for enabling communication between people and the surrounding world by surpassing the muscle activity and utilizing direct brain signal instead. Utilization of invasive BCIs may benefit BCI users. Among several approaches allowing navigation for invasive BCI implantation, the most promicing can be considered the use of magnetoencephalography (MEG). MEG is a non-invasive technology allowing precise temporal and spatial resolution of neuromagnetic signal. The aim of the current study was to evaluate for the first time the possibility for non-invasive navigation of subdural electrode implantation with MEG needed for high accuracy P300 speller performance. Methods: The study was performed in a right-handed female patient (17 yo) with diagnosis of intractable epilepsy, undergoing evaluation for epilepsy surgery. Two main approaches were utilized to select 8 channels for invasive P300 speller: (1) Protocol #1 (MEG-based) used MEG source localization to navigate the choice of electrodes; and (2) Protocol #2 (ECoG-based) utilized statistical ECoG signal analysis. The localized P300 sources obtained from Protocol #1 were overlaid with the 3-D-rendered cortical and grid map. Eight electrodes in a close proximity with localized P300 sources were selected for P300 speller protocol. To choose 8 channels with Protocol #2, a Mann-Whitney U-test was utilized to test if TARGET and NON-TARGET samples originate from the same distribution and led to a p-value for each sample and channel. The channels were selected according to the longest period of significant difference ($p<0.05$) between TARGET and NON-TARGET trials. Results: The accuracy of subdural P300 speller was compared for 8 electrodes identified with MEG (Protocol #1) and for 8 electrodes identified by ECoG data (Protocol #2) after creating a classifier with 10 letter phrases. The accuracy of subdural P300 speller for MEG-identified sites was 80%, whereas for ECoG-identified sites it reached 90%. Conclusions: Our preliminary data suggest that MEG has a potential to serve as a non-invasive tool for navigating electrode implantation of P300 speller-based BCIs.

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Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Topic: D.18. Brain-Machine Interface

Support: NSF ERC CSNE Seed Grant: EEC-1028725

Gift from Medtronic

Title: Mobile closed-loop deep brain stimulation system for ambulatory patient tremor mitigation

Authors: *J. A. HERRON, H. J. CHIZECK;
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Abstracts: Deep brain stimulation (DBS) has become a widely adopted method for treating a variety of neurological and movement disorders. However, current clinically deployed systems are open-loop and do not take into account the potentially intermittent nature of symptoms such as tremor. By closing the loop with wearable sensors to directly sense symptoms, we can determine not only when stimulation may be necessary but also estimate what intensity of stimulation should be used. By limiting stimulation to only the level needed we will increase the battery life of the implanted devices and reduce exposure to unintended side-effects. We have developed a mobile and wireless platform for investigations closed-loop DBS applications in ambulatory patients. This system takes advantage of a personal-area-network to connect sensors and a smartphone to a real-time command link to the implanted device. The system is capable of fusing data from multiple sensor sources including inertial or electromyography data to make control decisions. These control decisions can include enabling or disabling stimulation or modifying individual stimulation parameters (voltage, pulse width, frequency) in response to changes in neurological symptoms. Our platform consists of a set of worn sensors communicating over Bluetooth to a smartphone. The smartphone is capable of performing digital signal processing and sensor data fusion in order to make control decisions. These control decisions are then sent over Bluetooth to a Medtronic Nexus system which relays packets and control decisions to an implanted Activa PC or PC+S Neurostimulator. We will be using this system for investigations into the clinical performance of closed-loop deep brain stimulation. These mobile systems will be useful in expanding our understanding of neurological movement disorders treatable with DBS by providing consistent data collection and monitoring while patients continue their lives outside of the clinic or hospital.

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Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Topic: D.18. Brain-Machine Interface

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The Boswell Foundation

Title: Grasp representations in the human posterior parietal cortex

Authors: ***C. KLAES**[1], S. KELLIS[1], T. AFLALO[1], B. LEE[1,2], Y. SHI[1], K. PEJSA[1], K. SHANFIELD[3], S. HAYES-JACKSON[3], M. AISEN[3], C. HECK[2], C. LIU[2,3], R. A. ANDERSEN[1]; [1]Biol., Caltech, Pasadena, CA; [2]USC, Los Angeles, CA; [3]Rancho Los Amigos Natl. Rehabil. Ctr., Downey, CA

Abstracts: The posterior parietal cortex (PPC) of the Rhesus monkey has been extensively studied electrophysiologically in the past 20 years. It has been shown to be involved in the planning of reaching and grasping movements and decision making amongst other functions. In this current brain machine interface (BMI) study two Utah electrode arrays (UEA) were implanted in the grasp (AIP) and reach (area 5) related areas of a tetraplegic human. While training the participant to brain-control a robotic limb we had the unique opportunity to study the human PPC electrophysiologically. To improve the decoding performance it is important to understand the properties of the neuronal population we are recording from. For grasp related neural responses, we developed a training paradigm based on the widely known 'Rock-Paper-Scissors'-game. The basic version of the task consists of a cue, delay and response phase. In the cue phase a symbol is presented to the participant either in form of an auditory or visual stimulus which is associated with one of the particular hand shapes (rock, paper or scissors) of the game. In the delay phase a blank screen is shown. Finally the participant had to imagine forming the hand shape that was cued and to verbally respond to what he imagined. This basic task was

modified in various ways allowing us to study different aspects of grasp processing. Although only few recorded units stayed stable over many days, we sampled the same cortical area and could therefore statistically compare neuronal tuning properties in different task configurations. Preliminary results suggest that most of the units recorded at a specific electrode site had stable preferences for specific symbols and their corresponding grasp types. The specificity was consistent with the coding of different grasps within a continuous space of grasp features. We found that extending the number of grasp types in the task can change the organization of these features. We could further decode the task required grasp types offline and online, including using the neural activity to control grasps with a robotic hand. Units that were well tuned to the task typically were tuned either in the cue or response phase, although some cells were tuned to both phases of the task. Interestingly we also found units that were tuned when the symbol was cued by auditory or visual stimuli during the cue phase suggesting integration of different modalities into a uniform task relevant encoding prior to or upon entering the PPC. These results provide novel insights into the process of task related neuronal coding in the context of grasp related activity and could help to improve future neuroprostheses.

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Poster

638. Brain–Machine Interface: Implanted Electrodes II

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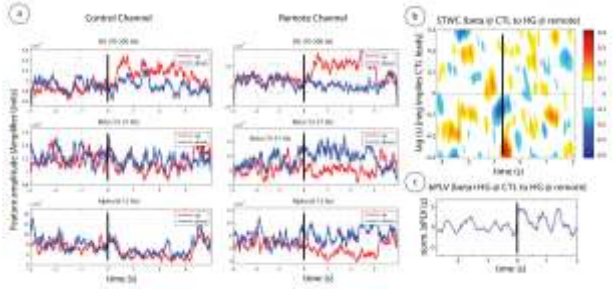
Title: Non-linear frequency coupling during brain computer interface (BCI) control - an ECoG study

Authors: *J. D. WANDER[1], K. WEAVER[2], R. P. N. RAO[3], J. OJEMANN[4], F. DARVAS[4]; [1]Bioengineering, [2]Radiology, [3]Computer Sci. and Engin., [4]Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstracts: Activation of large scale brain networks brain-computer interface (BCI) use has been a subject of considerable interest in recent years. Co-activation of separate regions during the operation of a (BCI) has been reported in prior studies, but characterization of direct interactions between these regions has not been widely addressed. Here we present results from a study with 11 subjects using an electrocorticographic (ECoG) BCI where we observe non-linear cross-frequency coupling between the control electrode and distant cortical sites during active modulation of high gamma power (70-200 Hz). We use the bi-phase locking value (bPLV) and short-time windowed correlation (STWC) as measures of interaction in a data-driven approach to identify lower (alpha/mu - beta range) frequency coupling to frequencies in the high gamma range. We find significant bPLV interactions between frequencies ranging from 7 to 25 Hz with a narrow band in the high gamma range (70-87 Hz) in the control electrode to a similarly narrow range (82-104 Hz) at distant electrodes in 10 out of 11 subjects. Further, we find significant STWC interactions between frequencies in the 15 to 31 Hz range at the control electrode to activity in the high gamma range (70-150 Hz) at electrodes in frontal motor networks. These interactions are specific to time periods where the subjects were executing direct BCI control, which suggests that such interactions are directly involved in the execution of the neuroprosthetic skill. Figure caption: Example band-limited power features and interaction metrics for a single subject. (a) Average feature time-series for a frequency band & electrode combination, calculated separately for trials requiring motor imagery and trials requiring rests. (b) Example Beta-to-HG STWC map for up trials at the same electrode pair. All shown STWC values have uncorrected $p < 0.05$. (c) Example plot showing normalized (rel. t=-1 to t=0) bPLV for electrodes from the same subject. In all cases, Black vertical line at t=0 corresponds to onset of the feedback period.



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Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 638.07/NN30

Topic: D.18. Brain-Machine Interface

Title: Micro cuff electrodes for very small peripheral nerves

Authors: J. ORDONEZ[1,2], T. STIEGLITZ[1,2,3], *J. RICKERT[4,2,3], M. SCHUETTLER[1,2,3,4]; [1]IMTEK, Albert-Ludwigs Univ., Freiburg, Germany; [2]Bernstein Ctr. Freiburg, Freiburg, Germany; [3]Brain Links-BrainTools Excellence Cluster, Freiburg, Germany; [4]CorTec GmbH, Freiburg, Germany

Abstracts: Cuff electrodes have settled down as the most appropriate method to interface nerves in the animal peripheral nervous system (PNS). However, the prerequisites set on cuffs are very difficult to meet if the dimensions are reduced to match nerve diameters smaller than 500 µm. Even the smallest defect in the seal along the cuff's length can avoid the detection of low amplitude neural activity. Furthermore, it is advantageous to have an inner diameter as close to the nerve diameter as possible in order to prevent current shunting through body fluids within the cuff, which leads to reduced recording amplitude and/or increased stimulation thresholds. At the same time the cuff must not compress the nerve, which results in reduced blood circulation of the small peripheral nerve blood vessels. A large disadvantage in miniaturized interfaces is also the reduced ability to transfer current without triggering toxic corrosion of the electric contacts. Using only medical grade silicone rubber (polydimethylsiloxane, PDMS) and noble platinum metal, we developed a miniaturized bipolar or tripolar electrode to interface nerves sized between 75µm and 300µm in diameter. Different to most other cuff electrode concepts, the device is fabricated in a planar fashion. A nut within the cuff defines the position of the nerve, which is exposed at predefined sites to the metallic contacts. The PDMS body of the electrode contains patterned chambers that expose a larger area of the metal to the electrolyte, reducing the corrosion risk. However, the chambers filled with electrolyte are channeled through the PDMS to allow all current transfer (from contact to contact) to happen across the nerve. This novel approach does not compromise the stimulation ability of the electrode's contact sites allowing higher stimulation amplitudes even when interfacing very small nerves. At the same time, the cuff contains a self-closing flap system to provides a tight seal towards the surrounding electrolytic environment without applying pressure on the delicate nerve. A picosecond laser is used to pattern the planar device. This allows a simple and controlled adaptation of the dimensions to match the targeted nerve's diameter. Furthermore, the contact's surface can be roughened also by laser patterning to increase the charge injection capacity of Pt up to 285 µC/cm^2 (measured by voltage transient detection during pulse testing). The thin and soft

devices (1.5 mm in height) are great in handling, which makes the implantation procedure simple. The initial prototypes are encouraging and we are confident that these can provide a proper tool for interfacing small nerves in the PNS.

Disclosures: **J. Ordonez:** None. **J. Rickert:** A. Employment/Salary (full or part-time):; CorTec. **T. Stieglitz:** F. Consulting Fees (e.g., advisory boards); CorTec. **M. Schuettler:** F. Consulting Fees (e.g., advisory boards); CorTec.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

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Topic: D.18. Brain-Machine Interface

Support: NIH Grant K08NS060223

Title: A novel neurofeedback paradigm enables the decoupling of LFP high gamma activity from spike rate

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Abstracts: The neural substrates generating high gamma (HG) activity (70-300 Hz) in motor cortex are not fully understood. Further, HG power has been postulated to be a close correlate of spiking activity in M1. This postulate is based on the possibility of components of local action potentials leaking into and influencing HG power fluctuation. However, others contest that true HG rhythms are generated by reciprocal interactions of the local pyramidal and interneuron network. Here, we use a novel brain-machine interface paradigm to help determine whether motor cortical high gamma activity and spikes are generated by the same neural substrate. We trained two rhesus macaque monkeys, implanted with 96-channel electrode arrays in M1, to attempt independent modulation of multi-unit spike (MSP) rate and HG power using a novel orthogonal neurofeedback (ONF) paradigm. Monkeys first learned to control a cursor in one-dimension using neurofeedback (1D-NF) of HG power (200-300 Hz) and then MSP rate (using 50 ms time bins) from the same electrode. Monkeys then controlled the cursor using a vector sum of both components (ONF task), with MSP and HG mapped to X and Y components, respectively. The task required the monkey to acquire targets along the X or Y axes and hold there for 100 ms. The degree to which the spike rate and the power in HG band can move the

cursor along orthogonal axes tells us the extent to which these signals can be decorrelated, and thus are generated by different neural substrates. We measured the dependence between the spike rate and HG power using spike-field coherence during the hold time on each electrode for each monkey. We found that both monkeys were able to modulate HG power and MSP rate independently. As the monkeys learned the task, the coherence between MSP and HG significantly decreased over a week of performing the task to a mean of 0.03 ($p < .001$, ANOVA, in both monkeys). Further, coherence was significantly (19% and 75%) lower during ONF than during 1D-NF of HG power ($p < .001$). These results demonstrate for the first time that spikes and high gamma activity on the same electrode can be decoupled and modulated independently. As a consequence, spikes and high gamma may represent two distinct physiological phenomena. The orthogonal neurofeedback paradigm thus provides a way of distinguishing correlated cortical signals.

Disclosures: M.W. Slutzky: None. **M.R. Scheid:** None. **R.D. Flint:** None. **Z.A. Wright:** None.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Fondazione Neurone

Title: Identifying selective auditory attention to speech from electrocorticographic signals

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Abstracts: People affected by severe neuro-degenerative diseases (e.g., late-stage amyotrophic lateral sclerosis (ALS) or locked-in syndrome) eventually lose all muscular control. Thus, they cannot use traditional assistive communication devices that depend on muscle control, or brain-computer-interfaces (BCIs) that depend on visual stimulation or feedback. For this population, auditory and tactile BCIs are two of only few remaining means of communication. All currently used auditory or tactile BCIs require a relatively artificial mapping between unnatural stimuli and the intended message. Thus, such systems are cumbersome to learn and use. One way to address this issue is to replace unnatural stimuli that require an artificial mapping with natural speech stimuli that do not. In such a system, the user would communicate simply by directing attention to the speech stimulus that matches his/her intent. Recent studies provided the physiological basis for such a BCI by showing that the envelope of attended speech is tracked by electrocorticographic (ECoG) signals in the gamma band (i.e., 70-170 Hz). To what extent this effect may support BCI communication remained to be determined. In this study, we explored this avenue by determining whether it is possible to use brain signals to learn the identity of an attended speech stimulus. Specifically, we recorded electrocorticographic (ECoG) signals from twelve human subjects while they selectively attended to one of two simultaneously and binaurally presented speech stimuli. We recorded 40 trials of 15-23 sec duration (12.5 min total) in which we interleaved and counter-balanced the attended conversation and its aural location. We hypothesized that the envelope of activity in the gamma band would be reflective of the acoustic envelope of the attended conversation. To test this hypothesis, we first calculated, for each trial, each electrode, and each conversation the correlation between the power envelope of the gamma band and the acoustic envelope of the conversation. We then evaluated the potential communication performance of this relationship in a BCI context. Our results show that a single cortical location over superior temporal gyrus or pre-motor cortex can support the identification of the subject's communication intent within 15 sec and with at least 90% accuracy. These results lay the groundwork for future studies that determine the real-time performance of BCIs based on selective auditory attention to speech.

Disclosures: P. Brunner: None. **K. Dijkstra:** None. **A. Gunduz:** None. **W.G. Coon:** None. **A.L. Ritaccio:** None. **J. Farquhar:** None. **G. Schalk:** None.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

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Topic: D.18. Brain-Machine Interface

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NIH/NCATS Grant 8KL2TR000146-07

DARPA N66001-10-C-4056

UPMC Rehab Institute

Title: Activation of the human primary motor cortex by sensory inputs in individuals with limb paralysis and implications for brain computer interfaces

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Abstracts: Introduction and objective: Brain Computer Interface (BCI) technology has the potential to benefit individuals with severe disabilities. One challenge for clinical applications of BCI technology in individuals with paralysis is to train a neural decoder, which maps brain activity to a BCI control command, in the absence of overt movement. Previously, researchers have proposed the action observation paradigm for neural decoder training. In this study, we evaluated the use of somatosensory input to activate the motor cortex for neural decoder training in individuals with largely intact sensory function but minimal motor function. Methods: Testing was performed in two participants with upper limb paralysis (P1 and P2) who had an electrocorticography (ECoG) array (P1) and a penetrating microelectrode array (P2), respectively, over their primary motor cortex. P1 was diagnosed with amyotrophic lateral sclerosis. P2 was diagnosed with spinocerebellar degeneration with no indication of cerebellar involvement. Participants performed two video-cued tasks: attempting hand/arm movement, and passive movement of the paralyzed limb by an experimenter while the participants were blind-folded. P2 also performed a third task of passively observing videos of hand/arm movements. The videos included 13 body movements of shoulder, elbow, wrist and fingers. Neuronal data included high-gamma band features from P1 and P2 (local field potential - LFP), and single/multi-unit activities from P2. The decoding analysis used Naïve Bayes algorithm to predict the thirteen movements. Results and Discussion: Somatosensory and visual input induced motor cortical activity in P2. ECoG data analysis classified attempted and passive movements with an accuracy of 33.8% and 68.2%, respectively. Spatial patterns of high-gamma band activity across all ECoG electrodes over the motor cortex were similar between passive and

attempted movements. In P2, attempted, observed and passive movements were classified using single/multi-unit activity with an accuracy of 59.2%, 28.5%, and 32.3%, respectively. LFP data analysis was able to classify attempted, observed and passive movements with an accuracy of 56.9%, 46.2%, and 47.7%, respectively. Based on the results, we postulate that somatosensory input can potentially be used for neural decoder training.

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Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Topic: D.18. Brain-Machine Interface

Support: EU Grant NEBIAS FET EVLIT 611687

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Title: Understanding the effects of transversal intraneural stimulation in amputee: a combined experimental and computational study

Authors: *S. RASPOPOVIC[1,2], F. PETRINI[1,3,4], M. CAPOGROSSO[1,2], S. MICERA[1,2]; [1]Ecole Polytechnique Federale De Lausanne, Lausanne, Switzerland; [2]Biorobotics Inst., Scuola Superiore Sant'Anna, Pisa, Italy; [3]Lab. of Biomed. Robotics and Biomicrosystems, Campus Bio-Medico di Roma Univ., Rome, Italy; [4]IRCCS S.Raffale-Pisana, Rome, Italy

Abstracts: To achieve a close to natural replacement for the lost hand in case of amputation, the user should be provided with the rich sensations that we naturally perceive when we explore the environment. After the first demonstrations that is possible to induce physiologically plausible sensations in amputees, by electrical stimulation of residual nerves, even years after the amputation, efforts were carried out to make it a viable solution for chronic implantation. However, there is a lack of systematic characterization of stimulation effects and of deep understanding regarding the mechanisms underlying the chronic body response to electrode implanted within the nerves, which would eventually allow for the development of a personalized approach for each patient. For this reason, we performed extensive trials during

which a subject with transradial amputation has been systematically stimulated by means of four transversal intrafascicular electrodes (TIMEs) implanted within his residual median and ulnar nerves. We performed single-site and multi-site stimulations with 56 active sites. He reported a variety of different sensations, such as touch, pressure waves, tingling, proprioceptive, twitch-like and hot. These were located in areas over phantom limb that corresponded to the innervations territories of the implanted nerves. Modulations of the injected current amplitude, frequency and pulse width were performed in order to gain knowledge about the modulability of such sensations. Furthermore, we showed that by combining different active sites in multi-site configurations, even across nerves, it is possible to elicit complex non-linear relationships resulting into a modified position, extension and quality of each perceived sensation with respect to single-site stimulation. These experimental findings were then compared with a hybrid electromagnetic-biophysics model of human nerve that we developed, based on realistic anatomy and histology, with a realistic representation of sensory fibers population, able to statistically reproduce the experimental data. Model results suggest that the fibrosis response developed during the chronic implantation of the electrodes does not induce significant perturbations of the electric potential elicited by the stimulation. Instead fibrosis cells would most probably tend to push away sensory fibers from the electrode (instead of killing them or inhibit their activity) explaining the increase in the threshold necessary to stimulate over time. This model and experimental findings will guide the design of the next generation of optimized implantable devices, for the long-term neuroprosthesis.

Disclosures: **S. Raspopovic:** None. **F. Petrini:** None. **M. Capogrosso:** None. **S. Micera:** None.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

Location: Halls A-C

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Program#/Poster: 638.12/NN35

Topic: D.18. Brain-Machine Interface

Support: Ford Foundation Predoctoral Fellowship

Title: Data-driven model comparing the effects of glial scarring and tip metallization loss on chronic neural recordings

Authors: *K. A. MALAGA[1], K. E. SCHROEDER[1], P. R. PATEL[1], Z. T. IRWIN[1], D. E. THOMPSON[1], C. A. CHESTEK[1], P. G. PATIL[1,2];
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Abstracts: The ability to record single-unit activity with chronically implanted microelectrode arrays is hindered by low recording signal-to-noise ratio (SNR). Low SNR can result from glial scarring or materials failures (e.g., loss of tip metallization, degradation of electrode insulation). The objective of this project is to quantify the effects of glial scarring and tip metallization loss on electrode impedance and recording quality using a data-driven model. A finite element method (FEM) electric field model with detailed representation of a single Utah array electrode (Blackrock Microsystems, Salt Lake City, UT) was developed to analyze electrode impedance as a function of encapsulation resistivity, encapsulation thickness, and tip impedance (modeled as a constant phase element [CPE]). Impedance measurements from two Utah arrays implanted in a rhesus macaque for twelve weeks were reconciled with the model. A cable model of a layer V pyramidal cell was then coupled with the FEM model to analyze waveform amplitude as a function of the aforementioned parameters. From the experimental data, mean electrode impedance increased significantly ($p < 0.05$) while mean waveform amplitude remained stable. By week twelve, mean electrode impedance and mean waveform amplitude increased by 81% and 50%, respectively. Histology at four months showed a glial scar thickness of 16 μm around the electrodes. For the default model parameters (ρ_{en} = 600 Ω-cm [Grill, 1994], encapsulation thickness = 20 μm, ρ_{CPE} = 1.92e5 Ω-cm), encapsulation and CPE impedance were 25 kΩ and 369 kΩ, respectively (Z_{tot} = 431 kΩ). CPE impedance was consistent with manufacturer and measured values, and dominated the encapsulation impedance (e.g., ρ_{en} would have to increase by a factor of fifteen for the encapsulation impedance to equal that of the CPE). Electrode impedance increased as a function of encapsulation resistivity, encapsulation thickness, and CPE resistivity. Waveform amplitude decreased as a function of encapsulation resistivity and CPE resistivity, and increased as a function of encapsulation thickness when the neuron model remained stationary and decreased when it moved with respect to the encapsulation boundary (Moffitt, 2005). Thus, to explain the 81% increase in electrode impedance from the experimental data, ρ_{en} or ρ_{CPE} would have to increase by a factor of fourteen or two, respectively. Electrode impedance was mostly insensitive to encapsulation thickness. While glial scarring may still lead to inflammation and neuron displacement, our results suggest that large increases in electrode impedance are more easily explained by tip metallization loss than glial scarring.

Disclosures: K.A. Malaga: None. **K.E. Schroeder:** None. **P.R. Patel:** None. **Z.T. Irwin:** None. **D.E. Thompson:** None. **C.A. Chestek:** None. **P.G. Patil:** None.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Topic: D.18. Brain-Machine Interface

Support: NSF Grant EFRI1137211

Title: Complete cognitive state estimation for asynchronous brain-computer interface systems

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Abstracts: One can imagine that an individual permanently connected to a neuroprosthetic will not want to continuously operate their device. Thus, it is critical to know in real time when neural activity of a user is intended for neuroprosthetic control (control state) and when it is not (no-control state). The proposed solution to this problem is to incorporate an asynchronous control mechanism to a brain computer interface (BCI) system as this will enable users to freely control their neuroprosthetic of their own volition. A variety of approaches have been applied to this problem such as using a salient event related potential to switch between the control/no-control states and utilizing the presence of motor planning activity as an indication of an intention to control. None of these approaches, though, have been implemented in a realistic scenario where subjects naturally transition between the control state and the no-control state, which comprises of a sleep state and an idle state where the user is awake but not attempting to engage their BCI. Here we present the design and performance of a cognitive state estimation algorithm that uses the electrocorticographic (ECoG) signal from freely behaving humans to predict the control, idle, and sleep states, and subsequently decide when and when not to control a neuroprosthetic. Data was collected from two human subjects with intractable epilepsy that were implanted with subdural ECoG grids for seizure foci localization. Subjects were implanted with electrodes for approximately one week during which they behaved freely and naturally. In two sessions on separate days subjects were asked to perform trials of a 3D motor reaching task for 1 to 2 hours at a time. Video recordings capturing the behavior of the subjects were acquired and manually examined to identify epochs when the subjects were asleep, performing the reaching task (control state), or awake but not performing the reaching task (idle state). Cognitive state estimation was done hierarchically in two steps using two probabilistic models. The first model learned the features in the ECoG signal that best differentiated the sleep state from the awake state, which encompassed both the control and idle state. The second model learned the features in the ECoG signal that best delineated the control state from the idle state. The models were implemented in succession to predict the three cognitive states and ultimately

decide when to control or not. The algorithm predicted states accurately and with a low false positive rate. The results suggest that this algorithm can be used as a mechanism for asynchronous control in ECoG based BCIs

Disclosures: **M. Pahwa:** None. **D.T. Bundy:** A. Employment/Salary (full or part-time):; Neurolutions. **C.D. Hacker:** None. **M. Kusner:** None. **K. Weinberger:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions.

Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Topic: D.18. Brain-Machine Interface

Support: National Natural Science Foundation of China 61031002

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National Natural Science Foundation of China 31371001

National Natural Science Foundation of China 61305146

Title: Comparison of direction information encoded in local field potentials from motor cortex during ipsilateral and contralateral arm movement

Authors: ***Q. ZHANG**[1], D. WANG[2], S. ZHANG[2], Y. LI[2], Y. WANG[2], X. ZHENG[2]; [1]Qiushi Acad. for Advanced Studies, [2]Zhejiang Univ., Qiushi Acad. For Advanced Studies, Hangzhou, China

Abstracts: Brain machine interfaces (BMIs) aims to help people restore their lost motor function. Currently, most of BMIs use the neural signals from contralateral motor cortex associated with the movement of limb. However, these signals are not available in the case that contralateral cortex has physical injury or neurological disease. Thus, many studies explored whether the ipsilateral cortex encoded movement information and they found that individual neurons in motor cortex may encode the direction information of ipsilateral arm movement. However, most previous studies focused on spikes, relatively little researches had directly compared the direction information encoded in multi frequency band of LFPs from motor cortex

during ipsilateral and contralateral arm movement. In this study, three monkeys were trained to perform 2D center-out task using left and right hand respectively. LFPs were collected from primary motor cortex (M1) and dorsal premotor cortex (PMd) of one hemisphere via two 96-channel Blackrock arrays. The results showed that the energy of multi frequency band in LFPs changed significantly during the both ipsilateral and contralateral movement, in which the variation trend was similar. These multi frequency band in LFPs also encoded the ipsilateral movement information through mutual information analysis. Direction tuning analysis showed the same neural signals had significant different prefer directions between ipsilateral and contralateral movement. Furthermore, neural decoding revealed that multi frequency band performed well in decoding ipsilateral kinematics and high frequency bands provided the best decoding performance. These results indicated that LFPs in ipsilateral motor contex, especially high frequency bands, could be a potential control signal source for the patients with unilateral cortex injuries.

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Poster

638. Brain–Machine Interface: Implanted Electrodes II

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Program#/Poster: 638.15/OO2

Topic: D.18. Brain-Machine Interface

Support: HFSP RGP0054/2014

SCOPE 131203015

Title: Modulation of brain function by transcranial extracellular impedance control (tEIC)

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Abstracts: EEG is a voltage drop of extracellular currents. Each extracellular current is continuously connected to the source current, or the corresponding intracellular dendric current. The voltage sources of these currents are believed to be the membrane potentials of the

pyramidal cells, the EEG-detectable activities of which are spatiotemporally locked. Now, when a shunt resistor is attached to two EEG electrodes, this is a parallel connection to a peripheral branch and hence the impedance seen from the voltage sources decrease. Therefore, this connection can potentially modulate membrane potentials. Although the connection of a positive shunt resistor may only cause slight modulation effects, that of a negative shunt resistor should cause observable modulation effects. The brain circuit seen from the electrode can be modeled with Ho-Thevenin's equivalent circuit, showing that the extracellular current is positively (called Type I setting) and negatively (called Type II setting) amplified. On the other hand, the brain circuit seen from each neuronal voltage source can be modeled with the superposition principle, showing that each source increases with Type I setting (decreases with Type II setting) the current in whose direction it applies. Thus, we call this technique transcranial extracellular impedance control (tEIC). Note that the above-mentioned operating principles are based on the linear electric circuit, which is usually used as EEG forward models and negative resistors for Types I and II were implemented with operational amplifier circuits. We performed a visually selective response task (speed priority) to evaluate tEIC performance. The tEIC resistors were connected to F3 and the right earlobe and the sham, Type I, and Type II conditions randomly switched every 16 trials of the task. The current flowing through the tEIC circuits was of the order of 10 nA, so that EEG recording could be done with tEIC application. The subjects gave written informed consent after they were given a detailed explanation of this study. The study received approval from the Ethics Committee for human and Animal Research of the University of Tokyo. EEG observations implied that tEIC operation was in accordance with Ho-Thevein's theorem and even single channel tEIC application worked as spatial filter (Type I: high-pass, Type II: low-pass). Moreover, Type I significantly improved reaction times. Therefore, tEIC, just a resistor attached to the scalp, can modulate a brain function.

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Poster

639. Neurosteroids

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Program#/Poster: 639.01/OO3

Topic: E.01. Neuroendocrine Processes

Support: NSF CAREER Award IOS-1053716

Tulane Start-up Funds to NV

Title: Membrane glucocorticoid receptor activation initiates rapid nuclear localization of cytosolic glucocorticoid receptor in hypothalamic neurons

Authors: *J. R. RAINVILLE[1], V. K. VALSARAJ[1], A. HUGHES[2], J. G. TASKER[3], N. VASUDEVAN[3];
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Abstracts: Stress activates signaling through the HPA-axis, which involves negative feedback from adrenal glucocorticoids through receptors in the hypothalamus. Classic glucocorticoid receptor (GR) signaling involves ligand binding, release from chaperone proteins in the cytosol, and translocation into the nucleus where GR dimerizes and acts as a transcription factor to modulate genes downstream of glucocorticoid response elements. Treatment of cells with ligand, such as the synthetic cortisol dexamethasone (Dex), rapidly induces nuclear localization of GR. Although the mechanism of this translocation is not known, there is evidence that ligand binding and participation of the chaperone complex are necessary for entry into the nucleus. However, recent data from a murine hypothalamic cell line (mHypoE-N11), that endogenously expresses GR, demonstrate that Dex conjugated to bovine serum albumin (Dex-BSA) is also able to rapidly induce translocation of the cytoplasmic GR (cGR). Dex-BSA is limited to the plasma membrane by BSA, which is too large and hydrophilic to cross the lipid layers of the membrane. Intensity analysis of the nuclear to cytoplasmic ratio of GR-immunoreactivity is increased by both Dex and Dex-BSA treatment within 10 minutes of treatment, and persists for at least 3 hours. The data suggest that a membrane glucocorticoid receptor (mGR) exists on the cell surface that is able to bind Dex-BSA and relay an intracellular signal that results in translocation of cGR to the nucleus in its unliganded form. MAPK, PKA, and PKC activators and inhibitors were used to identify possible roles of kinase pathways in mGR initiated cGR nuclear localization. Further investigation into alternate pathways, or phosphorylation studies may help elucidate the details of this process.

Disclosures: J.R. Rainville: None. **V.K. Valsaraj:** None. **J.G. Tasker:** None. **N. Vasudevan:** None. **A. Hughes:** None.

Poster

639. Neurosteroids

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Program#/Poster: 639.02/OO4

Topic: E.01. Neuroendocrine Processes

Title: Novel antidepressant-like activity of Caffeic acid phenethyl ester mediated by enhanced glucocorticoid receptor function in the hippocampus

Authors: *S. HER[1], Y.-H. KIM[1], B.-R. LEE[1], O.-K. PARK[1], K. MORITA[2];
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Abstracts: Caffeic acid phenethyl ester (CAPE) is an active component of propolis that has a variety of potential pharmacological effects. Although we have previously demonstrated that propolis potentiated antidepressant-like activity, but the effect of CAPE on the activity remains unknown. The present study assessed whether CAPE treatment (5, 10 and 20 μmol/kg, 21 days) has an antidepressant-like effect on the tail suspension test (TST) and forced swim test (FST) in mice subjected to chronic unpredictable stress. CAPE administration induced antidepressant-like behaviours, evidenced by decreases in immobility time in the TST and FST without affecting serum corticosterone secretion. Western blots conducted after testing the behaviours revealed that CAPE significantly decreased glucocorticoid receptor phosphorylation at S234 (pGR(S234)), thereby resulting in an increased ratio of pGR(S220/S234). We found also negative correlations of the pGR(S220)/(S234) with the p38 mitogen-activated protein kinases (p38MAPK) phosphorylation which was decreased by CAPE treatment. These findings suggest that CAPE treatment has antidepressant-like activity via the down-regulation of p38MAPK phosphorylation contributing to the enhanced GR function.

Disclosures: **S. Her:** None. **Y. Kim:** None. **B. Lee:** None. **O. Park:** None. **K. Morita:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.03/OO5

Topic: E.01. Neuroendocrine Processes

Support: JST Bioinformatics Project

Title: Hippocampus-synthesized estrogen and androgen rapidly modulate dendritic spines and LTP

Authors: *S. KAWATO[1], Y. HOJO[1], Y. HASEGAWA[2], H. MUKAI[1];
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Abstracts: We have demonstrated (1) hippocampal synthesis of estrogen, androgen and (2) synaptic modulation by these sex-steroids. [Synthesis] We showed expression as well as neuronal/synaptic localization of essential enzymes (mRNA and protein) in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that exact levels of estradiol (E2), testosterone (T), dihydrotestosterone (DHT) were 8 nM, 18 nM and 7 nM, respectively, which are much higher than their levels in plasma. Castration significantly decreased T and DHT in the hippocampus, indicating that plasma-derived T is efficiently converted to DHT within the hippocampus. Even after castration to deplete circulating T, the male hippocampal E2 level was not decreased, indicating that E2 is mainly synthesized from hippocampal T. Female hippocampal levels of E2 (0.5-4 nM), and T (1 nM) were less than those of male, and much higher than those in plasma. [Synaptic Modulation] E2-induced rapid modulation (1- 2 h) was demonstrated by analysis of spinogenesis and LTP of adult male rat hippocampal slices (steroid-depleted slices after recovery incubation). LTP analysis showed that 1 nM E2 completely rescued the 1 μM corticosterone-induced suppression of LTP. ERalpha agonist induced the same effect as that of estradiol. These results imply that *in vivo* hippocampal estradiol at 8 nM could rescue acute stress-induced LTP suppression. Spine analysis was performed for pyramidal neurons in hippocampal slices. The density of spines and their head diameters were obtained by mathematical and automated software Spiso-3D which identifies spines by calculating geometrical parameters (Mukai et al., Cerebral Cortex, 2011). E2 at 1 nM rapidly increased the density of small-head (0.2-0.4 μm) spines, in CA1 pyramidal neurons. T and DHT at 10 nM increased the density of middle-head (0.4-0.5 μm) spines and large-head (0.5-1.0 μm) spines, respectively. Signaling pathways are: synaptic ERalpha or AR→ PKA, PKC, MAPK, LIMK → cortactin or coffilin → actin polymerization→ new spine. References: Kawato et al., 2002 Methods in Enzymol, Hojo et al., 2004 PNAS, Mukai et al., 2007 J. Neurochem, Hojo et al., 2009 Endocrinology, Kimoto et al. 2010 Endocrinology, Higo et al., 2010 PLoS-ONE, Mukai et al. 2011 Cerebral Cortex, Ooishi et al. 2011 Cerebral Cortex, Komatsuzaki et al., 2012 PLoS-ONE, Kato et al., 2013, Frontier Neural Circuit, Okamoto et al., 2012, PNAS.

Disclosures: **S. Kawato:** None. **Y. Hojo:** None. **Y. hasegawa:** None. **H. Mukai:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.04/OO6

Topic: E.01. Neuroendocrine Processes

Support: AFAR Grant A12428

AHA Grant 13SDG17050059

NIH Grant AG022550

NIH Grant AG027956

Title: Pgrmc1/klf4 signaling- triggered glia-neuron crosstalk plays a critical role in progesterone neuroprotection

Authors: *C. SU[1], F. SUN[2], T. NGUYEN[3], X. JIN[3], M. SINGH[2];
[1]UNTHSC at Fort Worth, Fort Worth, TX; [2]Univ. of North Texas - Hlth. Sci. Ctr., Fort Worth, TX; [3]Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstracts: Abundant experimental and preclinical data suggest that progesterone (P4) is a potent neuroprotectant that may exert beneficial effects in a variety of neurodegenerative disorders, including Alzheimer's disease (AD) and stroke, though the mechanism(s) involved are still unclear. Recent findings from our laboratory have demonstrated that 1) P4 induces the expression of BDNF, and 2) BDNF signaling is an essential mediator of P4's protective effects in neuron- and glia-containing organotypic (slice) cultures of the cerebral cortex. Interestingly, P4 did not result in robust cytoprotection in "pure neuronal cultures", suggesting that an indispensable component of the protective program of P4 is glia. We have recently found that Pgrmc1, a novel membrane-associated progesterone receptor unrelated to the classical nuclear progesterone receptors (PR), mediates P4-triggered BDNF release specifically from glia. To date, downstream signaling transduction consequent to Pgrmc1 activation has not been revealed. Here we provide evidence that P4 elicits a Pgrmc1/ERK5/KLF4 (a transcription factor) signaling cascade, which in turn, orchestrates glia-neuron communication via a BDNF (Brain-derived neurotrophic factor)-mediated intercellular crosstalk. Pgrmc1 and KLF4 are abundantly expressed in both glia and neurons, although their functions within the brain are largely unknown. We show that P4 triggered a significant release of mature BDNF from glia, and this effect was abolished by RNAi-mediated knock-down of Pgrmc1 or KLF4. Treatment of neuronal cultures with conditioned media from P4-treated astrocytes (P4-CM) induced a robust increase of synaptic marker expression, while blocking neurotrophin signaling can attenuate this effect, supporting that glia-derived BDNF induced synaptogenesis in neurons. In addition, P4-CM from glia significantly protected neurons against oxidative stress. Interestingly, over-expression of KLF4 in neurons resulted in an increase of TrkB / p75 ratio, supporting that neuronal activation of the KLF4 pathway "prepares" the neurons to interpret the glia-derived mature BDNF signaling as favorable to survival. Finally, we determined that the levels of Pgrmc1, KLF4 and

BDNF expression were decreased in the hippocampi of aged mice, as well as in the 5×FAD mouse model of AD when compared to age-matched controls, suggesting that both "normal" and "pathological" aging (i.e., Alzheimer's disease) may diminish the sensitivity of the brain to the protective effects of P4 through down-regulating the Pgrmc1/KLF4/BDNF signaling system.

Disclosures: C. Su: None. **F. Sun:** None. **T. Nguyen:** None. **X. Jin:** None. **M. Singh:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.05/OO7

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant NS037324

Title: Basal and seizure-induced neurosteroid estradiol production in the hippocampus of freely moving rats

Authors: *S. M. SATO, C. S. WOOLLEY;
Neurobio., Northwestern Univ., Evanston, IL

Abstracts: Electrophysiological studies show that 17β-estradiol (E2) acutely modulates synaptic physiology in hippocampal slices from both male and female rats. E2 potentiates excitatory synaptic transmission within minutes in both sexes, and suppresses inhibitory synaptic transmission specifically in females. Furthermore, steroidogenic enzymes required for de novo synthesis of E2 are present in hippocampus, and E2 production in the hippocampus has been demonstrated *in vitro*. While these studies suggest that locally produced E2 could rapidly modulate synaptic function, hippocampal E2 production has not been demonstrated *in vivo*. In the current study, we investigated endogenous E2 synthesis in the hippocampus of awake, freely moving rats. We also evaluated changes in hippocampal E2 concentration during kainic acid (KA)-induced seizures because hyperexcitation observed during limbic seizures resembles the condition used to demonstrate hippocampal E2 synthesis *in vitro*. Adult male and female rats were gonadectomized and implanted with guide cannulae for *in vivo* microdialysis in the dorsal hippocampus. Seven to 10 days later, a dialysis probe was inserted, and Ringer's solution with BSA was perfused through the probe. In a first experiment, androstenedione (4-dione), an E2 precursor, was retrodialyzed for 1 hr after baseline sampling, followed by 2 more hrs of sample collection. Dialysate was assayed using a commercial E2 EIA. We found that 4-dione slowly

increased E2 concentration, reaching ~10 pg/ml above baseline ($F=2.63$, $p=0.041$) after 1 hr, indicating endogenous aromatase activity in the hippocampus. In a second experiment, we injected KA (1.25-5 mg/kg) after baseline sampling and dialysate was collected for 2 hrs while seizure behaviors were recorded on video. Overall, KA-induced seizures more than doubled hippocampal E2 concentration ($F=6.48$, $p<0.001$). Interestingly, the increase in E2 was most pronounced in rats with moderate seizures, in which E2 concentration tripled relative to baseline ($F=6.28$, $p<0.001$), whereas E2 did not increase in animals with the mildest or most severe seizures. Possibly, limbic seizures increase hippocampal E2 production, while extra-limbic seizures may inhibit E2 production. These results parallel our prior demonstration that acute intra-hippocampal administration of an aromatase inhibitor significantly attenuates both electrographic and behavioral KA-induced seizures. Thus, increased local E2 production during seizures likely contributes to seizure progression. These results suggest acute inhibition of hippocampal aromatase activity may be beneficial in preventing seizure progression.

Disclosures: S.M. Sato: None. **C.S. Woolley:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.06/OO8

Topic: E.01. Neuroendocrine Processes

Support: NIH MH0676980

Title: Knocking down expression of PXR in the midbrain ventral tegmental area of female rats attenuates actions of 3α,5α-THP via NMDA and GABA receptors for lordosis

Authors: *C. A. FRYE[1,3], C. J. KOONCE[3,2], J. C. RUSCONI[2], A. A. WALF[3,4,2];
[1]Psychology, [2]Univ. Albany, Albany, NY; [3]Univ. Alaska Fairbanks, Fairbanks, AK; [4]Cognitive Sci. Dept, Rensselaer Polytechnic Inst., Troy, NY

Abstracts: Pregnane xenobiotic receptor (PXR) is traditionally known as a liver factor involved in xenobiotic clearance and cholesterol metabolism, but it is expressed in brain regions, such as the midbrain ventral tegmental area (VTA). Mating induces synthesis of pregnane neurosteroids from cholesterol in the VTA of female rodents. Reducing PXR in the VTA attenuates mating as well as mating-induced biosynthesis of the neurosteroid, 5α-pregnan-3α-ol-20-one (3α,5α-THP). Although these data suggest that PXR is important for the synthesis of 3α,5α-THP in the

midbrain, a related research question is the downstream factors for 3α,5α-THP's actions. 3α,5α-THP has actions in the VTA for lordosis via gamma-aminobutyric (GABAA) and N-methyl-D-aspartate (NMDA) receptor. A question remains about whether PXR may have requisite actions upstream of GABAA and NMDA receptors in the VTA for mating. The hypothesis tested was that, in the midbrain VTA, PXR mediates biosynthesis of 3α,5α-THP, which has subsequent effects at GABAA and/or NMDA receptors for reproductive behavior of rats. Proestrous, ovariectomized (OVX) and estradiol-primed, or OVX and adrenalectomized (OVX/ADX) estradiol-primed rats were compared. Rats were infused to the VTA with saline or PXR antisense oligodeoxynucleotides (AS-ODNs to locally knock down expression of PXR), and then were infused with saline, GABAA receptor (bicuculline), or NMDA receptor (MK-801) to the VTA. Rats were tested in a behavioral battery assessing exploratory (open field), anxiety (elevated plus maze), social (social interaction), and reproductive (paced mating) behavior. Results indicate that knocking down expression of PXR in the midbrain VTA attenuates actions of 3α,5α-THP via NMDA and/or GABAA receptors for lordosis. Few differences were noted in other behavioral tests assessed, suggesting specificity for these effects. There were greater responses among proestrous rats than OVX/ADX rats. This pattern suggests an interactive role of 3α,5α-THP biosynthesis in the brain via PXR and downstream actions at GABAA and NMDA receptors, which is currently being investigated further.

Disclosures: C.A. Frye: None. **C.J. Koonce:** None. **J.C. Rusconi:** None. **A.A. Walf:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.07/OO9

Topic: E.01. Neuroendocrine Processes

Support: NIH

Title: Deletion of estrogen receptor beta is associated with attenuated responses to androgenic neurosteroids to reduce depression-like behavior and lower brain derived neurotrophic factor among mice

Authors: *A. A. WALF[1,2], C. J. KOONCE[2], C. A. FRYE[2];
[1]Cognitive Sci. Dept, Rensselaer Polytechnic Institute,, Troy, NY; [2]Psychology, Univ. Albany, Albany, NY

Abstracts: Differential distribution patterns and whole animal studies investigating activation and knockdown of estrogen receptor (ER) subtypes suggest that ERβ, rather than ERα, may be a target for some of the beneficial trophic effects of estrogens in the brain, without coincidently increasing growth in peripheral steroid-sensitive tissues. How experience, stress, and capacity for neurosteroid synthesis may be factors underlying individual differences in steroids' trophic effects are of interest. The pregnane neurosteroid, 5α-pregnan-3α-ol-20-one (3α,5α-THP), has anti-depressant-like effects and is formed in the brain in response to challenges. Estrogens with activity at ERβ enhance expression of 5α-reductase, which is a rate-limiting steps for 3α,5α-THP formation, and increase levels of 3α,5α-THP coincident with anti-depressant-like effects. Reduced capacity to form 3α,5α-THP is observed in female mice that are ERβ knockout (βERKO). A related question is the role of androstane neurosteroids, such as 5α-androstane,3α,17β-diol (3α-diol), via ERβ, particularly given robust expression of ERβ in the prostate. 3α-diol has rapid actions, which are similar to those of 3α,5α-THP, to modulate stress responses, and may involve actions at ERβ. Effects of a 5α-reductase inhibitor (finasteride), or 3α-diol administration, for depressive behavior in the forced swim test among male mice were assessed. Whether individual differences in responses to androgens may be attributed to current/past exposure to androgens was investigated by comparing mice with typical capacity to produce neurosteroids (C57BL6, wildtype) to those with reduced capacity (5α-reductase knockouts, βERKOs). Results indicate that finasteride increased, and 3α-diol reduced, depression-like behavior of mice, particularly in those that had less prior exposure to androgens. 3α-diol increased brain-derived neurotrophic factor levels in the prefrontal cortex of wildtype, more so than βERKO, mice, without increasing prostate growth. Thus, differences in prior exposure to steroids as well as actions involving ERβ may underlie individual variability in trophic effects in the brain that can be parsed from undesirable growth effects in the prostate.

Disclosures: **A.A. Walf:** None. **C.J. Koonce:** None. **C.A. Frye:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.08/OO10

Topic: E.01. Neuroendocrine Processes

Support: NIH MH0676980

NIGMS P20GM103395

Title: Androgen-mediated effects on seizure activity among male rats involves pregnane xenobiotic receptors

Authors: *C. J. KOONCE[1,2], A. A. WALF[1,2,3], J. C. RUSCONI[1], C. A. FRYE[1,2];
[1]Univ. Albany, Albany, NY; [2]Univ. Alaska- Fairbanks, Fairbanks, AK; [3]Rensselaer Polytechnic Inst., Troy, NY

Abstracts: Androgens have well-known important physiological functions throughout the lifespan. Testosterone is decreased with aging in male rodents, and seizure activity following a chemoconvulsant is increased coincident with age-related decline in androgens or decline following castration. Furthermore, blocking synthesis of testosterone to its neuroactive metabolite, and neurosteroid, 3α-diol (via pharmacological techniques or in 5α-reductase knockout mice) produces similar increases in seizure activity following chemoconvulsant administration to male rodents, as is observed with aging. The pregnane xenobiotic receptor (PXR) plays a putative role in biosynthesis of neurosteroids through its actions involving cholesterol metabolism. Little is known how PXR mediates androgen synthesis in the male brain and functional effects, such as those involving seizure processes. We hypothesized that genetic knockout of PXR would influence seizure activity and responses to testosterone treatment following chemoconvulsant administration. Adult male Sprague Dawley rats (SD WT) and PXR knockout rats (PXR KO) were injected with vehicle or testosterone prior to pentylenetetrazol (PTZ). Immediately after PTZ administration, seizure behavior (myoclonic twitches, forelimb and hindlimb clonus, tonic clonic, barrel rolls) and mortality of rats was assessed. Results demonstrate that PXR KO rats had increased latencies to intial myoclonic twitch, forelimb, and hindlimb clonus compared to SD WT rats. The latency to, and incidence of, myoclonic twitch, tonic clonic seizures, barrels rolls, and death increased following testosterone administration. There were interactions with testosterone administration and genotype, such that testosterone increased seizure activity among SD WT rats (forelimb clonus and barrel rolls), but decreased it among PXR KO (forelimb and hindlimb clonus). Together, these findings suggest that PXR regulation of androgenic neurosteroids influences ictal activity.

Disclosures: **C.J. Koonce:** None. **A.A. Walf:** None. **J.C. Rusconi:** None. **C.A. Frye:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.09/OO11

Topic: E.01. Neuroendocrine Processes

Support: NIH MH0676980

NIGMS P20GM103395

Title: Pregnane xenobiotic receptor deletion among female rats and mice attenuates responses to progestogens for mating

Authors: *J. RUSCONI[1], C. J. KOONCE[1,2], A. A. WALF[1,2,3], C. A. FRYE[1,2];
[1]Univ. Albany, Albany, NY; [2]Univ. Alaska- Fairbanks, Fairbanks, AK; [3]Rensselaer Polytechnic Inst., Troy, NY

Abstracts: Pregnane steroids, such as progesterone (P4) and 5α-pregnan-3α-ol-20-one (3α,5α-THP), have actions in the midbrain ventral tegmental area (VTA) to mediate reproductive behaviors of female rodents. Mating itself promotes production of 3α,5α-THP in the VTA via sequential actions of several well-recognized factors involved in cholesterol metabolism. Pregnane Xenobiotic Receptor (PXR) may be involved in 3α,5α-THP's actions in the VTA. PXR is a nuclear receptor that regulates gene transcription for cytochrome P450 enzymes, which are involved in 3α,5α-THP biosynthesis. PXR has been identified in the midbrain of female rodents. Knocking down PXR in the VTA, with infusions of antisense oligodeoxynucleotides, attenuate lordosis of rats and mating-induced biosynthesis of 3α,5α-THP. An important question is the role of lifelong knock down of PXR, which can be addressed with comparisons of PXR knockout (KO) rats and mice, which are expected to have attenuated responses to progestogens for mating. Experiment 1: wildtype (WT) and PXR KO rats were assessed for lordosis (mating posture) in a mating task when in proestrus (positive control, high endogenous levels of estradiol (E2), P4, and 3α,5α-THP) or following ovariectomy, E2-priming, and administration of vehicle, P4, or 3α,5α-THP. Experiment 2: C57BL6Tac (WT) and PXR KO mice were assessed for lordosis when in proestrus or following ovariectomy, E2-priming, and administration of vehicle, P4, 3α,5α-THP, or other ligands of PXR that do not increase 3α,5α-THP biosynthesis (MPA or RU486). P4 produced similar rates of lordosis as proestrous WT, but not PXR KO, rats; 3α,5α-THP increased lordosis of both WT and PXR KO rats. The same pattern of results was observed among mice, supporting species similarities in this mechanism. Additionally, mice did not respond to MPA or RU486 as they did for P4 or 3α,5α-THP. These results corroborate findings that acute knockdown of PXR reduces lordosis of female rats. These data suggest a specific deficit in synthesis of 3α,5α-THP with PXR deletion, rather than a deficit in binding of 3α,5α-THP to PXR, a promiscuous receptor with many other positive modulators. Thus, PXR may be a novel brain target involved in 3α,5α-THP biosynthesis and action.

Disclosures: J. Rusconi: None. **C.A. Frye:** None. **A.A. Walf:** None. **C.J. Koonce:** None.

Poster

639. Neurosteroids

Location: Halls A-C

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Program#/Poster: 639.10/OO12

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant 2T32GM008471-21

NIH Grant R01DA035008

NSF Grant 1146016

Title: Palmitoylation of Caveolin-1 has a role in membrane-initiated estrogen signaling

Authors: *K. R. TONN, P. G. MERMELSTEIN;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstracts: Through membrane-localized estrogen receptors (ERs), estradiol influences a variety of behaviors including learning and memory, cognition, sensorimotor control, and female sexual receptivity. In most cases, membrane-initiated hormone actions are caused by the activation of a subpopulation of the same receptors known to carry out nuclear activity and regulate gene expression. These membrane receptors transactivate metabotropic glutamate receptors, thus initiating multiple intracellular signaling cascades. In order to traffic to the plasma membrane, ERs undergo posttranslational modification. The entire process behind these alterations remains to be elucidated, but recent studies have demonstrated that palmitoylation is essential for transforming nuclear ERs to membrane proteins. Specifically, palmitoylation of ERα and ERβ is necessary for localization and function of these receptors at the membrane. Mutation of the palmitoylation site eliminates the physical association of surface ERs with caveolin (Cav) proteins required for rapid membrane estrogen signaling. To date, it is unclear if or how the processes of palmitoylation and caveolin association interact to regulate the capability of a cell to respond to estradiol at the membrane. Preliminary findings from our laboratory indicate that caveolin must also be palmitoylated in order for estrogen receptors to be present at the membrane. To further investigate the role of caveolin palmitoylation in rapid estrogen signaling, we mutated the palmitoylation sites on the Cav1 protein and probed the effects on membrane estrogen receptors. Experiments were performed using transient transfection of HEK293 cells followed by protein analysis via membrane fractionation, co-immunoprecipitation, and Western blotting. We found that co-transfection of ERα and non-palmitoylatable Cav1 (Cav1*) resulted in decreased levels of membrane ERα. This was somewhat surprising, as previous reports demonstrate that Cav palmitoylation is not required for the trafficking of this protein to the

membrane. Contrastingly, overexpression of Cav1* did not interfere with the Cav1*-ER association. These data suggest that Cav1 must be palmitoylated for ERα to be present at the membrane, but that palmitoylation is not required for the physical association of ERα and Cav1. We are currently exploring whether or not Cav1 must be palmitoylated in order for ERα to functionally couple to mGluR1.

Disclosures: K.R. Tonn: None. **P.G. Mermelstein:** None.

Poster

639. Neurosteroids

Location: Halls A-C

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Program#/Poster: 639.11/OO13

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R21 HD070611-01

Tourette Syndrome Association

KU-Strategic Initiatives Grant

NIH Grant P20 GM103638

NIH Grant UL1 TR000001

Title: Inhibition of allopregnanolone synthesis via 5α-reductase type 2 counters sensorimotor gating deficits induced by dopamine D1 receptor agonists in mice

Authors: *L. J. MOSHER, S. C. GODAR, M. BORTOLATO;
Pharmacol. and Toxicology, Kansas Univ., Lawrence, KS

Abstracts: Schizophrenia and other neuropsychiatric disorders feature deficits of sensorimotor gating, the perceptual process that suppresses the execution of motor responses to irrelevant perceptual stimuli. The main operational measure of sensorimotor gating is afforded by the prepulse inhibition (PPI) of the acoustic startle reflex, consisting in the reduction of the startle response by a weak prestimulus immediately preceding the startle-eliciting burst. The neurochemical regulation of this endophenotype is primarily orchestrated by dopamine neurotransmission; accordingly, activation of dopamine D1 receptors induces robust PPI impairments in C57BL/6 mice, which are reversed by antipsychotic agents. We recently showed

that the PPI deficits induced by D1 receptor agonists in mice are countered by pharmacological inhibition of 5α-reductase (5αR), the enzyme catalyzing the key rate-limiting step in neurosteroid synthesis. The functions of 5αR in brain steroidogenesis are primarily served by its isoforms 1 and 2; the specific contributions of these enzymes to the antipsychotic-like properties of finasteride, however, remain unknown. To address this issue, we studied the combined effects of finasteride (50mg/kg, ip) and the potent D1 receptor agonist SKF82958 (0.3mg/kg, sc) on the PPI response in wild-type (WT), 5αR1 knockout (KO) and 5αR2KO mice. While SKF82958 significantly disrupted PPI in all genotypes, these effects were prevented by finasteride in WT and 5αR1KO, but not 5αR2KO mice. Furthermore, we found that the effects of finasteride in WT and 5αR1KO mice were prevented by systemic administration of the neurosteroid allopregnanolone (15mg/kg, ip). Taken together, these data suggest a critical role for both 5αR2 and allopregnanolone in PPI regulation and the antipsychotic-like properties of finasteride. Further studies are warranted to determine the role of allopregnanolone in schizophrenia and other neuropsychiatric disorders characterized by sensorimotor gating perturbations.

Disclosures: L.J. Mosher: None. **S.C. Godar:** None. **M. Bortolato:** None.

Poster

639. Neurosteroids

Location: Halls A-C

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Program#/Poster: 639.12/OO14

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R21 HD070611-01

Tourette Syndrome Association

KU-Strategic Initiatives Grant

NIH Grant P20 GM103638

NIH Grant UL1 TR000001

Title: Sleep deprivation-induced manic-like behaviors are modulated by 5α-reductase type 1

Authors: *R. PES[1,3], S. C. GODAR[2], L. J. MOSHER[2], M. BORTOLATO[2];
[1]pharmacology and Toxicology, [2]Pharmacol. and Toxicology, Kansas Univ., Lawrence, KS;
[3]Biomed. Sci., Univ. of Cagliari, Cagliari, Italy

Abstracts: Ample evidence shows that sleep deprivation induces manic and psychotic-related behaviors. We previously found that sleep-deprived rats exhibit hyperlocomotion and sensorimotor gating deficits in a dopamine receptor antagonist-sensitive fashion. Dopamine receptor antagonists, however, are suboptimal therapies for mania and psychosis in view of their mixed efficacy and severe side effect profile, highlighting an urgent need for novel therapeutic targets. Our group has previously shown that blockade of the 5α reductase (5AR), the key enzyme for catalyzing the rate-limiting step of neurosteroidogenesis, elicits antidopaminergic properties and attenuates sensorimotor gating deficits without accompanying extrapyramidal symptoms. Moreover, we recently found that 5AR inhibition by finasteride (FIN) countered sleep deprivation-induced sensorimotor gating deficits by blocking the production of the 5-alpha reduced neurosteroid allopregnanolone. Although finasteride blocks 5AR, this enzyme is primarily comprised of two isoforms (type 1 and 2), that differ in substrate affinity and brain distribution. In order to probe the mechanism of finasteride in mediating sleep deprivation-induced mania and psychotic-like phenomena, we investigated the effects of sleep deprivation in 5AR type 1 knockout (5AR1 KO) mice. Our preliminary data show that sleep deprivation significantly enhances exploratory behavior and risk-taking/impulsivity in WT mice. In marked contrast to WT animals, sleep deprivation did not affect either behavioral domain in 5AR1-deficient mice. Studies are currently ongoing to examine sensorimotor gating and anxiety and depression-related behaviors following sleep deprivation in our animal models. In summary, our preliminary data suggest that sleep deprivation may affect manic-related behaviors through alterations in 5AR1 activity.

Disclosures: **R. Pes:** None. **S.C. Godar:** None. **L.J. Mosher:** None. **M. Bortolato:** None.

Poster

639. Neurosteroids

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 639.13/OO15

Topic: E.03. Behavioral Neuroendocrinology

Support: Louisiana Board of Reagents Fellowship LEQSF(2012-2017)-GF-15

NSF CAREER grant IOS-1053716

Title: Chronic GPER1 antagonism improves short term memory in male and female mice

Authors: *K. J. POLLARD, C. GERMANY;
Neurosci., Tulane Univ., New Orleans, LA

Abstracts: In addition to their many roles in organizing the developing brain and activating sexually dimorphic behaviors in adults, sex hormones can also influence day to day asexual cognitive functions such as memory retention. Estrogens in particular act in the brain through an array of specific receptors including the classic nuclear estrogen receptors alpha (ERα) and beta (ERβ) as well as the extra-nuclear G-protein coupled estrogen receptor (GPER1). Using the Y maze task to assay spatial memory, it has recently been demonstrated that administration of the GPER1 selective agonist G-1 to ovarectomized female rats 48 and 24 hours prior to the learning trial can enhance retention of the consolidated memory 48 hours later (Hawley et al. 2014). However, since g-protein signaling can also alter neuronal physiology on a shorter, pre-transcriptional time scale we also hypothesized a role for GPER1 in the retention of short term memories. Therefore we chronically treated gonadectomized male and ovarectomized female mice with G-1 and the GPER1 specific antagonist G-15 and adapted the y-maze and novel objects tasks to a short term memory paradigm with an inter trial delay of only 30 minutes. Surprisingly, G-1 had no effect while the GPER1 antagonist G-15 improved short term memory retention. This data suggests that GPER1 plays opposing roles in retention of short and long term memories and likely engages both post-translational and transcriptional mechanisms to exert differential effects on differential time scales.

Disclosures: K.J. Pollard: None. **C. Germany:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.01/OO16

Topic: E.01. Neuroendocrine Processes

Title: Proliferative cells and newborn GnRH3 neurons induced by androgen in female mozambique tilapia

Authors: *Y. NARITA[1], N. OKADA[1], A. TSUTIYA[1], T. KANEKO[2], R. OHTANI-KANEKO[1];
[1]Toyo Univ., Gunma, Japan; [2]The Univ. of Tokyo, Tokyo, Japan

Abstracts: We found sexual dimorphism of gonadotropin-releasing hormone type III (GnRH3) neurons in Mozambique tilapia Oreochromis mossambicus; males have a greater number of

GnRH3 neurons in the terminal nerve than females (Kuramochi et al., 2011). Treatment with androgen (11-ketotestosterone (11-KT) or methyltestosterone (MT)) in females increased the number of GnRH3 neurons to a level similar to that in males, although the mechanism remains unsolved. Since adult neurogenesis has recently been shown to occur in various vertebrate species, we aimed in the present study to examine whether adult neurogenesis is involved in the androgen-induced increase of GnRH3 neurons in female Mozambique tilapia. We first examined whether androgen increased proliferative cells in adult females by detecting cells with the expression of a proliferation-related marker, proliferating cell nuclear antigen (PCNA). Next, we studied the fate of newly generated cells by double labeling with antibodies against 5-bromo-2'-deoxyuridine (BrdU) and one of markers of interest including GnRH3. After adult females were treated with 11-KT, PCNA-positive cells were increased in various regions of the brain, compared to control females injected with oil. We further studied Hu-positive immature neurons and GnRH3-positive cells in combination with BrdU labeling in the brain at the level of the terminal nerve. BrdU- and Hu-positive cells were increased around the midline/ventricular zone, compared to control females. BrdU- and GnRH3-positive neurons were also increased by 11-KT treatment. These results revealed that androgen treatment increased proliferative cells and newborn GnRH3 neurons in females, indicating the androgen-induced increase of GnRH3 neurons in females is mediated partly through adult neurogenesis. Reference: Kuramochi et al., Sexual dimorphism of gonadotropin-releasing hormone type-III (GnRH3) neurons and hormonal sex reversal of male reproductive behavior in Mozambique tilapia. Zoological Science 28 (2011): 733-739.

Disclosures: Y. Narita: None. **N. Okada:** None. **A. Tsutiya:** None. **T. Kaneko:** None. **R. Ohtani-Kaneko:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.02/OO17

Topic: E.03. Behavioral Neuroendocrinology

Support: FDCT 012/2012/A1

Title: Dissociation of androgen effects in different tissues by regulation of androgen receptor genes in a polymorphic fish

Authors: *D. GONÇALVES[1], M. BARUZZO[2], C. POLASTRO[2], R. F. OLIVEIRA[2,3];
[1]USJ, Univ. of St. Joseph, Macau, Macao; [2]ISPA-IU, Lisbon, Portugal; [3]Inst. Gulbenkian de Ciência, Oeiras, Portugal

Abstracts: Across vertebrates, androgens exert a wide range of actions in multiple tissues, including stimulating the development of male secondary sexual characters and promoting sperm development and maturation. Variation in circulating levels of androgens helps to coordinate changes in male-related traits in multiple tissues, including the gonads, the brain and secondary sexual characters. However, in some species, the development of a functional male reproductive system is dissociated from the development of masculine behavioral and morphological traits, either permanently or during certain developmental periods. One possible mechanism allowing for this dissociation is variation in tissue sensitivity to circulating androgens, namely through changes in the expression of androgen receptors genes. We tested this hypothesis in a fish species, the peacock blenny Salaria pavo. Large nesting males of this species have fully developed male morphological traits and display courtship displays towards females to try to attract them towards their nests. However, a second class of smaller males reproduces by mimicking the female morphology and behavior in order to approach the nests of the larger males and parasitically fertilize some of the eggs inside the nest. These males have fully functional testes to allow parasitic fertilization of eggs but do not develop the secondary sexual characters typical of nesting males nor exhibit courtship displays towards females during the parasitic phase. Between the first and second breeding season, parasitic males irreversibly transition to the nesting male morphotype. To understand if variation in androgen-tissue sensitivity could contribute to the apparent dissociation between the effects of androgens in the testes and in other tissues in parasitic males, the expression levels of the two androgen receptors described for the species, ARalpha and ARbeta, were measured by qPCR in the testes, brain macroareas and secondary sexual characters. As predicted, parasitic males had lower androgen receptor expression levels in the brain and in male secondary characters, but not in the testes, when compared with nesting males. The results agree with the hypothesis that, in S. pavo, the observed dissociation in the effects of androgens across tissues is achieved by a differential regulation in the expression of androgen receptor genes.

Disclosures: D. gonçalves: None. **M. Baruzzo:** None. **C. Polastro:** None. **R.F. Oliveira:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.03/OO18

Topic: E.01. Neuroendocrine Processes

Support: NIH COBRE 5P20GM103653-02

Title: Comparative expression of GPR30 in the brains of goldfish and zebrafish

Authors: *J. WHITE, T. ADEBANJO, T. SZABO-MAAS;
Delaware State Univ., Dover, DE

Abstracts: Although receptors for steroid hormones are known classically to be nuclear receptors whose major function is regulation of gene expression, recent studies have shown that estrogen receptors (ERs) can be expressed outside of the nucleus in the plasma membrane as well as in the membrane of internal organelles, including the Golgi apparatus and endoplasmic reticulum. Localization of ERs to the membrane strategically positions them to rapidly transduce effects of estrogen. Since expression of ER protein is regulated in a complex manner at multiple time points, including during transcription, translation or splicing of protein isoforms, most studies examining ER expression have focused on ER mRNA. Therefore, little is known about expression and distribution of ER protein in circuits other than a few intensely studied mammalian systems, like the hippocampus. Recent studies have characterized novel estrogen receptors that are not 'classic' nuclear receptors (ERα and ERβ) but are coupled to G proteins, including G protein-coupled receptor 30 (GPR30). To date, few studies have examined expression of GPR30 in the nervous system and none have looked at expression in non-mammalian model systems. We therefore examined expression of GPR30 protein in two well-characterized teleost model systems, the goldfish, Carassius auratus and the zebrafish, Danio rerio. We found that GPR30 is 1) expressed in most brain regions albeit at variable intensities, 2) is highly regulated according to sex and breeding state and 3) is more strongly expressed in peripheral nerves vs. central brain tracts. In the central nervous system, GPR30 labeling appears as discrete puncta that colocalize with neuronal markers, while in the peripheral nervous system, GPR30 appears primarily on myelin. Since this is the first study examining expression of GPR30 protein in a non-mammalian model system, we believe this work will clarify evolutionarily conserved roles of this novel receptor for estrogen.

Disclosures: J. White: None. **T. Adebanjo:** None. **T. Szabo-Maas:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.04/OO19

Topic: E.01. Neuroendocrine Processes

Support: NIH COBRE 5P20GM103653-02

Title: Multiple distinct estrogen receptor-alpha protein isoforms in the central nervous system of adult teleost

Authors: *T. T. ADEBANJO, J. WHITE, T. SZABO-MAAS;
Delaware State Univ., Dover, DE

Abstracts: Estrogens exert multiple effects on the brain including neuroprotection, and improvements in learning and memory; however, the mechanisms through which they exert their effects are not well understood. Estrogenic effects are transduced via multiple receptor subtypes and isoforms, with estrogen receptor alpha (ERα) being the best characterized. ERα protein expression is known to be regulated at multiple levels, including: 1) during transcription, 2) during translation, 3) by truncation of the full length protein, 4) by its ligand, estradiol and 5) via interactions with other receptor isoforms. As a result, most studies examine distribution and expression of ERα mRNA, while expression of the protein remains unknown. In this study, we examined ERα protein expression in two teleost model systems: 1) the goldfish, Carassius auratus, which exhibits enhanced production of brain aromatase and alterations in sex hormone levels that occur annually in the spring, and 2) the zebrafish, Danio rerio, which does not possess elevated levels of brain aromatase and is an opportunistic breeder that can spawn every 1-2 days. We hypothesized that expression of ERα protein would positively correlate with breeding cycle and estradiol levels for both zebrafish and goldfish. While our results for the full-length ERα dimers and truncated receptor isoforms was more complex. Our study highlights the complexity of steroid hormone receptor expression and its regulation and the importance of examining the mechanism through which estradiol acts at the level of the protein.

Disclosures: T.T. Adebanjo: None. **J. White:** None. **T. Szabo-Maas:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.05/OO20

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant 0849102

Title: Non-nuclear distribution patterns of estrogen receptors in the brains of goldfish

Authors: R. R. THOMPSON[1], *L. A. MANGIAMELE[1,3], T. NICHOLSON[2], D. MICHAUD[2], M. CHEN[2], J. GOMEZ[2], D. DARDEN[2];
[1]Psychology, [2]Bowdoin Col., Brunswick, ME; [3]Dept of Biol. Sci., Smith Col., Northampton, MA

Abstracts: Steroid hormones, most notably estradiol (E2), have rapid, non-genomic effects on physiology and behavior in vertebrates. In order to learn more about the receptors that mediate those effects, we used immunohistochemistry to localize different estrogen receptors (ERs) in the brains of goldfish, particularly those with non-nuclear distribution patterns. GPR30, a membrane-bound G protein-coupled estrogen receptor, extensively co-localized with isotocin in the soma and fibers in the preoptic area. ERbeta immunoreactivity was observed in numerous regions, including those associated with visual processing (the retina and optic tectum), as well as most nodes within the social brain network. There was evidence for nuclear and non-nuclear staining patterns; non-nuclear staining was primarily observed in processes, some of which co-localized with tryrosine hydroxylase (retina), and some of which co-localized with GFAP (telencephalon). Western blots with the ERbeta antibody identified a 62 kD band, the same approximate size as ERbeta that has been identified in other species, in both cytosolic and plasma membrane preparations, suggesting that both cell membrane and nuclear receptor proteins result from expression of a single transcript. We will examine ERalpha distribution patterns in the future. Together, these results suggest that GPR30 likely influences processes associated with isotocin in goldfish, whereas ERbeta may mediate some of E2's rapid effects on sexual behaviors, particularly its ability to modulate responses to female visual stimuli.

Disclosures: R.R. Thompson: None. **T. Nicholson:** None. **D. Michaud:** None. **M. Chen:** None. **J. Gomez:** None. **D. Darden:** None. **L.A. Mangiamele:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.06/OO21

Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR postdoctoral fellowship

MSFHR postdoctoral fellowship

CIHR operating grant 67087

Title: Rapid effects of aggressive interactions on DHEA, testosterone, and estradiol levels in the male song sparrow brain: A seasonal comparison

Authors: *S. A. HEIMOVICS[1], N. H. PRIOR[2], C. Q. MA[2], K. K. SOMA[2];
[1]Univ. of St. Thomas, Saint Paul, MN; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstracts: Across vertebrates, aggression is robustly expressed during the breeding season, when circulating testosterone (T) levels are elevated. T activates aggression during the breeding season either directly or after its local conversion into 17beta-estradiol (E_2) in the brain. In some species, such as the song sparrow, aggressive behavior is also expressed at high levels during the non-breeding season, when circulating T levels are non-detectable. During the non-breeding season, the androgen precursor dehydroepiandrosterone (DHEA) is metabolized within the brain into T and/or E_2 to promote aggression. DHEA can be synthesized in the adrenal glands or in the brain itself. In the present study, we used captive male song sparrows to test the hypothesis that acute agonistic interactions during the non-breeding season, but not during the breeding season, would alter steroid levels in the brain. Non-breeding and breeding subjects were exposed to either a simulated territorial intrusion (STI) or an empty cage (CON) for only 5 min. Immediately afterwards, the brain was rapidly collected and flash frozen. The Palkovits punch technique was used to microdissect specific regions implicated in social behavior. Solid phase extraction followed by radioimmunoassay was used to quantify DHEA, T, and E_2. Overall, brain levels of DHEA, T, and E_2 were higher in brain tissue than in plasma. Local T and E_2 levels in the preoptic area (POA), hypothalamus (Hy), and nucleus taeniae of the amygdala (TnA) were significantly higher in the breeding season than the non-breeding season and were not affected by the STI. As expected, the STI rapidly increased DHEA levels in the POA in the non-breeding season only, suggesting that the POA synthesizes DHEA in response to social cues. Surprisingly, achieving social dominance during the STI was associated with decreased DHEA levels in Hy in both seasons. In addition, social dominance was associated with decreased DHEA levels in TnA in the breeding season only. Taken together, these data suggest that DHEA is involved in the neuroendocrine regulation of aggression throughout the year, but the specific role of DHEA in aggression is season, social status, and brain region-specific.

Disclosures: S.A. Heimovics: None. **N.H. Prior:** None. **C.Q. Ma:** None. **K.K. Soma:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

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Program#/Poster: 640.07/OO22

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH/NINDS RO1 Grant 35467

IAP Grant SSTC PAI P7/17

Title: Evidence for fast, non-genomic-like actions of estrogens in the regulation of birdsong

Authors: *B. A. ALWARD[1], T. T. CHAN[1], J. BALTHAZART[2], C. CORNIL[2], G. F. BALL[1]; [1]Psychological & Brain Sci., The Johns Hopkins Univ., Baltimore, MD; [2]GIGA Neurosciences, Univ. de Liege, Liege, Belgium

Abstracts: Steroid hormones cause long-term changes in behavior by acting at the level of the genome. A plethora of recent evidence, however, has shown that steroids can also have rapid, short-term effects on behavior by acting in a non-genomic manner. Song behavior is well known to be regulated by steroid hormones such as testosterone. Castrated canaries (*Serinus canaria*) substantially reduce song output and treatment with exogenous testosterone restores singing after approximately three days. Moreover, in many songbirds treatment with an inhibitor of aromatase, the enzyme that converts testosterone into estradiol, substantially reduces song output. Interestingly, recent studies in male Japanese quail (*Coturnix japonica*) indicate that brain-derived estradiol acts on a fast time scale (within minutes) to regulate sexual behavior and these effects dissipate within 1-2 hours, suggesting these effects are non-genomic. Here, we investigated the fast-acting, presumably non-genomic influence of estrogens in the regulation of song in male canaries. Male canaries begin singing within approximately 15 minutes after lights on. Five minutes after lights on, each bird was injected intraperitoneally with either fadrozole (30mg/kg), a potent aromatase inhibitor, or its vehicle. Using a within-subjects design, they received the opposite treatment three days later. Results obtained so far indicate that estrogen depletion decreases numbers of songs as well as their loudness, pitch and stereotypy in a rapid manner (in most birds within 45 minutes to 3-4 hours) indicating that estrogens exert an acute control of these aspects of behavior. Importantly, song rate and structure were restored to normal levels by the next day. Hence, estrogens synthesized from testosterone in male canaries regulate song on a rapid time scale in a presumably non-genomic fashion. As both motivational and acoustic measures of song were affected by aromatase inhibition, it is likely that distinct brain regions (e.g., the medial preoptic nucleus and the auditory telencephalon, both of which have dense aromatase expression) play a role in the fast regulation of song by estrogens.

Disclosures: B.A. Alward: None. **T.T. Chan:** None. **J. Balthazart:** None. **C. Cornil:** None. **G.F. Ball:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.08/OO23

Topic: E.01. Neuroendocrine Processes

Support: ROI NS35467

Title: Investigating possible intraspecific variation in testosterone-induced neuroplasticity by comparing two canary breeds

Authors: *F. N. MADISON, B. A. ALWARD, G. F. BALL;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstracts: Our previous work has shown that male canaries of the American Singer (AM) strain did not exhibit photoperiodic responses characteristic of wild-type canaries or of other strains such as the Border (BDR) canary. AMs do not regress their gonads when exposed to long days and exhibit photorefractoriness. We also found that castrated males responded to high exogenous testosterone (T) in a variable manner. For example, various doses of T did not consistently induce an increase in the volume of the song nucleus HVC. These findings suggest that domestication of the AM canary, via human selection, perhaps to 'lock in' a certain level of song quality and quantity, has led to a decrease in neuroplasticity associated with changes in photoperiod and hormonal action. In this study, we investigate possible strain differences in response to T on song production and HVC volume in adult male AM and BDR canaries. 15 male AM and 15 male BDR canaries were housed on 8L:16D (light:dark) for at least eight weeks, rendering them photosensitive and were placed into groups of intact control, castrated control, and castrated male implanted subcutaneously with one Silastic implant (12mm) filled with crystalline T. Immediately after implantation, birds were individually housed in sound-attenuated chambers on the 8L:16D light cycle for 3 weeks. After 3 weeks of T treatment, brains were collected. We found that HVC volume was larger in response to T treatment in BDR than in AM. Preliminary data suggest T stimulates castrated AM to sing sooner (1 day post-T treatment) than castrated BDR (3 days post-T treatment). Interestingly, castrated AM treated with T sang as much as their intact counterparts throughout the experiment while castrated BDR treated with T sang more than their intact counterparts from day 9 to day 17 and were indistinguishable from both groups of AM that were exposed to T. Castrated BDR treated with T sang songs with lower entropy (noise or variance) than intact BDR as well as both groups of AM exposed to T. Importantly, castrated AM treated with T sang songs with entropy that was indistinguishable from their intact counterparts. All groups showed a linear increase in the

energy (loudness or amplitude) of their songs. These results suggest T differentially stimulates song features in these two strains of canaries. In AM, song is stimulated relatively quickly, but HVC volume is smaller as compared to male BDR. These findings are consistent with the hypothesis that high rates of singing have been selected for in AM and can occur in the absence of marked T induced neuroplasticity.

Disclosures: **F.N. Madison:** None. **B.A. Alward:** None. **G.F. Ball:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.09/OO24

Topic: E.01. Neuroendocrine Processes

Support: NS 042767

NS 080585

Title: Estradiol, synthesized by reactive glia, is a potent anti-inflammatory in the injured vertebrate brain

Authors: *A. L. PEDERSEN, L. H. NELSON, C. J. SALDANHA;
Biology, Psychology, & Ctr. for Behavioral Neurosci., The American Univ., Washington, DC

Abstracts: Neuroinflammation following traumatic brain injury (TBI) may have detrimental and beneficial effects that likely differ between the acute and chronic periods post-trauma. In birds and mammals, traumatic brain injury increases the expression of cytokines in microglia and aromatase (estrogen synthase) in astroglia. In the songbird, TBI-induced synthesis of estrogens by glial aromatization is neuroprotective as aromatase inhibition and replacement with estradiol (E2) exacerbates and mitigates the extent of damage and apoptosis, respectively. The effect of glial estrogens on inflammation, however, remains unstudied. We hypothesized that induced astrocytic aromatization may affect neuroinflammation following TBI, via the synthesis of neural E2 around the site of damage. In three separate experiments on adult zebra finches (Taeniopygia guttata) of both sexes we tested the influence of (a) mechanical TBI, (b) inhibition of induced aromatase expression, and (c) inhibition of induced aromatase with central E2 replacement, on the expression of the pro-inflammatory cytokines TNFα, IL-1β, and IL-6, and aromatase. At 2hr post-injury, in both sexes, TBI increased ($p<0.05$), and tended to elevate, TNFα and IL-1β

respectively. At 24hr post-injury, also in both sexes, cytokines appeared to have returned to baseline, but aromatase is robustly elevated in the lobe that sustained TBI ($p<0.01$). Pharmacological inhibition of induced aromatization resulted in persistent neuroinflammation, as administration of fadrozole increased IL-1β (in females ($p=0.0007$)) and TNFα (in males ($p=0.0003$)) 24hr following MBI. This prolonged neuroinflammation following aromatase inhibition appears to be due to a failure to synthesize E2 locally, since E2 replacement lowered TNFα and IL-1β relative to fadrozole alone ($p<0.01$). IL-6 was not affected by TBI, aromatase inhibition or E2 replacement in either sex. These data suggest that astrocytic E2 synthesis following TBI is a potent and inducible anti-inflammatory signal, with specific modulation of discrete cytokine signaling. Induced neural provision of E2 following damage and compromise of central pathways may protect the brain from the deleterious effects of prolonged neuroinflammation.

Disclosures: **A.L. Pedersen:** None. **L.H. Nelson:** None. **C.J. Saldanha:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.10/OO25

Topic: E.01. Neuroendocrine Processes

Support: CRDF Grant

Title: Cholinergic regulation of aromatase in brain

Authors: ***J. LI**, D. NELSON, R. GIBBS;
Univ. of Pittsburgh, Sch. of Pharm., Univ. of Pittsburgh Sch. of Pharm., Pittsburgh, PA

Abstracts: Our goal is to understand mechanisms by which estrogens can influence brain function and cognition. Estrogens have been shown to influence neuronal plasticity and cognitive performance. Recent studies suggest that, in some cases, local estrogen synthesis can have a greater impact on neuronal survival and plasticity than systemic estrogen administration. Cholinergic projections also have a significant impact on neuronal plasticity in the brain, and recent studies demonstrate critical links between effects of estrogens and effects mediated by cholinergic inputs. In this project we are investigating whether aromatase expression and activity in specific regions of the adult brain are regulated by cholinergic activity. In one experiment, ovariectomized (OVX) rats were treated with the cholinesterase inhibitors donepezil (3 mg/Kg)

or galantamine (5 mg/Kg) daily for one week prior to tissue collection. In a second experiment, OVX rats received intraseptal infusions of 192IgG-saporin (SAP) to selectively destroy cholinergic inputs to the hippocampus. Tissues were collected two weeks following the infusions. Different groups of rats were used to evaluate effects on aromatase mRNA and aromatase activity. Effects on aromatase mRNA were evaluated using qRT-PCR. Effects on aromatase activity were evaluated using a novel microsomal assay in which brain tissue microsomes were extracted and activity was measured *in vitro* by measuring conversion of testosterone to estradiol. Results show an increase in aromatase mRNA in the preoptic area following treatment with galantamine, but no effect in the hippocampus, frontal cortex, or amygdala. Galantamine also produced an increase in aromatase activity in the amygdala, but no significant effect in other brain regions. Donepezil had no significant effects on either aromatase mRNA or activity. Effects of the cholinergic lesions are still being evaluated; however, preliminary results suggest no significant effect on relative levels of aromatase mRNA in the hippocampus. These results indicate that cholinergic manipulations can affect aromatase expression and activity in specific regions of the brain such as the preoptic area and amygdala, with little or no effect in the hippocampus and frontal cortex. This could have important implications for the effects of cholinergic and anticholinergic medications on local estrogen production in the brain.

Disclosures: **J. Li:** None. **D. Nelson:** None. **R. Gibbs:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.11/OO26

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant DA035008

NSF Grant IOS-114616

NSF Grant No. 00006595

Title: Estradiol recruits the cannabinoid system to enhance psychostimulant locomotor sensitization in female rats

Authors: *B. PETERSON, P. G. MERMELSTEIN, R. L. MEISEL;
Neurosci. Dept, Univ. of Minnesota, Minneapolis, MN

Abstracts: Estradiol enhances psychostimulant responses in females, however the neurobiological mechanisms underlying this effect are largely unknown. Recently our lab demonstrated that in ovariectomized female rats, estradiol treatment enhances cocaine-induced locomotor sensitization through an mGluR5-dependent mechanism. These data are in agreement with our model that within the female striatum, estradiol activation of membrane-localized ERα leads to transactivation of mGluR5. Interestingly, previous work has shown that both estradiol treatment and mGluR5 can each independently mobilize endogenous cannabinoids (endoCBs) within the nervous system. Given that the endoCB system is emerging as key mediator of drug related behaviors, we hypothesized that estradiol recruits the endoCB system to enhance psychostimulant responses in female rats via mGluR5. Consistent with previous studies, we found that five days of experimenter-administered cocaine (15mg/kg) induced locomotor sensitization in estradiol-, but not in oil-treated, ovariectomized animals. Consistent with our hypothesis, this locomotor sensitization in estradiol-treated females was attenuated by pretreatment with a cannabinoid receptor type 1 (CB1r) inverse agonist, AM251 (1mg/kg). Unexpectedly, cocaine-induced locomotor sensitization was observed in ovariectomized females that received AM251 treatment in the absence of estradiol. We are currently investigating whether structural plasticity within the striatum following these pharmacological treatments will explain these behavioral data.

Disclosures: B. Peterson: None. **P.G. Mermelstein:** None. **R.L. Meisel:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

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Program#/Poster: 640.12/OO27

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant DA035008

NIH Grant DA035008-S1

Title: Estradiol facilitation of cocaine-induced behaviors in female rats requires activation of mGluR5

Authors: *L. A. MARTINEZ, B. M. PETERSON, R. L. MEISEL, P. G. MERMELSTEIN; Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstracts: In comparison to men, women exhibit enhanced responsiveness to the stimulating and addictive properties of cocaine. A growing body of evidence implicates the steroid hormone estradiol in mediating this sex difference, yet the mechanisms underlying estradiol enhancement of behavioral responses to cocaine specifically in females are not known. Recently, we have found that estrogen receptor alpha (ERα) functionally couples with the metabotropic glutamate receptor 5 (mGluR5) to mediate the effects of estradiol on both cellular activation as well as dendritic spine plasticity in brain regions involved in the behavioral responses to cocaine. Thus, we sought to determine whether mGluR5 activation is required for the facilitative effects of estradiol in females on cocaine-induced behaviors (i.e., locomotor sensitization and self administration). We tested this hypothesis through a series of experiments. In the first experiment, ovariectomized (OVX) female rats were tested for locomotor activity on the first and fifth days of daily systemic injections of cocaine. For the two days prior to each locomotor test, females were injected with the mGluR5 antagonist MPEP (or vehicle) and estradiol (or oil). In the second experiment, OVX females were administered estradiol on a 2 days on, 2 days off schedule during sucrose pellet self-administration training (FR1 schedule). Females were then removed from estradiol treatment, implanted with IV catheters, and trained to self-administer cocaine on an FR1 schedule. At the completion of cocaine training, females were injected with MPEP (or vehicle) and estradiol (or oil) on a 2 days on, 2 days off schedule throughout 21 days of extended access to cocaine self administration (6 hrs per day, FR1 schedule). MPEP treatment blocked the facilitative effects of estradiol on cocaine-induced locomotor sensitization, without affecting acute locomotor responses to cocaine or the inhibitory actions of estradiol on weight gain. The effects of MPEP with estradiol on cocaine self administration are presently under investigation. Thus far, these data indicate that mGluR5 activation is critical for the actions of estradiol on cocaine-induced behaviors in females, and suggest novel therapeutic targets for treating psychostimulant addiction in women.

Disclosures: L.A. Martinez: None. **B.M. Peterson:** None. **R.L. Meisel:** None. **P.G. Mermelstein:** None.

Poster

640. Steroids and Plasticity

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Program#/Poster: 640.13/OO28

Topic: E.01. Neuroendocrine Processes

Support: NIH R01DA035008

NIH National Institute of Drug Abuse T32DA007234

Title: Interactions between estradiol and group 1 mGluR signaling influence dendritic spine density in the female rat nucleus accumbens

Authors: *K. GROSS, R. L. MEISEL, P. G. MERMELSTEIN;
Univ. of Minnesota, Minneapolis, MN

Abstracts: Estrogen receptors (ERs) can be localized to the cell membrane where their activity can result in alterations in neurotransmission, neural structure, and behavior. These effects are often mediated by group 1 mGluRs associated with surface ERs. Previous research in our lab has found that 17β-estradiol (17βE) influences spine density in the female rat nucleus accumbens (NAc). In the NAc core (NAcC), 17βE decreases dendritic spine density in an mGluR5 dependent mechanism. In contrast, in the NAc shell (NAcSh), 17βE increases spines via mGluR1. Given that these contrasting effects are a result of 17βE-mediated activation of disparate group 1 mGluRs, we wanted to determine if independent activation of individual group 1 mGluRs would bidirectionally change dendritic spine density in the NAc. Ovariectomized female rats were given a systemic injection of an mGluR5 positive allosteric modulator (PAM), CDPPB, at either 5 or 10 mg/kg and sacrificed 24 hours later. Neurons in the NAc were ballistically labeled with DiI, and spine densities were determined. At both doses, CDPPB decreased dendritic spine density throughout the NAc, paralleling the effect of 17βE in the NAcC. Experiments are currently underway to determine if an mGluR1 PAM will increase spine density throughout the NAc, similar to the increase within the NAcSh after 17βE administration. These data would suggest that within the NAc different group 1 mGluRs are functionally coupled to opposing signaling pathways that result in bidirectional effects on dendritic spine density. Furthermore, 17βE produces opposing effects on spine density within subregions of the NAc by activating distinct group 1 mGluRs within the NAcC and NAcSh. The ability of 17βE to differentially regulate plasticity within subregions of the NAc may have consequences for the functional output of this brain region.

Disclosures: K. Gross: None. **P.G. Mermelstein:** None. **R.L. Meisel:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.14/OO29

Topic: E.01. Neuroendocrine Processes

Support: NIH INBRE Grant 2P20RR016462

Title: Effects of testosterone on stages of neural development in the dentate gyrus of adult male rats

Authors: *M. D. SPRITZER[1], K. M. K. CALHOUN[1], E. A. ROY[1], Z. E. SCHNEIDER-LYNCH[1], J. M. BARKER[2], L. A. M. GALEA[2];
[1]Dept. of Biol. and Program in Neurosci., Middlebury Col., Middlebury, VT; [2]Dept. of Psychology and Grad. Program in Neurosci., Univ. of British Columbia, Vancouver, BC, Canada

Abstracts: Within the mammalian brain, neurogenesis occurs throughout adulthood along the subgranular zone of the dentate gyrus. We previously showed that castrating a male rat causes a decrease in hippocampal neurogenesis and injections with testosterone restore normal neurogenesis levels. However, some studies have found no effects of testosterone replacement on adult neurogenesis. Discrepancies among studies may be partly due to the timing of hormone injections relative to the stages of cell development. Another key variable is testosterone dose, as we have found that some doses of testosterone do not consistently improve neurogenesis. Therefore, the present experiment tested the effects of two testosterone doses given during three different stages of neural development. Adult male Sprague Dawley rats (N=8/group) were bilaterally castrated and give a one week recovery period. All subjects were given a single injection of bromodeoxyuridine (BrdU; 200 mg/kg) on the first day of the experiment to label actively dividing cells, and animals were euthanized sixteen days later to assess BrdU labeling. Subjects were assigned to three groups based on injection doses of testosterone propionate: oil-injected control group, 0.250 mg/rat, or 0.500 mg/rat. All subjects received five consecutive days of injections during one of three stages of neural development: Days 1-5 (cell proliferation and migration), Days 6-10 (neurite growth), or Days 11-15 (neuron maturation). Rats were transcardially perfused, brains sectioned (40 μm), and peroxidase immunohistochemistry was used to visualize BrdU-labeled cells. Light microscopy (1000x) was used to count all labeled cells in every 10th section throughout the dentate gyrus. Testosterone injections had a significant effect on the number of BrdU-labeled cells ($P = 0.022$), with the 0.500 mg/rat dose causing a significant increase in the number of BrdU-labeled cells compared to the 0.250 mg/rat dose and the control group. There was no difference in the number of BrdU-labeled cells between the 0.250 mg/rat group and the control group. The timing of injections had no significant effect on BrdU labeling. The effects of testosterone did seem to be strongest for the later stage (11-15 days) of neural development, but there was no statistically significant interaction between time and dose. Additionally, the effect of dose was found to be stronger for the ventral portion of the dentate gyrus ($P = 0.024$) than for the dorsal portion ($P = 0.13$). These results add to past

evidence that high physiological doses of testosterone increase adult neurogenesis, and suggest that only a short-term surge (5 days) of testosterone is necessary to see this effect.

Disclosures: M.D. Spritzer: None. **K.M.K. Calhoun:** None. **E.A. Roy:** None. **Z.E. Schneider-Lynch:** None. **J.M. Barker:** None. **L.A.M. Galea:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.15/OO30

Topic: E.01. Neuroendocrine Processes

Support: P01NS045260-01

R01NS057128

Title: GPR30 activation stimulates mTOR-dependent protein synthesis in hippocampal slices through BDNF release

Authors: *V. BRIZ, M. BAUDRY;
Western Univ. of Hlth. Sci., Pomona, CA

Abstracts: Estrogen is an important modulator of hippocampal synaptic plasticity through its rapid action on membrane-associated receptors. Stimulation of mammalian target of rapamycin (mTOR)-dependent protein synthesis is a key event required for consolidation of hippocampal long-term potentiation and hippocampus-dependent learning and memory. We recently showed that estradiol stimulates mTOR phosphorylation in hippocampal neurons through activation of G-protein coupled receptor 30 (GPR30), but the underlying molecular mechanisms are not yet fully understood. In the present work, we used acute rat hippocampal slices to analyze the mechanisms underlying the rapid changes in mTOR signaling elicited by estradiol and the GPR30 agonist G1. Both estradiol- and G1-induced mTOR phosphorylation were blocked by the novel and specific tropomyosin related kinase B (TrkB) receptor antagonist ANA-12. Similarly, estradiol- and G1-induced phosphatase and tensin homolog (PTEN) degradation, Akt activation and increase in calcium/calmodulin-dependent protein kinase II levels were prevented by ANA-12. Furthermore, the effects of G1 on PTEN/Akt/mTOR signaling pathway were also abolished by pre-incubating slices with recombinant TrkB-Fc chimera, suggesting that GPR30-mediated stimulation of mTOR signaling involves brain-derived neurotrophic factor (BDNF) release.

Immunohistochemistry studies revealed that G1 rapidly enhanced dendritic Arc protein synthesis in the CA3 area of hippocampus, an effect that was suppressed both by ANA-12 and TrkB-Fc. Overall, the present study indicates that estrogen exerts some of its effects on synaptic function and plasticity through the release of BDNF as a result of GPR30 activation.

Disclosures: V. Briz: None. **M. Baudry:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.16/OO31

Topic: E.01. Neuroendocrine Processes

Title: Right or left hemiovariectomy effects on dendritic length of CA1 and CA3 neurons of ventral hippocampus

Authors: D. A. BRAVO[1], *A. B. SILVA[2];
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Abstracts: Hemiovariectomy is an experimental paradigm recently used to study neural connections between the ovaries and the central nervous system. Due to this procedure it has been suggested that sensorial innervation has an important role in the biosynthesis of steroids which is different between ovaries. The aim of the present study was to determine the effects of hemiovariectomy (Hovx) on the morphology of CA1 and CA3 pyramidal neurons of ventral hippocampus, regarding the *in situ* ovary and the cerebral hemisphere analyzed. A total of 48 female rats of CIIZ-V strain with at least three regular estrous cycle monitored by cytological examination of daily vaginal smears were used. All rats were housed in light-dark cycle (12:12 h) with access to food and water ad libitum. We performed the surgery during the estrus phase of the estrous cycle in all the rats. Twenty four animals were anesthetized, laparotomized and underwent hemiovariectomy (Right Hovx= 12, Left Hovx=12), the remaining rats were used with Sham surgical treatment (Right Sham=12, Left Sham= 12) as control group. After surgery, the estrous cycle was monitored and after presenting three consecutive 4-d cycles, the animals were sacrificed. The brains were collected and processed by Golgi-Cox method. Twenty neurons of CA1 and twenty of CA3 regions (Right Hemisphere = 10, Left Hemisphere = 10) were drawn with camera lucida. Using Sholl analysis the total dendritic length was calculated. Also dendritic spine density was determinated. In Left Hovx animals a decrease in basolateral and apical

dendritic length of CA1 neurons in left hippocampus (LH) was observed, while CA1 neurons of Right Hovx rats showed a decrease in the basolateral dendritic length in LH. Neither Left Hovx nor Right Hovx group showed changes on spine density of CA1 neurons. On the other hand, Left Hovx rats showed an increase in basolateral total dendritic length of CA3 neurons in LH. In Right Hovx animal a decrease in basolateral dendritic length of CA3 neurons in both LH and right hippocampus (RH) was observed. Spine density showed an increase in apical trees of CA3 neurons in LH. It has been described that gonadal steroids have an important activity in pyramidal cells of hippocampus, since a depletion of estradiol after an ovariectomy promotes a decrease in spine density mainly in CA1 pyramidal neurons. These results support previous data about the different neural innervation between ovaries and the central nervous system and suggest that left hemiovariectomy produces neural changes mainly in basolateral and apical tress of CA1 neurons in LH; meanwhile right hemiovariectomy affects basolateral tress of CA3 neurons.

Disclosures: D.A. Bravo: None. **A.B. Silva:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.17/OO32

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant MH095248

Title: Evidence for an acute estradiol-induced increase in postsynaptic sensitivity to glutamate at synapses in the hippocampus

Authors: *J. G. OBERLANDER, C. S. WOOLLEY;
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Abstracts: Estradiol (E2) is well-known to acutely potentiate excitatory synaptic transmission in the hippocampus of both males and females. In ovariectomized E2-primed adult female rats, paired-pulse ratio decreases in parallel with E2-induced synaptic potentiation, suggesting that potentiation occurs at least partly through increased glutamate release probability (Smejkalova and Woolley, 2010). Whether E2 also acutely increases postsynaptic sensitivity to glutamate is unknown. To investigate this, we used 2-photon laser uncaging of glutamate at individual CA1 pyramidal cell dendritic spines combined with whole-cell voltage clamp recording before,

during, and after 10 minute application of 100 nM E2 to hippocampal slices. We tested the effects of E2 in 5 groups of rats: juvenile females and adult males and females that were either gonadectomized or gonadally intact. We found that E2 significantly increased the amplitude of uncaging-evoked EPSCs (uEPSCs) in approximately 20% of spines (t test, within spine) with similar prevalence in all groups; no spines showed a significant decrease in uEPSC amplitude. The magnitude of E2-induced uEPSC amplitude potentiation ranged from 25-65%, and spines on the same cell (in some cases even the same dendritic branch) typically showed differential responsiveness to E2. These results indicate that E2 acutely potentiates the postsynaptic response to glutamate at a subset of synapses. Between uncaging pulses, we also recorded miniature EPSCs (mEPSCs) and measured their amplitude and frequency in each cell. Similar to the E2-induced increase in uEPSC amplitude, E2 also increased the amplitude of mEPSCs, in approximately 31% of cells, by 30-80%; this E2-related increase occurred with a similar prevalence in cells from all groups. In fewer than 5% of cells, and all from females, mEPSC amplitude decreased after E2, by 30% or less. In addition, and consistent with our previous results, the frequency of mEPSCs, which is an indirect measure of presynaptic glutamate release probability, was significantly increased after E2. E2 increased mEPSC frequency in more than 50% of cells, by 25-100%. As with EPSC amplitude increases, the E2-induced increase in mEPSC frequency occurred in all groups with similar prevalence. Importantly, increases in mEPSC amplitude and frequency after E2 rarely occurred in the same cells, fewer than 10%, indicating that higher mEPSC frequency measured after E2 is not likely to be an artifact of larger mEPSCs. Together, these results demonstrate that E2 acutely potentiates synapses in the hippocampus of males and females through both pre- and postsynaptic mechanisms that are likely to be independent.

Disclosures: J.G. Oberlander: None. **C.S. Woolley:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.18/PP1

Topic: E.01. Neuroendocrine Processes

Support: NIH/NIGMS 2T32GM008541-16

Title: Pregnenolone sulfate as a modulator of synaptic plasticity

Authors: *K. SUGUNAN[1], J. I. LUEBKE[2], V. KUMARESAN[1], D. H. FARB[1];
[1]Pharmacol., [2]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstracts: Neurosteroids such as pregnenolone have been implicated in the pathophysiology of neuropsychiatric disorders such as schizophrenia and cannabinoid addiction (1, 2). Our laboratory discovered that the unique negatively charged steroid pregnenolone sulfate (PregS), the product of pregnenolone metabolism via a single sulfation step, modulates fast excitatory synaptic transmission by potentiating NMDA receptor activity in a fashion that is balanced by AMPA receptor inhibition at micromolar concentrations. We recently reported finding a novel high affinity response to PregS (EC50 = 2 pM), that increases intracellular [Ca2+] and CREB activation in cultured rat cortical neurons. Here, we report that PregS increases glutamate receptor and PSD95 colocalized puncta, presumably on dendritic spines of hippocampal neurons, suggesting that PregS increases surface-postsynaptic AMPARs and NMDARs. Whole cell recordings of primary rat hippocampal neurons revealed that PregS increases the frequency of spontaneous excitatory postsynaptic currents, suggesting an increase in glutamate release from presynaptic terminals or an increase in the number of activatable synapses. The results indicate that PregS acts within the physiological range of endogenous concentrations (3) to recruit AMPA receptors to postsynaptic sites, thereby altering synaptic plasticity. This mechanism may well underlie the cognitive enhancing pharmacological properties of PregS first reported 20 years ago (4). (1)Marx et al. (2009) Neuropsychopharmacology 34:1885-1903 (2)Vallée et al. (2014) Science 343(6166):94-8 (3)Rustichelli et al. (2013) J Chromatogr B Analyt Technol Biomed Life Sci. 930:62-9 (4) Flood et al. (1995) PNAS 92(23):10806-10

Disclosures: K. Sugunan: None. **J.I. Luebke:** None. **V. Kumaresan:** None. **D.H. Farb:** None.

Poster

640. Steroids and Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 640.19/PP2

Topic: E.01. Neuroendocrine Processes

Support: SiS Branco Weiss Fellowship

Title: Estrogen mediates fractional anisotropy changes in the hippocampus during the menstrual cycle - A pilot DWI study

Authors: *C. BARTH[1], C. J. STEELE[1], K. ARELIN[1,2,3], K. MUELLER[1], I. BURMANN[1], J. KRATZSCH[4], A. VILLRINGER[1,2,3,4,5], J. SACHER[1,2,5];
[1]Max Planck Inst. Human Cognitive and Brain Sci., Leipzig, Germany; [2]Clin. of Cognitive Neurology, Univ. of Leipzig, Leipzig, Germany; [3]Leipzig Res. Ctr. for Civilization Diseases, Univ. of Leipzig, Leipzig, Germany; [4]Integrated Res. and Treatment Ctr. Adiposity Diseases, Univ. of Leipzig, Leipzig, Germany; [5]Berlin Sch. of Mind and Brain, Mind and Brain Inst., Berlin, Germany

Abstracts: Introduction: Several lines of evidence from animal models and human studies suggest sex hormones to be important modulators of neuroplasticity. The menstrual cycle offers a unique natural setup to study whether physiological sex hormone fluctuation can influence brain morphology. However, data on the potential impact of the menstrual cycle phase on female brain architecture are sparse, especially regarding white matter microstructural organization. Here, we utilize diffusion weighted imaging (DWI) to investigate the impact of fluctuating hormone levels during the menstrual cycle on fractional anisotropy (FA). Methods: DW-images were acquired during 30 scanning sessions across four menstrual cycles with a 3-Tesla Magnetom Verio scanner (Siemens, Erlangen, Germany). Diffusion imaging data were processed with the FMRIB's software library (www.FMRIb.ox.ac.uk/fsl). Based on previous work that links the menstrual cycle to gray matter changes and to changes in functional connectivity in the hippocampus, we chose this structure as our Region-of-Interest (ROI). Hand drawn ROIs were derived from the thresholded (FA > 0.2) mean FA image. Mean FA values of mask regions were extracted, correlated with hormone levels and fed into partial correlation analysis (scan acquisition time) using SPSS Statistics 22 ($p < 0.05$). To investigate correlations between estrogen and progesterone levels in plasma and FA on a whole brain level, voxel-wise statistical analysis of the diffusion imaging data was carried out using tract-based spatial statistics (TBSS) and whole-brain smoothed FA. Results: In the ROI analyses, the extracted mean FA values of the left hippocampus showed a positive correlation with estrogen ($r = 0.504$, $p = 0.005$). No significant FA changes were mediated by progesterone in the mask regions. No regions in the TBSS and voxel-wise whole-brain approach reached significance after correction. Conclusion: Our findings suggest that estrogen-fluctuations across the menstrual cycle modulate FA in the hippocampus. Our results are in line with well-established evidence from rodents demonstrating hippocampal-plasticity to be mediated by sex steroids and reports in humans that suggest estrogen replacement therapy to be associated with changes in hippocampal volume. To our knowledge, this exploratory single-subject study is the first to link the subtle hormonal fluctuations that occur during the menstrual cycle to changes in FA. Our study demonstrates the feasibility of such a longitudinal DWI design and represents a step towards creating a personalized map of the human brain by integrating potential mediators of brain states, such as menstrual cycle phase.

Disclosures: C. Barth: None. **C.J. Steele:** None. **K. Arelin:** None. **K. Mueller:** None. **I. Burmann:** None. **J. Kratzsch:** None. **A. Villringer:** None. **J. Sacher:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 641.01/PP3

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant HD065604-01

Title: Pre- and post-wean early life social environments interact to shape socio-spatial memory in prairie voles

Authors: *G. S. PROUNIS[1,2], L. FOLEY[2], A. REHMAN[2], A. OPHIR[1,2];
[1]Cornell Univ., Ithaca, NY; [2]Zoology, Oklahoma State Univ., Stillwater, OK

Abstracts: Several studies have demonstrated that the dynamic influence of pre-weaning social environments shape behavioral and neural development across taxa. For example, maternal interactions influence oxytocin-mediated social behavior. It is important to consider how socio-environmental influences may interact during pre- and post-weaning development to finely shape the neurobehavioral phenotype. The social world in which an animal is raised conveys both the identity of conspecifics, and where they are in space. These two forms of socio-spatial information can have important implications on the reproductive decisions that an animal will make as an adult. Prairie voles provide an opportunity to understand the influence of social environments on neurobehavioral development. They are a bi-parental, socially monogamous species in which complex early-life manipulations are possible. They also demonstrate a large degree of intraspecific variation in both the social brain and behavior. Here we aim to determine if pre- and post-weaning early life social environments influence social and spatial memory. Male prairie voles were reared in a bi-parental or fatherless pre-weaning environment, and then weaned into isolated or group housing until reaching sexual maturity. Males were then tested in a modified social discrimination test, which allowed us to measure social and spatial memory simultaneously. Whereas the presence or absence of the father had no effect on social recognition of group housed males, isolated males raised without fathers did not demonstrate social recognition but isolated males raised with fathers did. Additionally, unlike the males raised with fathers or those that were group housed, males that were both fatherless and then isolated appeared to exhibit an overdependence on spatial rather than social information. Our results indicate that post-wean social isolation interferes with how animals use socio-spatial information, but the presence of a father buffers this effect. The expression of oxytocin (OTR) and vasopressin (V1aR) receptor in the social brain network suggests that in some cases, early life social environment can have profound effects on the behavioral and neural phenotype. We

speculate that the changes in brain and behavior induced by an interaction between pre- and post-weaning development may predispose animals to adopt different strategies in life.

Disclosures: **G.S. Prounis:** None. **A. Ophir:** None. **L. Foley:** None. **A. Rehman:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 641.02/PP4

Topic: E.03. Behavioral Neuroendocrinology

Title: Social status affects metabolic activity patterns of the social decision making network

Authors: *S. MAGUIRE, H. A. HOFMANN;
Univ. of Texas At Austin, Austin, TX

Abstracts: Animals continuously evaluate external cues from a stimulus in relation to internal information such as their past experience, current condition, and hormonal state. In the context of social behavior, decision-making is governed by a network of twelve evolutionarily conserved brain areas, the Social Decision-Making (SDM) network. Behavioral decisions vary across physiological contexts. For example, an animal may approach other individuals during the breeding season but may avoid the same stimulus in the non-breeding season. We hypothesized that this variation in response is regulated by changes in the patterns of activity across the SDM network that co-vary with behavioral phenotype. The African cichlid fish Astatotilapia burtoni is a model system in social neuroscience and ideally suited to study the mechanisms underlying social decision-making. Males of this species display several distinct phenotypes: dominant, subordinate, and intermediate. These phenotypes are associated with differences in behavior, hormone levels and brain gene expression patterns. We quantified the behavior of males in a social transition paradigm and found that transitioning males showed marked increases in aggression, sexual behavior, gonad size and coloration. We then measured metabolic activity in the SDM network using histochemistry of cytochrome oxidase (COX), an enzyme critical for energy metabolism whose activity correlates with neuronal activity. We used linear models to identify the brain areas where COX activity co-varied with social status and behavioral patterns. Furthermore, we constructed co-variance networks across the nodes of the SDM network and used the quadratic assignment procedure to assess how these patterns relate to social status. Ongoing studies are testing how these status-dependent activity patterns influence neural activity and behavior induced by social stimuli.

Disclosures: **S. Maguire:** None. **H.A. Hofmann:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 641.03/PP5

Topic: E.03. Behavioral Neuroendocrinology

Support: NIMH RO1 MH47538

Title: Decreased number and mean frequency of ultrasonic vocalizations in juvenile Brattleboro rats

Authors: ***M. J. PAUL**[1], N. V. PETERS[2], J. WHYLINGS[2], C. BADEAU[2], G. DE VRIES[2]; [1]Psychology, Univ. at Buffalo, SUNY, Buffalo, NY; [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstracts: Many neurodevelopmental disorders are characterized by deficits in social behaviors. For example, children with Autism Spectrum Disorders exhibit deficits in social interactions (including social play) and social communication. The neurobiological basis for these deficits in social development is not known. Recently, the neuropeptide arginine vasopressin (AVP) has been implicated in the development of social behaviors (e.g., social play and social recognition). Here we ask whether AVP also contributes to the development of social communication using Brattleboro rats, which contain a single base pair deletion in the vasopressin gene that disrupts the production of AVP in homozygous mutants. Male and female pairs of juvenile wild type, heterozygous, and homozygous Brattleboro rats (34±1 and 44±1 days of age) were single-housed then reunited 24 h later in a social behavior test, during which ultrasonic vocalizations (USVs) were recorded. All genotypes predominantly produced ~50 kHz USVs when reunited in the social behavior test. Preliminary data, however, indicate that the absence of a functional vasopressin gene in male and female homozygous pairs reduced the number and mean frequency of USVs. These data indicate that AVP is necessary for the expression of typical levels and call quality of USVs in male and female juvenile rats. They further indicate that AVP plays a broad role in social development, impacting several juvenile social behaviors including social communication.

Disclosures: **M.J. Paul:** None. **N.V. Peters:** None. **J. Whylings:** None. **C. Badeau:** None. **G. de Vries:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

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Program#/Poster: 641.04/PP6

Topic: E.03. Behavioral Neuroendocrinology

Support: University of Louisville College of Arts and Sciences

University of Louisville Intramural Research Incentive Grants

Title: Investigation of sex differences in wheel running and social behavior in pre-pubertal mice

Authors: *E. A. GORDON, S. F. BAMJI, K. N. BENCKER, D. N. PATEL, C. CORBITT; Dept. of Biol., Univ. of Louisville, Louisville, KY

Abstracts: The idea that sex differences in social behaviors exist in mammals during adulthood is well accepted, and further evidence suggests that sex differences in behavior are present even before sexual maturity (e.g., play behaviors). In order to model behavioral disorders in animals, it is important to assess baseline sex-related behavioral differences, especially when studying disorders for which sex-related behavioral effects are expected, such as autism. We undertook a study to measure potential sex-related behavioral differences in C57BL/6 and CFW mice (P27-P33), investigating the effect of sex on distinct behaviors in pre-pubertal males and females using a series of wheel running assays developed to evaluate autism-like behaviors. Specifically, we examined the animals' ability to gain and maintain a routine, cognitive rigidity and social interactions. We found no significant differences in latency to run on the wheel or total duration of wheel interaction between males and females. We also evaluated stereotypical behaviors, such as burrowing and grooming. During the social interaction test, there were no differences between the sexes in latency or total duration of contact or following between a subject and novel mouse. In addition, no play behavior was noted, but brief mutual grooming was displayed between mice. Both sexes showed characteristic wheel running behavior, spending the majority of their time interacting with the wheel when it was free and more time performing other activities (e.g., stereotypical behaviors, general locomotion) when it was jammed. Given that pre-pubertal sex differences were not detected in this assay, the results suggest that baseline pre-pubertal sex-related differences are not strong enough to influence behavior in wheel running studies. We conclude that sex-related behavioral differences can be discounted when designing future experiments to measure the effects of developmental insults on this assay in juvenile mice.

Disclosures: E.A. Gordon: None. **S.F. Bamji:** None. **K.N. Bencker:** None. **D.N. Patel:** None. **C. Corbitt:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

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Program#/Poster: 641.05/PP7

Topic: E.03. Behavioral Neuroendocrinology

Support: Polish Ministry of Science #UMO-2011/01/B/HS6/06442

Title: The development of play fighting: Do interactions with siblings before weaning matter?

Authors: *B. T. HIMMLER[1], R. STRYJEK[2], K. MODLINSKA[2], S. M. HIMMLER[1], B. KOLB[1], W. PISULA[2], S. M. PELLIS[1];
[1]Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Polish Acad. of Sci., Warsaw, Poland

Abstracts: Play fighting behavior begins to develop in the week preceding weaning, but is incomplete at this stage, not attaining its completed form until the week after weaning. What is unknown is whether experiencing immature patterns play fighting is necessary for the development of the fully mature form. Previous literature has shown that if rats or cats are weaned early, they exhibit heightened levels of play, suggesting the earlier experiences with play may be important. The objective of the present experiment was to evaluate the contributions of the siblings to the development of play behavior in a wild strain of rat--Warsaw Wild Captive Pisula Stryjek rats (WWCPS). On postnatal day 15, both male and female rats were randomly selected to be reared in one of three conditions, (1) Mother-only rearing, (2) Sibling-Only rearing, or (3) Mother and Sibling-reared. It must be noted that in the sibling-only condition, the rats received adequate nutrition. It was predicted that the rats reared in the mother-only condition would show the biggest deviation in juvenile typical play, because with the mother, the animals receive little-to-no playful experiences. Surprisingly, our results show that in all conditions, the rats developed juvenile typical play, suggesting that peer-peer interactions at this age are not necessary for the development of normal play fighting. Indeed, the only group that showed some inadequacies in the sensory-motor coordination of playful movements was the sibling-only rats, suggesting that even though the mother does not engage in play with the pups, she must be providing some experiences which contribute to the development of some important brain mechanisms that are engaged during play.

Disclosures: B.T. Himmler: None. **R. Stryjek:** None. **K. Modlinska:** None. **S.M. Himmler:** None. **B. Kolb:** None. **W. Pisula:** None. **S.M. Pellis:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 641.06/PP8

Topic: E.03. Behavioral Neuroendocrinology

Support: Farris Family Foundation Award

Whitehall Foundation Grant

Title: Reduced social anxiety and elevated fear expression in mice lacking NMDA function in CRF neurons

Authors: *J. DAMERT, T. GILMAN, J. D. MEDURI, A. M. JASNOW;
Dept. of Psychological Sci., Kent State Univ., Kent, OH

Abstracts: Corticotropin-releasing factor (CRF) is a neurohormone involved in mediating behavioral and neuroendocrine responses to various stressors. Here, we employed a cross of two transgenic mouse lines to better understand the regulation of the CRF system and how its activation influences stress-related behaviors. Mice expressing Cre recombinase (Cre) specifically driven by the CRF promoter (CRF-Cre mice) were crossed with floxed NR1 (fNR1) mice allowing for selective ablation of NMDA receptor function only in CRF-expressing neurons. Male fNR1xCRF-Cre mice positive for Cre (fCC+) were first trained along with their Cre negative littermates (fCC-) in cued fear conditioning. Though both groups of mice acquired training similarly, fCC+ mice displayed increased fear retention as well as impaired fear extinction. These fear traits have been observed previously in C57BL/6 mice characterized as resilient following social defeat stress (i.e., displaying unperturbed social investigation following defeats). Consequently, a cohort of these fCC mice was exposed to social defeat stress involving 8 total defeats (4/day) by different male CD-1 aggressor mice. A social interaction assessment was performed 24 h after the last defeat. Though we hypothesized fCC+ mice would display greater resilience, we observed no genotype-dependent differences in social investigation by resilient or susceptible (i.e., displaying reduced social investigation following defeats) fCC mice. Surprisingly, only in the control group was any indication of a genotype difference observed, with fCC+ mice showing a trend for increased social investigation. Indeed, this was confirmed by examining baseline sociability and social novelty behavior in defeat naïve fCC+ mice and comparing to their fCC- littermates. Due to this enhanced social propensity of fCC+ mice, we then hypothesized that the 8 defeats may have masked any genotype-related differences in social stress responsiveness. Therefore, social investigation was examined 24 h after a single defeat

stress. Interestingly, all fCC+ mice displayed a resilient phenotype, while fCC- mice were more likely to be characterized as susceptible. These data support previous literature indicating a behavioral link between defeat resilience and enhanced fear expression/impaired extinction. Furthermore, they suggest activation of CRF neurons via NMDA receptors modulate behavioral susceptibility to social stress and the ability to adequately attenuate fear responses over time.

Disclosures: J. Damert: None. **T. Gilman:** None. **J.D. Meduri:** None. **A.M. Jasnow:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 641.07/PP9

Topic: E.03. Behavioral Neuroendocrinology

Support: Fapesp

Title: Effect of stress on the anxiety-like behavior in an animal model of perimenopause

Authors: *P. D. BARROS[1], N. PESTANA-OLIVEIRA[1], C. LEITE-PANISSI[2], J. A. ANSELMO-FRANCI[2];
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Abstracts: Introduction: It is known that during perimenopause, a period of transition from reproductive to non-reproductive life several hormone and behavioral alterations arise. During this time, the rates of mood disorders, such as depression and anxiety, are highest when compared to any other phase in woman life. A suitable animal model of perimenopause can be produced by the administration of the chemical 4-vinylcyclohexene diepoxide (VCD) which lead to ovarian failure through gradual depletion of primordial and primary follicles, allowing studies on this transition period of reproductive life. Preliminary data of our laboratory show that this perimenopause animal model exhibit anxiety-like behavior. Since is well established that previous stresses can intensify the anxiety behavior of male rats, we aimed at investigating if previous stresses applied in this animal model could also increase this behavior, evaluated in the elevate plus maze (EPM). Material and Methods: 28-day old female rats were treated subcutaneously with VCD (160 mg/Kg) or corn oil during 15 days. Around 80 days after the beginning of VCD/oil administration, on metestrus, rats were submitted to restraint stress during one hour and then isolated from the other animals for 24 hours before the EPM test, performed on diestrus. The unstressed rats were not submitted to restraint or isolation stress. The number of

entries in open and closed arms as well as the time spent in open arms were counted. Moreover, risk assessment behaviors, such as stretched attend posture, rearing and end-arm exploration were also analyzed. Results: VCD-treated rats exhibited an increased anxiety-like behavior expressed by a lower number of entries as well as time spent (in percent) in the open arms when compared to the control rats (1.2±0.7 vs 3.4±1.2 and 4.2±2.6% vs 16.4±6.9% respectively), while the number of entries in closed arms was unchanged. There was no difference between control and VCD-treated rats regarding the risk assessment behaviors analyzed. Previous and associated stress did not modify any of the parameters studied. Conclusion: Although VCD-induced ovarian failure has led to increased anxiety-like behavior in female rats, previous stresses were not able to intensify this behavior.

Disclosures: **P.D. Barros:** None. **N. Pestana-Oliveira:** None. **C. Leite-Panissi:** None. **J.A. Anselmo-Franci:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

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Program#/Poster: 641.08/PP10

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant 0822041

Title: Organizational and behavioral effects of the early cultural environment in the highly social prairie vole

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Abstracts: The expression of high levels of prosocial behavior is the product of the interplay of underlying neural mechanisms, which include the neuropeptides, oxytocin (OT) and arginine vasopressin (AVP) and steroids, especially estrogen. Here we report of the effects of the early social/cultural environment on the organization of the brain and expression of social behavior using two populations of prairie voles (*Microtus ochrogaster*). While both populations Illinois (IL) and Kansas (KS) are socially monogamous they are behavioral, physiologically and neuroanatomically distinct, with IL prairie voles displaying much higher levels of prosocial behavior than KS. To separate genomic effects from cultural effects litters IL and KS breeding pairs were established and their litters were then in-fostered to a pair form the same population or

cross-fostered to pairs from the other population on postnatal day 1-2. Litters were reared using standard vole animal husbandry and then tested as adults for the expression of prosocial behavior using a partner preference test, as pair bond formation is a critical aspect of social monogamy. Test animals were cohabitated for six hours with an unrelated sexually naïve member of the opposite sex IL conspecific and then participated in a 3 hr test to determine social interaction with the familiar or sex- and age-matched novel individual. Brains were then collected and analyzed, using immunocytochemistry, for OT and AVP expression in the PVN and tyrosine hydroxylase and estrogen receptor alpha in the hypothalamus. The effects of fostering were populational and sexually dimorphic. As predicted there were significant differences in the levels of prosocial behavior between KS and IL, with in-fostered IL voles displaying higher level of social interaction than KS and with in-fostered males showing higher levels than females. The largest effects of cross-fostering were observed in KS males, who were the only group to form a partner preference, spending significantly less time exploring the novel female and significantly more time ($p < 0.05$) in the cage of and in physical, side-by-side, contact the familiar female than the novel female. We also report on significant differences in arginine vasopressin and oxytocin immunoreactive cells in the PVN and estrogen receptor expression and tyrosine hydroxylase in the hypothalamus by population and associated with cross-fostering and expression of differential social behavior.

Disclosures: B.S. Cushing: None. **L. Stetzik:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

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Topic: E.03. Behavioral Neuroendocrinology

Support: UCI Center for Autism Research and Translation

UCI Medical Scientist Training Program

Title: Endocannabinoid Regulation of Sociability

Authors: *D. WEI[1], C. MURRAY[1], D. LEE[1], A. ANGUREN[1], D. DINH[1], K.-M. JUNG[2], D. PIOMELLI[2];
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Abstracts: Social behavior is a hallmark of diverse species from invertebrates to humans and is essential to health and group survival. The neural systems that underlie the expression of sociability and encode the reward of social interactions remain largely unknown. Clues indicate that the endogenous cannabinoid ('endocannabinoid') system might be involved: (a) users of marijuana, a drug in which the psychoactive principle chemically resembles the endocannabinoids, report changes in sociability, and (b) endocannabinoids regulate mood and cognition through the activation of cannabinoid type-I (CB1) receptors, which are richly expressed in areas of the brain involved in social behavior. However, how the endocannabinoids system might regulate sociability and its possible dysregulation in social impairment are unknown. Initial studies suggest that enhancement of the endocannabinoid anandamide, through pharmacological blockade or genetic removal of the anandamide-hydrolyzing enzyme fatty acid amide hydrolase, promotes socially-conditioned place preference, while CB1 blockade decreases this preference. We also found that anandamide signaling is low in BTBR mice, a well-established model of social impairment. Anandamide enhancement in this model restores normal social approach, as evaluated using the three-chamber test. These studies suggest that anandamide is important for the regulation of social reward and its signaling can be targeted to correct social impairment. We hypothesized that anandamide might exert these effects through an interaction with oxytocin, a neuropeptide known to be crucial for social behavior. We found that oxytocin receptor antagonism attenuated both the andandamide-mediated enhancement in socially-conditioned place preference as well as social stimulation-induced increases in anandamide. Consistent with the expectations suggested by these findings, anandamide enhancement corrects aberrant oxytocin transcription in the socially-impaired BTBR mice. Together, these studies suggest that anandamide is important for the regulation of sociability through an interaction with oxytocin. This provides implications for the understanding of the neurobiology of sociability and social impairment, a cardinal feature of many neuropsychiatric disorders.

Disclosures: **D. Wei:** None. **D. Piomelli:** None. **C. Murray:** None. **D. Lee:** None. **A. Anguren:** None. **D. Dinh:** None. **K. Jung:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

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Grant Drunken Moon Lake Integrated Scientific Research Platform

Grant Aim for Top University Project from National Taiwan University

Title: Behavioral response and functional neuroanatomy of male golden hamsters during social eavesdropping

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Abstracts: Social eavesdropping is a special type of social learning and it is defined as the act of extracting information about the relative or absolute quality of signalers from social interactions between conspecifics. Social eavesdropping has advantage in information gathering and has attracted increasing attention. However, this behavioral phenomenon and its underlying neural mechanisms remain much unclear. Taking advantage of agonistic behaviors in male golden hamsters, we developed a new laboratory method to study social eavesdropping. After 3-day social learning, naïve male bystanders were attracted to the winning demonstrators whereas bystanders with defeated experience avoided approaching to the winning demonstrators in a U-maze. To further identify brain areas and related neural activity underlying social eavesdropping in hamsters with defeated experience, 3 groups of hamsters that exposed to either a fighting interaction, a neutral encounter, or an empty arena were tested. In experiment 1, compared with the arena controls, male bystanders in the other two groups displayed more time in the information gathering behaviors during the 3-day social learning. Using c-Fos immunohistochemistry to map neuronal activities in the brain, males in the fighting interaction group had more c-Fos labeled neurons in the piriform cortex and sub-regions of cingulate cortex compared with males in the other two groups. But no significant difference was found in the hippocampus and amygdala. In experiment 2, local field potential was recorded in the cingulate cortex of behaving hamsters to reveal neural activity during social eavesdropping. Data collection and analyses are still in progress. Collectively, our data suggest that hamsters are capable of social eavesdropping and cingulate cortex plays an important role in the information gathering and extracting process.

Disclosures: C. Liu: None. **W. Yu:** None. **C. Chang:** None. **W. Lai:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

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Program#/Poster: 641.11/PP13

Topic: E.03. Behavioral Neuroendocrinology

Support: NIEHS Grant R01 ES022759

Title: Dose-Response study of di-(2ethylhexyl) phthalate (dehp): Androgenic and anti-androgenic actions

Authors: *K. M. QUINNIES[1], E. P. HARRIS[1], E. F. RISSMAN[2];
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Abstracts: Di-(2ethylhexyl) phthalate (DEHP) is a man made endocrine disrupting compound (EDC) used in production of flexible plastics. The mechanism of action for this EDC is complex acting on both steroid receptors and steroidogenic enzymes. Much of the work on this compound has focused on the effects that high doses have on androgen-target tissues. However, in addition to gonadal hormones, effects of DEHP may be caused by its actions on lipid and cholesterol, the hypothalamic-pituitary-adrenal axis, and/or retinoic acid. Epidemiological studies have demonstrated correlations between metabolites in urine and a variety of behaviors in children, including higher urinary levels of DEHP in autistic as compared with control children. Here we use several doses of DEHP given orally to mice (5ug, 40ug, 400ug and of 200mg/kg body weight/day) during pregnancy and the first ten days of lactation. These doses represent a range from low (within levels found in humans) to rather high and others have suggested the low doses are androgenic while the highest dose is anti-androgenic. Preliminary data indicates that PN1 male offspring have a shorter anogenital distances than control males, suggesting an anti-androgenic effect. Furthermore, dams exposed to low doses of DEHP tend to display less maternal care than dams not consuming DEHP. This research has important implications for human social behaviors; and may be relevant to disorders with known sex differences, such as Autism Spectrum Disorders. Due to the interaction of DEHP with genes and steroid hormone pathways integral to neurodevelopment, as well as social behavior effects in human studies, we predict that animals exposed to DEHP *in utero* will have dose-related changes in behavior.

Disclosures: K.M. Quinnies: None. **E.P. Harris:** None. **E.F. Rissman:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Award 1257162

Title: Stress inhibits partner preference in same-sex pairs of female meadow voles

Authors: *A. M. ANACKER, K. M. REITZ, E. R. GUNZEL, A. K. BEERY;
Neurosci., Smith Col., Northampton, MA

Abstracts: Meadow voles (Microtus pennsylvanicus) provide an excellent model of social relationships between same-sex peers. While females of this species are territorial and solitary in the summer breeding season, they live in groups and nest communally in the winter. In the laboratory, they form partner preferences for other specific females under winter-like light conditions (10 hours of light : 14 hours of dark). Here we tested whether stress hormone levels differ under winter- and summer-like conditions, and whether stress inhibits partner preferences, in order to understand the factors that can mediate same-sex relationship formation. Adult female meadow voles housed under winter-like light conditions had lower levels of free serum corticosterone than those housed under summer-like light conditions. Previous studies in monogamous female prairie voles found that a forced swim stressor or an injection of the stress hormone corticosterone inhibited formation of a preference for a male partner (DeVries et al. 1996, 1997). We exposed adult female meadow voles housed under winter-like light conditions to a three-minute swim stress in room temperature water, and then paired the test subject with another female previously unknown to the subject (the partner). Following 24-hour cohabitation, we measured the amount of time the subject spent huddling with the partner or a stranger in a 3-hour partner preference test. We found that swim stress exposure decreased the amount of time spent with the partner in comparison to controls and decreased the likelihood that voles would exhibit a preference for the partner over a stranger. In a separate test we demonstrated that the three minute swim stress significantly increased corticosterone levels. Further studies will demonstrate whether corticosterone is necessary and/or sufficient to inhibit partner preference formation. These findings demonstrate that stress inhibits same-sex social bonding in female meadow voles, which may relate to their seasonal changes in social behavior.

Disclosures: A.M. Anacker: None. **K.M. Reitz:** None. **E.R. Gunzel:** None. **A.K. Beery:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC

NSERC

Title: Infection threat elicits assortative sociality in female mice

Authors: *M. KAVALIERS[1], C. J. CLOUTIER[2], K.-P. OSSENKOPP[3], E. CHOLERIS[4]; [1]Univ. Western Ontario, London, ON, Canada; [2]Psychology, [3]Univ. of Western Ontario, London, ON, Canada; [4]Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstracts: There is mounting evidence that pathogen threat affects social and sexual responses and underlies the evolution of disgust. In humans the reverse is also true with the presence of strangers heightening sensitivity to pathogen threat, promoting "in-group" bias and "out-group" avoidance. Whether or not a similar effect of social context on pathogen threat occurs in non-human animals is unclear. The present study shows that the responses of female mice to males are also rapidly affected by the presence of either unfamiliar or infected males in a manner consistent with the expression of disgust. In female mice, where odor cues determine the appetitive aspects of social responses and preferences, brief (1 min) exposure to the urinary odors of an unfamiliar male led to females subsequently discriminating more strongly against the odors of males subclinically infected with the nematode parasite, *Heligiomides polygyrus*. In a parallel fashion, brief exposure to the odors of infected, males attenuated the responses of females to the odors of the normally preferred unfamiliar males and enhanced their preferences for familiar males. These findings are consistent with the concept of "assortative sociality", whereby the presence of infection threat and unfamiliar individuals biases female preferences for uninfected and familiar individuals ("in-group" preference and in human terms "ethnocentrism") and leads to the avoidance of unfamiliar individuals ("out-group" avoidance and "xenophobia"). Hence, as in humans infection associated social information can rapidly bias the social preferences and elicit assortative sociality in female mice.

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Poster

641. Social Behavior: Drivers and Mechanisms

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant SC2DA034996

Title: Intrasexual dimorphism of the serotonergic system in a vocal fish with alternative reproductive tactics

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[1]Biol., Brooklyn College, City Univ. of New York (CUNY), Brooklyn, NY; [2]Ecology, Evolutionary Biol. and Behavior, [3]Behavioral and Cognitive Neurosci., CUNY Grad. Ctr., New York, NY

Abstracts: The plainfin midshipman, *Porichtys notatus*, is a seasonally breeding marine teleost that produces vocal signals for intraspecific communication. There are two different male reproductive morphs: Type I males establish nests and vocally court females while type II males neither build nests nor produce a mating call but instead sneak-spawn in competition with type I males. The morph phenotype is fixed once an individual is mature. Type I males are consequently the territorial, and aggressive morph, possessing a distinct vocal motor and hormonal profile relative to type II males. Circulating cortisol as well as CNS glucocorticoid receptor expression is greater in type II males. The highly conserved serotonergic (5-HT) system is known to modulate a range of socially motivated behaviors across taxa, including mating-related vocalization and audition, stress, and aggression. 5-HT reciprocally interacts with the hypothalamic-pituitary interrenal axis in teleosts; agonist binding at toadfish 5-HT1A receptors elevates circulating cortisol. Subsequent decrease in 5-HT1A expression indicates a negative feedback organization. The nuclei of the mammalian superior raphe (SRa) are functionally distinct as a result of specific projection patterns, receptor associations, and electrophysiological profiles, as well as distinctive axonal morphology. Similarly, recent studies in zebrafish demonstrate specialization of SRa nuclei in teleosts. In midshipman, ascending serotonergic fibers innervate the auditory thalamus, parvocellular preoptic area, and limbic homologs in the telencephalon. Intrasexual differences in the anatomy of the teleostean SRa may underlie morph-specific behavioral profiles, with the expectation of a more robust ascending serotonergic system in non-aggressive type II males. Reproductive adult males were collected from intertidal nests and processed for 5-HT immunofluorescence histochemistry. Midshipman SRa 5-HT-ir somata were quantified and assigned to one of three groups delineated strictly by anatomical landmarks: a dorsomedial, ventromedial, or ventrolateral group. Our results demonstrate that type II males have significantly greater total numbers of SRa 5-HT-ir neurons compared to type I males. We

also provide evidence (in both morphs) for heterogeneity between the proposed raphe groups, specifically the morphometric confirmation of a distinct 5-HT-ir ventromedial SRa group exhibiting significantly smaller somata. Our findings support the hypothesis of a divergent ascending 5-HT phenotype inversely related to aggressive territorial phenotype in a teleost model with fixed male alternative reproductive morphs.

Disclosures: **M. Timothy:** None. **Z.N. Ghahramani:** None. **A. Chernenko:** None. **M. Gorbonosov:** None. **P.M. Forlano:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

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Topic: E.03. Behavioral Neuroendocrinology

Support: RFA-MD-07-001

Title: Association of smoking-induced variations on hypothalamic pituitary adrenal axis activity and mental health stress in African Americans

Authors: ***P. A. ABRAHAM**, J. KAZMAN, S. ZENO, K. DENNIS, P. DEUSTER; Military and Emergency Med., Uniformed Services Univ., Bethesda, MD

Abstracts: Objective: Ample evidence suggests cigarette smoking causes neuroendocrine changes in humans. We investigated the possible influence of smoking on changes in cortisol and DHEAS and self-reported psychological status in adult African Americans (AA). Methods: AA aged 18 to 60 years were recruited from the community. Morning saliva samples were obtained to measure cortisol and DHEAS. Additionally, questionnaires were administered to assess self-reported depression, daily hassles and anxiety. Participants were classified into two groups based on smoking status: Smokers (S: n= 40) and Non-Smokers (NS: n=80). The p value was set at ≤0.01. Results: DHEAS levels did not differ by smoking status (S: 22.9±2.3 vs. NS: 20.2±1.6; p =0.4), but cortisol was slightly lower in smokers (S: 11.6± 2.4 vs. NS: 14.7± 1.7; p=0.3). Additionally, smokers had significantly higher scores on depression (S: 8.9±1.0 vs. NS: 5.5±0.7; p≤0.01), trait anxiety (S: 42.0±1.6 vs. NS: 35.8±1.1; p≤0.01), and state anxiety (S: 40.5±1.7 vs. NS: 30.7±1.2; p≤0.001) but not on daily hassles (S: 45.2± 4.2 vs. NS: 35.9± 3.0; p = 0.08). Conclusion: Although no significant differences in cortisol or DHEAS were noted as a function of smoking status, symptoms of anxiety and depression were significantly higher in

smokers than non-smokers in AA. The modulation of psycho-social-neurobiological variables must be considered when providing smoking cessation education programs. Whether other neuroendocrine markers contribute to the association between mood and smoking needs to be further investigated.

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Poster

641. Social Behavior: Drivers and Mechanisms

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Title: Validation of a partner preference test in coppery titi monkeys (Callicebus cupreus)

Authors: *E. ROTHWELL[1], S. CARP[3], S. FREEMAN[2], E. FERRER[1], K. BALES[1,2];
[1]Psychology, [2]California Natl. Primate Res. Ctr., Univ. of California- Davis, Davis, CA;
[3]Psychology Dept., Univ. of Nebraska-Omaha, Omaha, NE

Abstracts: The partner preference test is a behavioral paradigm that has been used extensively in rodents to quantify social preference in a variety of species. The development and validation of this behavioral task has given researchers the ability to study the factors that influence the formation of social attachments in monogamous species as well as social memory and mate choice in non-monogamous rodent species. However, an analogous partner preference testing paradigm for use in nonhuman primates has not yet been developed and validated. Thus, the goal of the current study was to establish an adapted partner preference test for nonhuman primates using the socially monogamous coppery titi monkey (*Callicebus cupreus*). Twelve established pairs of monkeys were tested in a three-chambered apparatus for three hours. Analogous to the paradigm used in rodents, the test subject was placed in the middle chamber, with its pair-mate

on one side and an opposite sex stranger on the other. The test animal was separated from the two stimulus animals by grated windows, and we recorded the duration of time that the test animal spent in proximity with and touching each window. All monkeys spent significantly more time near the partner's window compared to the stranger [$t = 3.88$; $p < .001$], but the time touching either window did not differ [$W = 335$; n.s.]. A dyadic mixed model analysis revealed partner preference did not change during the test. We found that females, but not males, spent significantly more time near the stranger's window as the test progressed [$t = 2.43$; $p = 0.017$]. This validated partner preference test can now be used as a standardized measurement for social preference in a variety of primate species to quantitatively study the formation and maintenance of pair bonding and to determine what factors influence the expression of social preference.

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Poster

641. Social Behavior: Drivers and Mechanisms

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant IOS-1253188

GSU Brains and Behavior Seed Grant

Title: Excitatory and inhibitory reciprocal connections between the anterodorsal and posterodorsal medial amygdala in the adult male Syrian hamster

Authors: *J. WHYLINGS[1], A. BURNS[2], P. BEHNIA[1], A. PETRULIS[1], B. M. COOKE[1]; [1]Neurosci. Inst., [2]Biol. Dept., Georgia State Univ., Atlanta, GA

Abstracts: The medial amygdala (MeA) is important for processing social odor cues and is critical for reproductive behavior in rodents. The MeA can be subdivided into anterior and posterior subdivisions; in male Syrian hamsters (Mesocricetus auratus), communication between these subdivisions is necessary for processing sexually relevant odor cues. Disconnecting the anterior MeA from the posterodorsal medial amygdala (MePD) abolishes opposite sex odor preference, and lesioning the anterior MeA reduces MePD Fos labeling in response to opposite-sex odors. In contrast, if the MePD is lesioned, anterior MeA Fos expression in response to

opposite-sex odors is unchanged. This suggests that the anterior MeA provides a stronger excitatory input to the MePD during odor processing than the MePD provides to the anterior MeA. Thus, we predicted that projections from the anterodorsal medial amygdala (MeAD) would be predominantly glutamatergic, and that projections from the MePD would be mainly GABAergic due to the large number of GABAergic neurons in the MePD. To test this, we quantified the excitatory and inhibitory connections between these areas in male Syrian hamsters. We injected the anterograde tracer biotinylated dextran amine (BDA) into either the MeAD or MePD, and then visualized BDA-labeled axons in sections immunolabled for either vGlut2 or GAD65, which are markers of glutamatergic and GABAergic presynaptic terminals, respectively. Confocal z-stack images were sampled from throughout both regions. We selected well-defined axons and determined the linear density of terminal bouton-like varicosities, defined as any enlargement greater than 1.5x the axon diameter, on ~16 axon segments per hamster (mean length 58 μm ± 31 μm). We then determined each bouton's colocalization with the presynaptic marker. Contrary to our expectation, preliminary results suggest that the MeAD and MePD project roughly equivalent numbers of excitatory and inhibitory efferents to each other. However, the extent of co-localization with either presynaptic marker is greater in MeAD efferents than in MePD efferents. It is unclear whether the disparity in colocalization is due to differences in terminal characteristics such as size, or likelihood to express vGlut2 or GAD65. More exploration will be needed to distinguish between these and other possibilities, as well as to determine the peptidergic content in these projections.

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Poster

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIMH Grant 1R01MH082833

NSF Grant 1306132

Title: Gene co-expression network analysis in a free-living, behaviorally polymorphic species

Authors: *W. M. ZINZOW-KRAMER[1], B. M. HORTON[1], G. K. THARP[2], D. L. MANEY[1]; [1]Psychology, [2]Yerkes Div. of Developmental and Cognitive Neurosci., Emory Univ., Atlanta, GA

Abstracts: Individuals of all species must balance parental investment and competitive behavior to successfully produce viable offspring. In this context, aggression is associated with prioritizing short-term goals (maximizing mating opportunities) over long-term goals (successfully rearing young). Our research takes advantage of a unique model species, the white-throated sparrow, to study the genetic underpinnings of individual differences in aggressive and parental behavior. This species exhibits a plumage polymorphism that correlates with alternative reproductive strategies. Individuals of the white stripe (WS) morph are more aggressive and adopt a more competitive, less parental strategy than individuals of the tan stripe (TS) morph. The plumage polymorphism is linked to a chromosomal rearrangement, presenting a unique opportunity to study the relationship between genes and social behavior. In this study, we employed genome-wide transcriptome analysis to identify clusters of genes associated with social behavior in a free-living population. At our study site, a higher song rate in WS males represents the greatest behavioral difference between the morphs. We therefore prioritized male song as the main behavioral focus. In order to correlate this behavior with gene expression, we measured song rate in response to simulated territorial intrusions during the breeding season. We then collected brains from those males and isolated RNA from two regions: the medial amygdala and the hypothalamus. We used RNA-sequencing to generate transcriptome data for each region, which we then used to identify genes that vary in expression with relation to both plumage morph and vocal aggression. Because social constructs such as aggressive behavior are mediated by many genes, we are ultimately interested in identifying genetic pathways associated with behavior. Therefore, we used weighted gene co-expression network analysis (WGCNA) to conduct a systems-level analysis of the gene networks that differed according to morph. Gene modules identified by WGCNA consist of groups of genes with expression profiles that are related across samples. Thus, we identified groups of genes with expression profiles that are associated with plumage morph and behavior. Because genes and pathways that regulate social behavior are highly conserved, our findings are likely relevant to the mechanisms underlying social strategies in all vertebrates.

Disclosures: W.M. Zinzow-Kramer: None. **B.M. Horton:** None. **D.L. Maney:** None. **G.K. Tharp:** None.

Poster

641. Social Behavior: Drivers and Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 641.19/PP21

Topic: B.08. Synaptic Plasticity

Support: FDCT 012/2012/A1

Title: Behavioral plasticity meets neuroplasticity: Neurogenesis associated with male polymorphism in the peacock blenny Salaria pavo

Authors: *P. VIEIRA[1], J. M. SIMÕES[3,4], R. F. OLIVEIRA[3,4], D. GONÇALVES[2];
[1]Univ. of St. Joseph, Macao; [2]Univ. of St. Joseph, Macao, Macao; [3]Inst. Gulbenkian de Ciência, Oeiras, Portugal; [4]ISPA-IU, Lisbon, Portugal

Abstracts: The adult brain of teleost fish is distinguished by a high degree of structural plasticity with extensive brain neurogenesis and gliogenesis persisting throughout life span. Fish are also the vertebrate class with the highest degree of behavioral plasticity and in many species individuals undergo major behavioral transitions during their lifetime, including changing sex or adopting alternative modes of reproduction. We hypothesized that these extreme behavioral changes in the adult stage rely on a significant capacity for structural rearrangements of neuronal networks that include the integration of new neurons. This idea was tested in a species with a high level of behavioral plasticity, the peacock blenny Salaria pavo. In this species, young males reproduce by mimicking female behavior and appearance in order to approach nests defended by larger males, deceive the males, enter the nests, and parasitically fertilize part of the eggs inside the nest. The small "sneaker" males transition to the nesting male phenotype, suffering extreme morphological and behavioral changes. An experiment was conducted to test if the behavioral transition was associated with an increase in cell proliferation levels. One outdoor pool was set up with 12 nesting males and respective nests, 24 females and 24 sneaker males. The 24 sneaker males were provided with 12 potential nests to induce the transition in approximately half of the sneaker males. In fact, at the end of the experimental period (10 days), some of the sneakers had occupied the available nests and started the morphological and behavioral transition while others had remained sneakers. To compare cell proliferation levels in the brains of nesting males, sneakers and transitional males, fish were injected with the mitotic marker BrdU and sacrificed 2h later. Animals were perfused, the brains extracted, sliced in a cryostat at 15µm and a BrdU immunohistochemistry protocol with DAPI counterstain was applied for detecting new cells. Cells were manually counted under a fluorescence microscope. A detailed map of the brain proliferation areas for this species was generated. For the most significant nuclei, differences in the number of proliferating cells between the male morphotypes were analyzed. The results are discussed in light of the hypothesis that periods of extreme behavioral transition are associated with an increase in cell proliferation levels. In conclusion, extensive adult neurogenesis may be a condition for extreme behavioral plasticity and may explain why, throughout vertebrates, adult behavioral plasticity seems to be associated with the capacity for adult neuroplasticity.

Disclosures: **P. Vieira:** None. **J.M. Simões:** None. **R.F. Oliveira:** None. **D. Gonçalves:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.01/PP22

Topic: E.03. Behavioral Neuroendocrinology

Support: CONACYT Ciencia Basica 127777

PAPIIT DGAPA IN218111

PAPIIT-DGAPA-UNAM IN216214

PAPIIT DGAPA IA202314

Title: Neonatal maternal separation impairs osmotic stress coping in adult rat: Mapping brain metabolic activity by [18F]-FGD positron emission tomography and c-Fos expression

Authors: *C. L. IRLES[1], V. M. LARA[2], M. C. AVILA[2], H. BARRIO[1,3], E. RAMOS[4], T. MORALES[4], M. A. AVILA-RODRIGUEZ[2], L. ZHANG[1];
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Abstracts: Hypothalamic AVP-containing osmosensitive neurosecretary neurons serve as important sources for AVP intracerebral innervation (1-5). We have previously demonstrated that neonatal maternal separation (MS, 3 hr daily, from postnatal day 2 to 15) potentiates hypothalamic vasopressin (AVP) system. Under the experimental conditions in which the AVP system was particularly up-regulated (e.g. hypertonicity), the adult MS rats exhibit high anxiety and spatial learning impairments. In light of these previous observations, we herein hypothesized that up-regulation of the VP system by an osmotic stimulus in MS rats would result in differences in brain metabolism in the neuropeptide hypothetical downstream regions. Hence, we used high resolution positron emission tomography for rodents (microPET) with [18F] 2-fluoro-2-deoxy-D-glucose (18F-FDG) to *in vivo* image the regional brain glucose metabolism, as an indicator of neuronal activities. Adult (postnatal day 90) male Wistar animal facility reared (AFR) and MS rats were injected with 18F-FDG under isoflurane anaesthesia. Dynamic scans of

90 min were acquired in the microPET. Hypertonic saline (900mM saline, 2% of body weight) was injected i. p. at min 20 post-injection of the tracer. The averaged values from the min 15-20 served as the baseline of the metabolic activity for each region/subject. Dynamic images were analysed post-hoc using the ASIPro VM software. Dynamic changes of 12 brain regions were plotted (time vs standardized uptake value). MS rats showed an increased metabolism in hypothalamus, locus coeruleus (LC), periaqueductal gray (PAG), dorsal raphe (DR), lateral septum (LS), striatum, amygdala, infralimbic prefrontal cortex (IL), motor cortex 1, cerebellum and ventral hippocampus (vHi). Using immunohistochemistry against the immediate early gene c-Fos, we found increased neuronal activation in some of the regions with increased metabolism, especially CeA, vHi, LS, LC, PAG and DR. These results suggest than MS modifies the stress-related neurocircuitry of the VP system downstream regions crucially involved in anxiety modulation, spatial learning and fight-of-flight response. 1. Zhang L. SfN 2008, abstract. 2. Zhang L.& Hernandez VS. Neuroscience (2013)228:139-62. 3. Hernandez VS & Zhang L. SfN 2012, abstract. 4. Zhang L., Vazquez-Juarez E., Hernandez V. S. SfN 2014, abstract. 5. Cui, Gerfen and Young, J. comp. Neurol, 2013: 521(8):1844-66.

Disclosures: **C.L. Irles:** None. **V.M. Lara:** None. **M.C. Avila:** None. **H. Barrio:** None. **E. Ramos:** None. **T. Morales:** None. **M.A. Avila-Rodriguez:** None. **L. Zhang:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.02/PP23

Topic: E.03. Behavioral Neuroendocrinology

Support: Pekary Trust

Title: Over-expression of TRH and/or a TRH-like peptide in the limbic system and adrenals of a spontaneous rat mutant

Authors: *A. SATTIN[1], A. E. PEKARY[2];
[2]Res., [1]VA Greater Los Angeles Hlth., LOS ANGELES, CA

Abstracts: Thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NH2) and TRH-like peptides (X-TRH, pGlu-X-Pro-NH2), where “X” can be any amino acid residue, are endogenous neuroprotective, antidepressant, anti-epileptic, analeptic, and anxiolytic peptides. During studies of the effects of ketamine on TRH and TRH-like peptide turnover in commercially sourced male

Sprague-Dawley rats, very high levels of TRH or a TRH-like peptide, eluting right after TRH during reverse phase HPLC, was observed in limbic brain regions, even in untreated animals. All animals were hyperglycemic (serum glucose: 213 ±44 mg/dl) but euthyroid (fT4: 1.67 ±0.04 ng/ml); T3: 71.4 ±1.6 ng/ml). The TRH-like peptide being over-expressed is likely to be Thr-TRH, but mixing synthetic TRH, Thr-TRH and tissues extracts from various brain and peripheral tissues resulted in coelution of the synthetic and endogenous TRH and Thr-TRH. The ratio (mutant/normal) of the TRH-immunoreactivity (TRH-IR) derived from various brain regions eluting in the region of TRH and Thr-TRH were: hippocampus (285), periform cortex (63), posterior cingulated (57), entorhinal cortex (46), adrenals (13), frontal cortex (11), nucleus accumbens (11), striatum (11), anterior cingulate (10), cerebellum (9), amygdala (3), medulla oblongata (2), and hypothalamus (0.6). Because the mutants were euthyroid, it is unlikely that TRH is the peptide being over-expressed in the limbic system, though feedback inhibition of hypothalamic TRH biosynthesis is suggested by the reduced hypothalamic TRH levels. The dams for these males are being used to establish a breeding colony for the purpose of: (1) studying the effects of TRH-like peptide over-expression on behavioral tests for depression (Porsolt forced swim test), anxiety (elevated plus maze), and reward seeking (running wheel), (2) identifying the progenitor protein for the over-expressed TRH-like peptide and the enzymes involved in its processing to the bioactive tripeptide, and (3) determining the therapeutic potential for TRH-like peptide over-expression in the treatment of neuropsychiatric disorders such as major depression, bipolar disorder, anxiety, Alzheimer's and Parkinson's diseases.

Disclosures: **A. Sattin:** None. **A.E. Pekary:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.03/PP24

Topic: E.03. Behavioral Neuroendocrinology

Support: CONACyT Grant 127777

CONACyT Grant 179616

PAPIIT DGAPA-UNAM IN218111

PAPIIT DGAPA-UNAM IN216214

PAPIIT DGAPA-UNAM IA202314

PAEP-UNAM Travel Grant

Title: Effect of neonatal maternal separation and adolescent ethanol exposure on adult preference for ethanol

Authors: *A. T. NAVA KOPP[1], C. IRLES[1], H. BARRIO[2], L. ZHANG[1];
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Abstracts: Mechanisms underlying physiological actions of ethanol are not fully understood. However, the involvement of different types of neurotransmitter systems, such as GABA and opioids is clear. For instance, alcohol is known to increase GABAa ionotropic receptor (GABAaR) activation, but its precise molecular mechanism remains unclear. On the other hand, there are evidences suggesting 1) adolescent exposure to ethanol (AEE) increases drinking in adulthood; 2) adverse environmental conditions during development, such as neonatal maternal separation (MS) potentiate ethanol preference. A down-regulation of GABAergic transmission via GABAaR in determined brain regions has been suggested to underlie the latter phenomenon. The potentiation of the intracerebral vasopressinergic (AVP) transmission has been associated to elevated ethanol consumption, since administration of V1bR specific antagonist has been demonstrated to decrease anxious- and depressive-like behaviors, as well as ethanol consumption in alcohol preferring rats. We have previously demonstrated that MS up-regulates the AVP hypothalamic system. Hence, it is interesting to test whether the exposition to alcohol in the MS subjects can cause permanent adaptational modifications inducing up-regulation or down regulation of V1bR in the brain. The objective of the present study is to evaluate the possible role of MS and AEE in the development of alcohol preference during adult life, and to elucidate if one of the underlying mechanisms involves AVP transmission. For this purpose, alcohol intake of male Wistar rats exposed to 4 different developmental environments was quantified, experimental groups comprised (i) different rearing conditions: animal facility reared (AFR) vs. MS, and (ii) differences in ethanol exposure during adolescence: naïve (n) vs. experienced (e). Alcohol preference was measured during adulthood using a 2-bottle free choice paradigm. MS and AEE increased ethanol intake, observed in AFRe and MSn groups; however, rats from the MSe group had a significant higher average alcohol intake compared to all other groups. These results suggest that MS and AEE provoke a higher preference for ethanol in adults. Protein signaling pathways underlying this phenomenon is being carried out.

Disclosures: **A.T. Nava Kopp:** None. **C. Irles:** None. **H. Barrio:** None. **L. Zhang:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.04/QQ1

Topic: E.03. Behavioral Neuroendocrinology

Support: VA NC-PTSD, WHSD

VA Boston Healthcare System

Title: Maximum load exercise induced increases in neuropeptide Y and allopregnanolone correlate with fitness and increases in pain threshold and tolerance

Authors: *A. RASMUSSON[1], E. R. SCIOLI-SALTER[2], J. D. OTIS[2], K. ALLSUP[3], D. E. FORMAN[4];
[1]NC-PTSD/WHSD, VA Boston Healthcare System/116B-3, Boston, MA; [2]Psychiatry, Boston Univ. Sch. of Med., Boston, MA; [3]VA Boston Healthcare Syst., Boston, MA; [4]Cardiol., Harvard Med. Sch., Boston, MA

Abstracts: The high comorbidity between chronic pain and posttraumatic stress disorder (PTSD) may be explained in part by a shared underlying molecular pathophysiology. Neuropeptide Y (NPY) and the GABAergic neuroactive steroid allopregnanolone (ALLO) play critical roles in pain gating at the spinal and supra-spinal levels, are lower in the blood and cerebrospinal fluid in PTSD, and correlate negatively with PTSD symptoms. Exercise in humans and rodents increases plasma NPY and ALLO. This pilot study assessed the effects of acute exercise on NPY and ALLO levels and their relationship to pain threshold and tolerance measured by the cold pressor test in two groups: a) healthy trauma-exposed male and female Veterans, and b) Veterans with PTSD comorbid with chronic pain. A symptom-limited maximum load cardiopulmonary exercise test was performed in accordance with guidelines of the American College of Cardiology. During exercise, rates of oxygen (O2) consumption and carbon dioxide (CO2) production were calculated from continuous recordings of ventilation rate and expired fractions of O2 and CO2. Blood sampling from an IV line was performed prior to exercise, during the last minute of each exercise workload, and at 5' and 30' after exercise for measurement of NPY, ALLO, and other hormones of interest. Pain threshold and tolerance were measured via the cold pressor test 30 minutes before and 30 minutes after testing. The pilot sample (N=12) was 58.3% male (n=7), 41.7% female (n=5) with mean age of 38 years; 42% identified themselves as Black, 33% White, 8% Asian and 16.7 as "other. Half of the participants had PTSD and comorbid

chronic pain; the rest were trauma-exposed but healthy. Across all participants, peak VO2 correlated with change from baseline in ALLO ($r=0.77$, $p<.01$), NPY measured at the anaerobic threshold ($r=.61$, $p<.05$), and NPY measured 5' post-exercise ($r=.81$, $p<.01$). Pain threshold measured after exercise by the cold pressor test correlated with changes in ALLO ($r=.61$, $p<.05$) and NPY ($r=.81$, $p<.01$), but not cortisol or DHEA. A regression model in which NPY and ALLO changes predicted post exercise pain threshold was significant: $F(2,9) = 13.38$, $p<.01$. Exercise-induced changes in NPY and pain tolerance were also correlated ($r=.64$, $p<.05$). This is the first study of exercise-associated increases in the anti-stress, anti-nociceptive compounds NPY and ALLO in healthy trauma-exposed participants compared to persons with comorbid PTSD and chronic pain. As exercise training increases VO2max, we are now investigating whether it will also increase the capacity for release of these protective anti-nociceptive molecules_with possible resultant reductions in pain in CP/PTSD.

Disclosures: A. Rasmusson: None. **E.R. Scioli-Salter:** None. **J.D. Otis:** None. **K. Allsup:** None. **D.E. Forman:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.05/QQ2

Topic: E.03. Behavioral Neuroendocrinology

Title: Gender differences in the effect of perinatal high sugar diet on spatial cognitive behavior of adolescent rats

Authors: *I. ZARCO DE CORONADO, S. MOSSO-MENDOZA;
Fisiologia, UNAM, Mexico DF, Mexico

Abstracts: We described that individuals perinatally exposed to high sucrose diet have an increased risk of obesity and dyslipidemia. This study was undertaken to determine whether the perinatal administration of high sugar diet would affect spatial learning/memory behavior. Also, we would like to know if the coffee can improve this cognitive function. Mothers and the after weaning tested offspring were exposed to water (controls) or 20% sucrose diets since gestation day 14 to the 34 postnatal (PN) day. From PN28 day the offspring were tested 3 days in a maze (8 arms) and the latency to go out was determined. After the second test, coffee (25mg/dl) was added for 24 hrs to the drinking solution. Male control and experimental rats did not show significant differences in the latency the first test day but the female experimental rats showed

significant (P= 0.03) increased latency in the maze solution. Second test day males and females showed similar significant (P= 0.01) decreased latency to go out (learning). After coffee both groups presented no significant increase in latency. Thus our results indicate that high sugar diet affects not just metabolic but also cognitive functions in rats in a sex dependent action.

Disclosures: I. Zarco de Coronado: None. **S. Mosso-Mendoza:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

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Program#/Poster: 642.06/QQ3

Topic: E.03. Behavioral Neuroendocrinology

Support: CONACyT Grant 127777

CONACyT Grant 179716

PAPIIT DGAPA-UNAM IN128111

PAPIIT DGAPA-UNAM IN216214

Title: Hypertonicity increases arousal and anxiety following predator exposure: The direct and indirect modulation of hypothalamic vasopressin containing magnocellular neurosecretory neurons on Fos expression in limbic regions in rat

Authors: *E. VAZQUEZ-JUAREZ[1], F. JÁUREGUI-HUERTA[2], V. S. HERNÁNDEZ[1], L. ZHANG[1];
[1]Fac. of Medicine, Physiol., Univ. Nacional Autonoma De Mexico, Mexico City, Mexico; [2]Dept. de Neurociencias, Ctr. Universitario de Ciencias de la Salud, Univ. de Guadalajara, Guadalajara, Mexico

Abstracts: Hypothalamic vasopressin containing magnocellular neurosecretory neurons (VP-MNNs) are potently upregulated by hypertonicity. Traditionally, it has been considered that this population of neurons almost exclusively projects to the posterior pituitary. However, recent results from our group, together with other few groups, have challenged this notion. We have found by *in vivo* juxtacellular labeling, retrograde tracing and anatomical analysis that a subpopulation of MNNs possess important axonal branches from/near MNN-somata projecting intracerebrally, especially to the limbic regions, such as ventral hippocampus (1), amygdala (2),

locus coeruleus (3) and lateral habenula (4). However, the functional implications of these intracerebral projections remain elusive. In order to assess whether a potent upregulation of this VP-MNN population modulates predator-fear processing and the neuronal activation weights in different nodes of the so called "survival circuit", we used a live-predator (cat) exposure test in rats the assess the changes in behavior and the neuronal activation weights in hypothalamus, amygdala, locus coeruleus, hippocampus, striatum and thalamus, under two conditions: basal and hypertonicity, i.e. 900mM saline, 2% b.w., injected under sevoflurane-inhalation as transient anesthesia, 30min before the behavioral test. Ethological analysis showed significant behavioral modifications in "hypertonicity" group, including a four-fold increase of freezing behaviour. Immunohistochemical assessment of C-Fos expression showed generally sharpened patterns in amygdala, thalamus and hypothalamus. The Fos expression in the basolateral (BLA) and corticomedial (CoMA) nuclei of the amygdala were significantly attenuated, while the expression of central and medial nucleus (the postero-dorsal division (MeApd)) were increased. Remarkable increase of Fos+ nucleus counts was also observed in the locus coeruleus (LC). Immunohistochemical analysis with light and electronic microscopy has showed increased VP immunopositve fibers in MeApd, CeA and LC, where VP containing type I synapse were found. Our data, together with recent findings reported in the literature, suggest that the up-regulation of the VP-MNNs exert strong modulatory effects on fear processing. 1. Zhang L.& Hernandez VS. Neuroscience (2013)228:139-62. 2. Hernandez VS & Zhang L. SfN 2012, abstract. 3. Zhang L. SfN 2008, abstract. 4. Zhang L., Vazquez-Juarez E., Hernandez V. S. SfN 2014, abstract.

Disclosures: E. Vazquez-Juarez: None. **F. Jáuregui-Huerta:** None. **V.S. Hernández:** None. **L. Zhang:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

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Program#/Poster: 642.07/QQ4

Topic: E.03. Behavioral Neuroendocrinology

Support: NIMH R01 MH084966

DARPA Grant W911NF-10-1-0059

Title: Does maternal ghrelin drive transgenerational change in emotional processing and stress responsivity?

Authors: *G. LEE[1], K. A. GOOSENS[2];
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Abstracts: Stress during pregnancy can have a profound impact on the emotional state of both the mother and offspring. A tremendous amount of work has focused on stress hormones of the hypothalamic-pituitary-adrenal (HPA) stress axis as the primary cause of such changes. However, the Goosens lab has recently identified a critical role for ghrelin and growth hormone in affective dysregulation following chronic stress, acting independently of the HPA axis. Because hunger, a stress state that elevates ghrelin in humans, has been closely linked to transgenerational changes in stress responsivity and health, we hypothesize that elevated maternal ghrelin signaling during pregnancy may give rise to depressive behaviors in the mother, and also alter aversive processing and stress responsivity in the offspring. Here, we exposed rat dams to an agonist of the ghrelin receptor (growth hormone secretagogue receptor, or GHSR), during pregnancy, mimicking the prolonged enhancement of ghrelin that is observed in chronically stressed animals. In the dams and adult offspring, we found behavioral deficits including anhedonia (sucrose preference), dysfunctional exploratory behavior in the open field and elevated plus maze, and deficits in fear learning (auditory Pavlovian fear conditioning). We are currently exploring changes in endogenous ghrelin-growth hormone signaling that may account for the dysfunction in neural circuits involved in emotional processing. These data indicate a role for circulating ghrelin in the emotional state of both mothers and their offspring. Ghrelin-growth hormone signaling in the brain is a potential developmental risk factor for emotional processing disorders, and provides a potential therapeutic target that may improve the quality of life of people that suffer with poor stress coping.

Disclosures: G. Lee: None. **K.A. Goosens:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.08/QQ5

Topic: E.03. Behavioral Neuroendocrinology

Support: FAPESP

Title: Activation of glucocorticoid and NMDA receptors in the prelimbic prefrontal cortex decreases the expression of contextual conditioned fear in rats

Authors: *F. M. REIS[1,2], R. C. ALMADA[3], M. V. FOGAÇA[3], M. L. BRANDÃO[1];
[2]Psychology, [3]Pharmacol., [1]Univ. of São Paulo, Ribeirao Preto, Brazil

Abstracts: Glutamatergic abnormalities and functional alterations of the medial prefrontal cortex (mPFC) have been associated to several psychiatric disorders such as anxiety. Decreased NMDA receptor activity and potentiation of glutamatergic neurotransmission at non-NMDA receptors in the mPFC have been linked to behavioral maladjustment. It is also recognized that stress and changes on circulating glucocorticoids concentrations induce changes in glutamate synapses and circuitry, and this in turn can modify mental states. Although it is known that glucocorticoids can influence glutamate release in the mPFC, the role of mineralocorticoid (MR) or glucocorticoid receptors (GR) and the interaction between corticosteroid effects and glutamatergic activity in the mPFC on the expression of conditioned fear remain to be elucidated. The present study investigated the role MR and GR located in the prelimbic cortex (PrL) on the expression of freezing response of rats subjected to contextual fear conditioning. The effects of enhanced glutamatergic transmission at NMDA receptors on fear expression were also evaluated. Wistar male rats received vehicle or different doses of MR or GR antagonists bilaterally into the PrL and were exposed to a context previously paired with footshocks. The results showed that administration of the MR antagonist reduced freezing expression while the GR antagonist produced no effects. Previous administration of GR antagonist into the PrL abolished the anxiolytic effects induced by MR antagonist in this region. Additionally, corticosterone administration in the PrL reduced the expression of conditioned fear, an effect abolished by previous GR antagonist administration. Administration of NMDA in the PrL prior to the test also decreased conditioned fear expression. Thus, these findings suggest that the modulation of conditioned fear in the PrL is partly influenced by GR activation highlighting the importance of this mechanism in behavioral adaptation. The results concerning NMDA effects in the PrL will be useful in future experiments to explore possible interactions between glucocorticoids effects and the glutamatergic system as an underlying mechanism involved in the non-genomic effects induced by GR activation.

Disclosures: F.M. Reis: None. **M.L. Brandão:** None. **R.C. Almada:** None. **M.V. Fogaça:** None.

Poster

642. Hormones and Cognition

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Program#/Poster: 642.09/QQ6

Topic: E.03. Behavioral Neuroendocrinology

Support: CONACYT grant 127777

CONACYT grant 179616

PAPIIT-DGAPA-UNAM IN216214

Title: Límbic-Region projections of vasopressin containing mangocellular neurosecretory neurons revealed by *in vivo* juxtacellular recording and anatomical analysis: its rhythmic changes under osmotic stress and implications on predator fear processing

Authors: *L. ZHANG, E. VAZQUEZ-JUAREZ, V. S. HERNÁNDEZ;
Facultad de Medicina, Physiology, Medicine, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstracts: Hypothalamic vasopressinergic mangocellular neurosecretory neurons (VP-MNNs) are well known to release their peptidergic contents from neurohypophyseal axonal terminals into the circulation as well as to release VP locally from their dendrites serving as powerful autocrine and paracrine signals to their neighboring neuronal populations. However, the possible crosstalk between these MNNs population and distant neuronal populations has not yet been explored. In order to assess whether this subcortical neuropeptidergic system also exerts fast/precise influence to the limbic regions, we used a combination of electrophysiological approaches, including *in vivo* juxtacellular recording, labeling and frequency analysis, *in vitro* acute slices patch-clamp recording and pharmacological tools, fluorogold retrograde tracing, immunohistochemistry, confocal imaging, electron microscopy and behavioral tests as our study strategy. We show an *in vivo* labelled PVN VP-MNN possessing multiple axons and other axons branching very near the soma. Beside of the neurohypophyseal axon, neurobiotin labelled axon segments/terminals were found in the preoptical area, anterior hypothalamic area (AHA), suprachiasmatic nucleus, in the thalamus and the lateral habenula (LHb). Frequency analysis of LFP recorded in the PVN showed clear theta rhythms (7-9 HZ) in the region and the osmotic stressor increased the theta power and shifted the frequency band to 8-10 Hz. To evaluate the functional consequence of those intracerebral projections of the MNNs, we used predator fear behavioural test and c-Fos expression to assess the neural circuits involved in fear processing under osmotic stress. Our data showed a four-fold increase of freezing behaviour and modified Fos expression patterns in lateral septum, AHA, ventromedial hypothalamus, amygdala (MeApd, CeC and BLA), locus coeruleus and LHb. These findings clearly indicate a regulatory role of VP-MNNs in stress coping.

Disclosures: **L. Zhang:** None. **E. Vazquez-Juarez:** None. **V.S. Hernández:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.10/QQ7

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH INBRE Grant 2P20RR016462

Title: Testosterone replacement restores spatial working memory in castrated adult male rats

Authors: *B. CULLEN[1], C. G. BATSON[2], S. C. SPILLANE[2], B. A. WAGNER[2], M. D. SPRITZER[2];
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Abstracts: Testosterone declines among men with increasing age, and some evidence indicates that low testosterone levels lead to an increased risk of dementia. Therefore, testosterone replacement may be useful for the treatment of age-related memory loss, but the role of testosterone in regulating memory remains controversial. We previously demonstrated that a high physiological dose of testosterone (0.500 mg/rat) improved spatial working memory among castrated male rats relative to a castrated control group. Using the water maze, we found that only a relatively low dose of testosterone was needed to restore spatial memory. It remained to be determined whether testosterone had dose-dependent effects on spatial working memory. Therefore, the current study involved testing a wide range of testosterone doses for their effects on the performance in a working-reference memory version of the 8-arm radial maze. Castrated adult male Sprague-Dawley rats (N = 13-15/group) were either given daily s.c. injections of drug vehicle (castrated control and sham-castrated control) or one of five doses of testosterone propionate (0.0625, 0.125, 0.250, 0.500, and 1.00 mg/rat). Injections started 7 days before the first day of maze testing and continued through the 25 days of acquisition trials on the maze. Four arms of the maze, chosen at random for each rat, were consistently baited on each day of testing. Testing proceeded until all food rewards were retrieved or 10 min had elapsed. Working memory errors were defined as repeated visits to the same arm within a day of testing, and reference memory errors were defined as first visits to arms that were never baited. Rats showed significant reductions in working memory errors and reference memory errors over the course of testing (both $P < 0.005$), indicating that all groups were able to learn the task. We found that testosterone had a significant effect on working memory errors ($P = 0.041$), with all groups except the 0.250 and 1.000 mg/rat doses performing significantly better than the castrated control

group. In contrast, there was no significant effect of testosterone on spatial reference memory. There were also no significant day x treatment interaction effects for either working memory or reference memory errors. These results indicate that testosterone replacement can restore spatial working memory among castrated male rats, but these effects showed complex dose-dependent effects which may explain some discrepancies among past studies. Importantly, the one supra-physiological dose of testosterone that we used (1.00 mg/rat) did not restore spatial memory function, suggesting that physiological doses may have the most therapeutic value.

Disclosures: B. Cullen: None. **C.G. Batson:** None. **S.C. Spillane:** None. **B.A. Wagner:** None. **M.D. Spritzer:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.11/QQ8

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH grant MH077687

Title: Neuroendocrine response to extreme challenge

Authors: *V. J. MEYER, Y. LEE, E. SHIRTCLIFF;
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Abstracts: Skydiving is an extreme challenge which activates stress-responsive biomarkers. Our aim was to broadly characterize the neuroendocrine response to skydiving, and to examine how reactivity may be moderated by previous experience or gender. Prior research on laboratory stressors has shown evidence for habituation to a stressor with repeated exposure. Sex differences also emerge, with males demonstrating greater cortisol reactivity to laboratory stressors than females. We hypothesized that participants would show reactivity to the challenge by demonstrating increases in cortisol, DHEA, and testosterone. We further hypothesized that males would have greater responses to the jump across these three stress-responsive biomarkers. We collected five saliva samples from 44 skydiving participants. Saliva was assayed using enzyme immunoassays. Hierarchical linear modeling was utilized to model the hormone response. Cortisol (β=.42, $p<.001$), testosterone (β=.17, $p<.001$), and DHEA (β=.32, $p<.001$) all demonstrated reactivity to the jump. Experienced jumpers showed less cortisol reactivity (β=-0.41, $p<.05$) and faster recovery (β=-0.37, p0.05). Sex differences emerged in testosterone levels

(β=-1.38, p<.001), but not reactivity or recovery (p's<0.05). There were no sex differences in cortisol or DHEA levels or responses (p's>0.05). Our findings of hormonal reactivity to skydiving show that, across several biomarkers, the body mounts a neuroendocrine response to deal with the stressor. This is in line with previous research which demonstrates the utility of skydiving as a model of an extreme challenge. Contrary to hypotheses, we did not find sex difference in cortisol response to the jump. The similarity of hormone response profiles in males and females may indicate that skydiving activates the HPA and HPG axes in a non-sex-specific manner. The slightly different profile among experienced jumpers indicates that skydiving is a reliable activator of the HPA and HPG axes, even with repeated exposures; however, experience may modulate the response.

Disclosures: **V.J. Meyer:** None. **Y. Lee:** None. **E. Shirtcliff:** None.

Poster

642. Hormones and Cognition

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Topic: E.03. Behavioral Neuroendocrinology

Support: Oberlin College Grant-in-Aid

Title: Blocking luteinizing hormone but not gonadotropin-releasing hormone in the dorsal hippocampus rescues spatial memory deficits in ovariectomized female rats

Authors: V. L. BURNHAM, A. GOLDBERG, *J. E. THORNTON;
Neurosci. Dept, Oberlin Col., OBERLIN, OH

Abstracts: Recent work has implicated the activity of luteinizing hormone (LH) in post-menopausal memory decline. In rodents, increasing physiological levels of LH has been shown to significantly decrease performance on spatial memory tasks. Previous research has also indicated that blockage of gonadotropin releasing hormone (GnRH) activity, which decreases LH levels, can cause amelioration of spatial memory deficits. However, it is unclear whether directly reducing activity of LH in the brain is able to rescue spatial memory deficits in individuals with high LH. To further investigate the role of hippocampal LH receptors in spatial memory deficits, female Sprague-Dawley rats were ovariectomized (ovx) and implanted with either an estradiol (E) or blank (blk) capsule. Bilateral cannulae were implanted into the dorsal hippocampus (DH). Animals received infusions of 0.9% saline (vehicle), the LH homologue hCG (human chorionic

gonadotropin), or deglycosylated-hCG (dg-hCG; an LH receptor antagonist), 3-5 hours prior to behavioral testing via Object Location Test (OLT). Consistent with previous results, estradiol enhanced spatial memory (ovx + E compared to ovx) and ovx + E animals receiving hCG infusions into the dorsal hippocampus showed a significant decrease in spatial memory compared to vehicle infusions. Importantly, infusion of the LH receptor antagonist dg-hCG into the dorsal hippocampus of ovx + blk animals caused a rescue of spatial memory deficits induced by ovariectomy. These data indicate that LH acts on the hippocampus to modulate spatial memory. Whether GnRH antagonism exerts its effects on spatial memory via its effects on LH or thru some more direct action on the dorsal hippocampus was also assessed. In the present experiments infusion of Antide via bilateral cannulae into the DH of ovx + E and ovx + blk animals 4-6 hours prior to administration of behavioral tests did not result in any significant alteration of OLT performance, when compared to vehicle infusion. These results support the hypothesis that the GnRH antagonist Antide affects spatial memory via regulation of LH levels. Together these data provide strong evidence that LH acts at the hippocampus as a key modulator of hippocampal-dependent spatial memory.

Disclosures: V.L. Burnham: None. **A. Goldberg:** None. **J.E. Thornton:** None.

Poster

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Topic: E.03. Behavioral Neuroendocrinology

Support: Ministry of Education, Singapore

Title: Toxoplasma-gondii infection shifts balance between approach-avoidance decisions towards impulsivity

Authors: *D. TAN, A. VYAS;
Nanyang Technological Univ., Singapore, Singapore

Abstracts: Foraging theories predict that foraging animals maximize their rate of energy intake per unit of time. In contrast, several empirical observations suggest that foraging behavior does not reach the adaptive perfection. Decisions made by animals are rather subject to probabilistic and reciprocal trade-offs between foraging, reproduction and predation. Interestingly, each of these trade-offs are essentially intertemporal decision problems. In this backdrop, we report our

observations about effects of a shift in trade-offs on intertemporal discounting or delay discounting. Male rats infected with a parasite *Toxoplasma gondii* are known to manifest a hyper-androgenic metabolism. This metabolic change is believed to institute behavioral changes in the host that are likely beneficial for the parasite transmission. This model has been previously used in context of parasitic behavioral changes in host. We posit that *Toxoplasma gondii* provides a serendipitous perturbation model to study effects of higher testosterone and concomitant shift to reproductive state on decision making process. We report that *Toxoplasma gondii* infection enhances preference for sooner but smaller rewards in comparison to larger but later available food. This represents a change in impulsivity of choice, but neither to hedonic value of food itself and nor in motor impulsivity. Moreover, this represents a coordinated and organized biological change, as demonstrated by specific structural change in neuronal arbors of nucleus accumbens core, but not in other brain regions important for decision making processes. Our observations provide support to the notion that life history choices and hormonal changes adaptively modulate decision making, constraining the decisions from adaptive perfection. These observations also provide putative biological substrates for modulation of economic decision making under uncertainty and delay.

Disclosures: D. Tan: None. **A. Vyas:** None.

Poster

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC Grant 400212

Title: Investigation of the roles of the hippocampus and basolateral amygdala in rapid estrogenic enhancement of social learning

Authors: *K. S. ERVIN[1], P. PALETTA[1], M. SAWULA[1], A. MOORE[1], K. SINCLAIR[1], A. PHAN[2], E. CHOLERIS[1];
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Abstracts: Social learning is a strategy by which animals acquire information from others in their social group rather than through potentially costly trial and error learning. The social transmission of food preferences (STFP) is a natural form of social learning, common in rodents,

including mice. In the STFP, a naïve observer interacts with a demonstrator that has recently fed on a novel flavoured food. The observer then tends to prefer the food it smelled on the demonstrator's breath over other novel foods. Estrogens modulate performance on this task via both genomic mechanisms (i.e. 48h after hormone manipulation) (Clipperton et al 2008, Neuropsychopharmacology: 2362-75) and rapid mechanisms. We previously found that systemic treatment with 17β-estradiol (E2) rapidly facilitated social learning within 45 min. However the brain regions involved in the rapid estrogenic enhancement of social learning in the STFP are currently unknown. The hippocampus is a likely candidate as systemic E2 rapidly enhances dendritic spine density in the CA1 hippocampus (Phan et al 2012, Neuropsychopharm: 2299), intrahippocampal E2 rapidly enhances learning in nonsocial learning tasks (Phan et al 2012, SfN abstracts: 92.12), and hippocampal lesions impair acquisition of the STFP (Alvarez et al 2001, Learn Mem: 79). Hence, we implanted female ovariectomized CD1 observer mice with bilateral guide cannulae aimed at the dorsal hippocampus. Observers were infused with 0.5µl per side of vehicle, 25, 50, or 100nM E2 15 min prior to a brief social interaction with a same sex demonstrator. Observers were immediately tested for a food preference and measurements were taken at the 30 min, 2h, 4h, 6h, and 8h intervals. The first measurement was therefore 45 min after treatment to focus on the rapid E2 effects. The STFP task was also modified to be difficult, such that vehicle treated observers typically show no social learning, in order to see any enhancing effects. Our results show that the hippocampus is likely not the site of rapid estrogen facilitation of social learning. Thus estrogens may enhance social and nonsocial learning by acting in different brain regions. Therefore in ongoing experiments, we are investigating the role of the basolateral amygdala, which has also been implicated in the STFP (Wang et al 2006, Learn Mem: 794) and other social behaviours. Using the same procedures described above, we are infusing E2 bilaterally into the basolateral amygdalae of female ovariectomized observer mice. Our aim is to further elucidate how estrogens act in the brain to facilitate social learning, as it is an adaptive and ubiquitous learning strategy. Supported by NSERC.

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Poster

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Topic: E.03. Behavioral Neuroendocrinology

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National Basic Research Program of China (2012CB945101)

Title: Patch clamp recording in hypocretin/orexin neurons in larval zebrafish (Danio rerio) *in vivo*: sensory responses

Authors: *X.-B. GAO[1], J.-L. DU[2];
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Abstracts: The zebrafish, Danio rerio, has long been a model organism widely used in the study of early development in animals. In recent years, zebrafish has emerged as a powerful model organism in the study of basic behaviors in animals highly conserved across the species from fish to mammals. The neuropeptide hypocretin/orexin (Hcrt), which was originally discovered in mammals, plays crucial roles in the regulation of homeostatic and cognitive functions in the brain. It is now clear that Hcrt expresses in the zebrafish and exerts functions relevant to what have been observed in mammals. Although ample studies have been performed to investigate the changes in activity and morphology in the Hcrt system in behaving larval zebrafish with molecular, structural and imaging approaches, the direct evidence is required to verify and understand these changes in Hcrt neurons *in vivo*. In this study, we performed whole-cell patch clamp recording in Hcrt neurons in larval zebrafish (3-7 days post-fertilization) *in vivo* and the electrophysiological responses of Hcrt neurons to sensory (light flash and sound) stimuli were examined. In larval zebrafish exclusively expressing GFP in Hcrt neurons driven by a selective Hcrt promoter (a gift from Dr. A. F. Schier at Harvard), whole-cell patch clamp recording of membrane potential, action potential and spontaneous excitatory postsynaptic currents was performed as reported by Mu et al (2012). The exposure to a light flash induced a significant inhibition in Hcrt neurons while the exposure to a sound stimulus excited these cells. The inhibition of Hcrt neurons by a light flash was due to a significant decrease in excitatory inputs onto Hcrt neurons as examined under voltage clamp mode. The responses to light and sound stimuli were not detected in a neighboring neuron without the expression of GFP, suggesting the specificity of the responses in Hcrt cells. In summary, our preliminary results indicate that the patch clamp recording in intact larval zebrafish is capable of providing an unparalleled insight into neural circuits centered on Hcrt neurons.

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Poster

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PAEP scholarship

Title: Vasopressin modulates lateral habenula network activity via both V1a and V1b receptors: Dual electrophysiological mechanisms revealed by *in vitro* whole-cell patch clamp recording

Authors: *V. S. HERNANDEZ, F. CHAY, C. IRLES, L. ZHANG;
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Abstracts: The lateral habenula (LHb) is an important epithalamic structure within the dorsal diencephalic conduction system, which controls the exchange of information between forebrain and midbrain structures. Its involvement is implicated in a variety of biological functions, including a strikingly powerful role in the regulation of midbrain monoamine release. Glutamatergic projections from the lateral hypothalamus targeting VTA-projecting neurons in the lateral habenula have recently been reported (Poller WC et al, 2013). We have also recently reported (Hernandez and Zhang, SfN, 2013) that vasopressin (VP) containing fibers from the hypothalamic paraventricular nucleus, specifically the medial magnocellular division (PVNmmd), strongly project to the medial division of the LHb. However, little is known about the vasopressin (VP) signaling pathway or the electrophysiological response in the LHb. Using whole cell patch clamp recording in an acute slice preparation of the rat LHb, we demonstrate that the application of VP (10nM) to the recording chamber induces a differential electrophysiological respons. 50% of recorded neurons increased their firing rate [post-hoc identification revealed some of these neurons to contain vesicular GABA transporter (vGAT)], 30% decreased their firing rate, and 20% had no evident response. Western blot analysis on fresh microdissected habenula revealed a remarkable expression of V1a protein (protein bands: ≈ 47 KDa as well as ≈ 70 KDa, Alomone Avr-010) and the presence of V1b protein as well (protein bands: ≈ 47 KDa as well as ≈ 70 KDa Enzo ADI-905-750), although the latter one was near the limit of detection. The ≈ 70 KDa protein band has been attributed to the glycosylation of the receptors these receptors. These data support the previously reported presence of vasopressin

receptors V1a and V1b mRNA in the habenula by *in situ* hybridization. Moreover, at the electron microscopic level, we found AVP containing fibers establishing asymmetric synapses with lateral habenular neurons (synapse n=10, 100% type I). Our data strongly suggests that the PVN VP population is an upstream element for a function of the LHb that mainly plays an activation role implicated in the promotion of negative rewards

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Poster

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Topic: E.03. Behavioral Neuroendocrinology

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Donald W. Reynolds Foundation

Title: Igf-1 regulates neuronal structure and function via rhoa/rock signaling

Authors: *N. ASHPOLE, J. E. LANDOLL, E. L. HODGES, M. C. MITSCHELEN, H. YAN, J. FARLEY, W. E. SONNTAG;
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Abstracts: Neuroendocrine regulated hormones such as insulin-like growth factor-1 (IGF-1) have an important role in learning and memory since they influence neuronal differentiation, enhance axonal path finding, and promote dendritic growth. It is generally accepted that IGF-1 exerts these actions via downstream activation of growth-stimulating signaling pathways, mainly Phosphoinositide 3-kinase/Akt. We hypothesize that in addition to the classical role of upregulating growth-stimulating pathways, IGF-1 also downregulates growth-limiting pathways such as the RhoA/Rho-associated coiled-coil containing protein kinase (ROCK) pathway. RhoA/ROCK signaling is associated with spine retraction, synapse elimination, decreased neuronal excitability, and impaired learning and memory. Thus, if IGF-1 serves as a negative regulator of RhoA/ROCK signaling, it may further promote learning and memory by preventing neurite retraction. To test this hypothesis, IGF-1 was inhibited in both cultured murine hippocampal neurons as well as in the hippocampus of IGF-1f/f mice. Following treatment,

Western blots, GTP-binding assays, and *in vitro* phosphorylation assays were used to assess RhoA/ROCK activity. Sholl analysis was employed to measure neuronal complexity following co-administration of IGF-1 modulators with pharmacological antagonists of ROCK. Spatial learning and memory was assessed using the Barnes maze and the TSE intellicage behavior testing system. Our results indicate that IGF-1 regulates the RhoA/ROCK signaling cascade. Our *in vitro* studies indicate that a reduction in IGF-1 resulted in the upregulation of RhoA/ROCK activity. No change in RhoA/ROCK protein expression was identified; however, antagonism of ROCK signaling restored neuronal growth to control levels even when IGF-1 signaling was impaired. Together, these results are consistent with a model suggesting that IGF-1 can influence neuronal structure by negatively regulating the RhoA/ROCK signaling pathway. To determine the influence this regulation may have on learning and memory, we knocked out circulating and hippocampal IGF-1 in our IGF-1f/f. This resulted in a significant decrease in spatial learning and memory. We are now examining the regulation of RhoA/ROCK in our *in vivo* mouse model.

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Poster

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Support: NIDCR grant T32 DE014320

Title: Sex differences in the response to intermittent hypoxia, a model for obstructive sleep apnea

Authors: *T. G. AUBRECHT, R. D. JENKINS, R. J. NELSON;
The Ohio State Univ., Columbus, OH

Abstracts: Obstructive sleep apnea (OSA) results from upper airway obstruction during sleep, causing repeated brief periods of low oxygen termed hypoxia. In addition to increased health risks, individuals with OSA experience impaired cognitive functions and ~50% of people presenting with OSA are also diagnosed with depression and anxiety. OSA is more prevalent in men than premenstrual women, but the mechanism for this sex difference remains unspecified. Gonadal hormones are one obvious mechanism underlying sex differences in OSA. Sleep apnea

is more frequently reported in postmenopausal women than premenopausal women, and both prevalence and severity of OSA increase after menopause, even after correcting for body mass index. Additionally, exogenous testosterone treatment has been associated with exacerbating OSA in men. Considered together, these data suggest a role for gonadal hormones in the development of OSA that could also affect OSA associated symptoms. OSA can be modeled in mice using intermittent hypoxia (IH). Such research indicates sex differences in the cellular response to IH, especially protection from IH in intact females that is negated by ovariectomy. Therefore, we hypothesize that female gonadal hormones may protect mice from IH induced behavioral and hippocampal morphological changes. To test this hypothesis we exposed intact or gonadectomized male and female mice to IH (15 cycles/h, 8 h/day, FIO2 nadir of 5%) for a total of 30 days. During the final four days of IH mice were tested for anxiety- and depressive-like behaviors during the dark phase. After cessation of IH exposure mice were also tested on the Barnes maze and passive avoidance tests to asses learning and memory. Ovariectomy in female mice paired with IH treatment impaired spatial learning and memory compared to all other female groups. Intact male mice in IH treatment also displayed significantly impaired learning and memory compared to intact or castrated male mice exposed to room air. These data suggest that female sex hormones provide protection against IH induced deficits, whereas male sex hormones partially exacerbate IH induced deficits. Spatial learning and memory deficits should be mirrored by changes in cornu ammonis (CA) 1 hippocampal morphology. Work is currently underway to assess the benefits of estrogen replacement in protecting against IH-induced cognitive deficits. Understanding the role of sex hormones in the development of OSA or the worsening of OSA symptoms could provide support for hormone treatment in a clinical setting.

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Poster

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Topic: E.03. Behavioral Neuroendocrinology

Title: Sex-differences in hippocampal ca1 intrinsic excitability and peripheral responses in a high-energy diet-induced prediabetic rat model

Authors: *E. UNDERWOOD, L. THOMPSON;
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Abstracts: Alarming increases in fat intake and rising obesity/insulin-resistance/type-II diabetes necessitate focusing on dietary effects on brain and cognitive function. Diabetes affects over 10% of the adult U.S. population, with an additional 35% considered prediabetic (50% when > age 65). Rats exhibit enhanced memory retention when intrahippocampal insulin is administered immediately post-acquisition (Babri et al., 2007). Acquisition and consolidation of many memory tasks reduces Ca2+ dependent afterhyperpolarizations (AHPs) of hippocampal pyramidal neurons (see Farmer & Thompson, 2012), increasing intrinsic excitability. Here we investigated the effects of a high-energy (HE) diet in a prediabetic state on measures of CA1 hippocampal neuron intrinsic excitability, on performance in a novel object recognition (NOR) task, and on peripheral blood chemistry measures associated with diabetes. Male and female adult Long-Evans rats (3-4 mo) were fed from weaning either a control (14% fat, 64.8% carbohydrate, 21.2% protein) or a HE diet (57.6% fat, 26.8% carbohydrate, 15.6% protein) for 6-10 wk prior to experimentation. The control diet was augmented with casein and coconut oil to achieve the desired HE ratios without altering other nutrients. Episodic memory was assessed in a NOR task, serum samples were collected for biochemical analyses, and vaginal cytology was performed on female subjects to assess estrus. *In vitro* current-clamp recordings were made to assess post-burst AHPs, accommodation, and passive membrane properties. After baseline recordings, slices were perfused with 12.5 nM insulin and all measures repeated to directly assess insulin-sensitivity of CA1 pyramidal neurons. Baseline sex-dependent differences were observed in weight gain and other measures, in NOR, and in hippocampal excitability. Females had reduced AHPs compared to males. After 6 wk on the HE diet, this sex-dependent intrinsic excitability profile reversed, with AHPs of CA1 neurons from both sexes significantly enhanced. HE diet female neurons had significantly larger mAHPs and longer duration sAHPs than those from males, which were also less excitable after 6 wk or more on the HE diet. Insulin-sensitivity was significantly reduced by the HE diet before traditional peripheral measures indicative of diabetes were altered. Since there is an increased prevalence of Alzheimer's in the diabetic population, these sex-dependent neurophysiological changes in hippocampus, occurring early in the pre-diabetic state may have significant consequences in brain development, brain aging and cognitive decline, which are being longitudinally followed in the next phase of our studies.

Disclosures: E. Underwood: None. **L. Thompson:** None.

Poster

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Title: Low-to-severe neurotoxicity and thyroxine deficits in the absence of behavioral changes in rats with developmental hypothyroxinemia

Authors: *P. R. MOUTON[1], G. S. TRAVLOS[2], C. J. PRICE[4], J. HARRY[3];
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Abstracts: Thyroid hormones (TH) regulate biological processes that are implicated in neurodevelopmental disorders and behavioral aberrations, yet animal models with less than 50% disruption in TH levels rarely manifest behavioral changes. To compare neuroanatomical and behavioral effects associated with tetrachloroazobenzene (TCAB)-induced developmental hypothyroxinemia, male rats were exposed to TCAB (0.1, 1.0, or 10.0 mg/kg/day) or vehicle (corn oil) from gestation day 6 to postnatal-day (PND) 21. Endpoints included serum TH levels in dams (PND 4) and offspring and a standard behavioral battery between ages PND 21 and 150. The left cerebrum was sectioned in the parasagittal plane and stained with histochemical and immunologic-based methods for qualitative and stereological evidence of neuropathology at PND 21 and 150 and the right cerebellum was Golgi-stained for morphological analysis of Purkinje cells (PC) at PND 21. TCAB significantly reduced serum thyroxine (T4) in dams and offspring in a dose-dependent manner at PND 21 with normalization by PND 150, and no changes in 3,5,3'- triiodothyronine (T3) or thyroid stimulating hormone (TSH) at either time point. Standard qualitative examination of the left cerebrum showed no TCAB-related neurodegeneration or neurogliosis for any TCAB dose at PND 21 and PND 150 while more rigorous qualitative examination at PND 21 revealed glial cell disturbances in hippocampus and frontal lobe, including thinner astrocytes processes with reduced density at the two higher doses and reduced complexity of microglia processes at the highest dose. Golgi-stained sections at PND 21 suggested about a 15% reduction in dendritic branching area of PCs at the highest TCAB dose (10 mg/kg/day). Blinded computerized stereology confirmed significant reductions in total numbers of neurons in hippocampus at both PND 21 and 150. Despite evidence of neurodegeneration and glial cell disturbances there were no behavioral changes related to TCAB exposure between PND 21 and 150. Reduced complexity of process morphology of astrocytes

and microglia indicate TCAB-mediated developmental delay, while adult deficits in total number of hippocampal neurons at PND 21 and 150 show developmentally initiated changes not mitigated by a return to normal T4 levels. These findings suggest that standard neurobehavioral and neuropathological assessments lack sensitivity to detect long-term changes induced by developmental T4 deficits in TCAB-exposed male offspring. The demonstrated sensitivity of hippocampal neurons and glial cells to TH-disrupting chemicals support inclusion of these endpoints in future assessments of developmental neurotoxicity.

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Poster

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Magnus Bergvalls Foundation

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Title: Genetic deletion of SNAP-25b: A new mouse model of genetic diabesity

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Abstracts: BACKGROUND: The relationship between the release machinery of neuroendocrine messengers and the development of diabesity has been poorly explored. The SNARE complex is conceived as a master regulator of exocytosis of neurotransmitters and other signaling molecules involved in the regulation of body weight, energy expenditure and glucose homeostasis. AIM: We therefore hypothesize that a genetic predisposition for an imbalance in the release machinery controlling the release of signalling molecules triggers cognitive and/or emotional disorders secondary to the onset of diabese phenotypes. METHODS: Five-week-old male and female SNAP-25b-deficient mice and C57Bl6 controls were assigned either to a high-fat/high-sucrose diet or to a control chow diet for 7 weeks. We monitored: 1) Body weight, food intake, and water intake; 2) Lipid homeostasis and the serum levels of adipokines released; 3) Glucose tolerance and the release pattern of insulin; 4) Leptin receptor content, AMPK-alpha1/2 and ERK1/2 phosphorylation in brain areas involved in the regulation of energy balance (hypothalamus) and in emotional aspects of eating behavior (hippocampus and frontal cortex); 5) Depression and anxiety processes linked to genetic- and/or diet-induced obesity. RESULTS: SNAP-25b deletion elicits vulnerability to develop early-onset diabesity with associated deranged lipid homeostasis, adipocyte hypertrophy, glucose intolerance, hepatic steatosis and hypothalamic dysfunction; all primary features of the human metabolic syndrome. The lipotoxic diabese phenotype, the consequence of SNAP-25b-deficiency, was associated with depression-like behavior and an anxiety-related phenotype. CONCLUSION: An intact release of signalling molecules involving SNAP-25b is essential to prevent major metabolic abnormalities related to eating habits as well as ensuing depression and anxiety disorders. These findings open up for novel therapeutic strategies to prevent development of major metabolic abnormalities and their secondary consequences, such as mood disorders.

Disclosures: I.V. Acebes: None. **T. Daraio:** None. **K. Brismar:** None. **S. Ögren:** None. **H. Tomas:** None. **C. Bark:** None.

Poster

642. Hormones and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 642.22/QQ19

Topic: E.03. Behavioral Neuroendocrinology

Support: Wellcome Trust

Medical Research Council UK

Title: Fetal testosterone is associated with brain white matter volumetric sex differences in adolescents

Authors: *A. N. RUIGROK[1], M.-C. LAI[1,2], R. J. HOLT[1], M. V. LOMBARDO[1,3], B. AUYEUNG[1,4], J. SUCKLING[5,6,8], K. TAYLOR[9], G. HACKETT[10], E. T. BULLMORE[7,8], S. BARON-COHEN[1,5];
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Abstracts: Testosterone is known to have organizational effects on brain structure and function in non-human species. Our previous research shows that variation in fetal testosterone (fT) predicts regional gray and white matter (GM; WM) volume in pre-pubertal boys (♂). In a follow-up study we investigate how variation in fT predicts regional WM volume in ♂ and girls (♀) aged 13-17 years old. We hypothesized that volume of areas associated with fT level overlap with regions showing sex differences (SD) in a congruent direction as found in the child WM volume study, higher fT in boys overlaps with SD regions larger in girls in an independent group of adolescents of the same age-range. T1-weighted scans were acquired of 21 ♂ and 17 ♀ (mean (M) age in years ♂ 15, ♀ 15) part of the Cambridge Child Development Project. FT was measured using radioimmunoassay from amniotic fluid samples collected between 13-20 weeks of gestation (fT range: ♂ 0.40-2.05 nmol/L; ♀ 0.15-0.40 nmol/L). Measures of handedness (H) and self-report puberty development stage (PDS) were also taken (right-H: ♂ 16; ♀ 15; PDS: ♂ 3.02, ♀ 3.51). Data from the NIH Pediatric Repository of adolescents (♂ 88; ♀ 98) between 13-

17 years (M age in years ♂ 15, ♀ 15; right-H: ♂ 75, ♀ 90; PDS: ♂ 2.74, ♀ 3.28) were used as an age-matched group to identify normative SD. The Template-O-Matic Toolbox was used to create sex- and age-specific tissue prior templates. SPM8 New Segment and DARTEL were run and statistical analysis was done using general linear model. Total WM volume effect was adjusted at an individual level. FT, PDS, age, and H were regressors for the separate fT regressions and sex, PDS, age, H and scanning sites in the NIHPR SD regression. Results were thresholded at a cluster-wise topological FDR $q < 0.05$, with voxel-level threshold set at $p < 0.01$. Overlap with WM tracts was identified using standard-space diffusion-based white matter tract atlas (Thiebaut-de Schotten, 2011). Areas overlapping with anterior corpus callosum, bilateral and inferior fronto-occipital fasciculi were larger in ♂ and areas overlapping with the medial fornix, internal capsule (IC), left and right medial arcuate fasciculi (AF), ILF, cortico-ponto cerebellar (CPC) and cortico-spinal (CS) tracts were larger in ♀. In ♀, no correlations between fT and WM were found. In ♂, fT positively predicted WM volume in a cluster encompassing the left IC, CS and CPC tracts, and the AF. These results (i.e., areas positively correlating with fT in ♂ overlap with an SD region larger in ♀) confirm the hypothesis. These findings provide more evidence that fT may act differently as an organizing mechanism in WM than in GM in brain development in ♂, but not in ♀.

Disclosures: **A.N. Ruigrok:** None. **M. Lai:** None. **R.J. Holt:** None. **M.V. Lombardo:** None. **B. Auyeung:** None. **S. Baron-Cohen:** None. **J. Suckling:** None. **K. Taylor:** None. **G. Hackett:** None. **E.T. Bullmore:** A. Employment/Salary (full or part-time):; GlaxoSmithKline plc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GlaxoSmithKline plc.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.01/QQ20

Topic: B.04. Ion Channels

Support: NIH grants GM88804

NIH grants GM80255

Title: Distinct membrane excitability control of Ca2+ handling in identified synaptic terminals in *Drosophila* neuromuscular junctions

Authors: *X. XING[1], C.-F. WU[2];
[1]Dept. of Biol., [2]Univ. of Iowa, Iowa City, IA

Abstracts: The *Drosophila* larval neuromuscular junction (NMJ) is ideal for investigating the genetic mechanisms for synaptic terminal excitability control and Ca2+ handling. By expressing the Ca2+ indicator GCaMPs in motor neurons, we performed genetic dissections to investigate how ion channels regulate synaptic Ca2+ dynamics. Our results demonstrate striking effects of several well-established Na+ and K+ channel mutations on synaptic Ca2+ accumulation. We found that Na+ channel mutations of the paralytic gene and K+ channel mutations of the Shaker locus exerted preferential effects on the tonic and phasic motor synaptic terminals. The hyperexcitable allele of paralytic (bss) as well as several alleles of Shaker enhanced the rise, but not the decay, kinetics of the Ca2+ transients. However, the decay time was impeded by inhibition of Ca2+ extrusion with high pH saline, confirming that ion channels control Ca2+ influx rather than clearance. At low external Ca2+ levels (0.1 and 0.5 mM), both types of mutations markedly enhanced the Ca2+ transients in phasic synaptic terminals at lower frequency stimulation (2 - 20 Hz), whereas the enhancement in tonic synapses were detected at higher frequency (20 - 40 Hz). Applications of available GCaMP variants (GCaMP1, GCaMP6 and myrGCaMP5) confirmed the distinct properties of tonic and phasic motor terminals, regardless of their subcellular localization and sensitivity to cytosolic Ca2+. Our findings reveal basic differences in Ca2+accumulation and frequency dependence of different *Drosophila* NMJ synapses, reflecting their distinct frequency domains of operation. These findings provide baseline information for inferences of GCaMP signals during *in vivo* studies of neuronal activity.

Disclosures: X. Xing: None. **C. Wu:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.02/QQ21

Topic: E.04. Autonomic Regulation

Title: BDNF's role in the excitatory-inhibitory imbalance within the solitary tract nucleus during the critical period of postnatal development in the rat

Authors: *X.-P. GAO, M. T. WONG-RILEY;
Cell Biology, Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstracts: Previously, we found a transient imbalance between suppressed excitatory postsynaptic currents (EPSCs) and enhanced inhibitory postsynaptic currents (IPSCs) on postnatal days (P) 12-13 in hypoglossal motorneurons of rats. This is a critical period when abrupt neurochemical, metabolic, ventilatory, and physiological changes occur in multiple respiratory-related nuclei of the rat's brain stem, but its mechanism is poorly understood. Interestingly, a reduced expression of brain-derived neurotrophic factor (BDNF) and its high affinity tyrosine kinase B (TrkB) receptors also occurred in the same nuclei during the critical period. As BDNF normally enhances excitatory and suppresses inhibitory synaptic transmission, we wished to test our hypothesis that *in vivo* application of a TrkB agonist (7,8-DHF) will partially reverse the imbalance during the critical period. Using whole-cell patch-clamp recordings, we studied nucleus tractus solitarius (NTS) neurons in brain slices of normal rats and those that received 7,8-DHF *in vivo*. Results indicate that: 1) the amplitude and frequency of spontaneous EPSCs (sEPSCs) in normal NTS neurons were significantly reduced at P12-13; 2) the amplitude and frequency of spontaneous IPSCs (sIPSCs) in normal NTS neurons were significantly increased at P12-13; 3) after 7,8-DHF application *in vivo* (5 mg/kg, i.p. once a day at P10 and P11), the amplitude and frequency of sEPSCs were increased by 35.22% and 44.71%, respectively, during the critical period (P12-P13); and 4) the amplitude and frequency of sIPSCs were decreased by 43.66% and 25.26%, respectively, during the critical period. Thus, our results documented the existence of an excitatory-inhibitory synaptic imbalance within the NTS of normal rats during the critical period. They also substantiated BDNF's role in the synaptic imbalance within respiratory-related nuclei during this time. This may have significant implication for Sudden Infant Death Syndrome (SIDS).

Disclosures: X. Gao: None. **M.T. Wong-Riley:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.03/QQ22

Topic: E.04. Autonomic Regulation

Support: NIH Grant NS087828

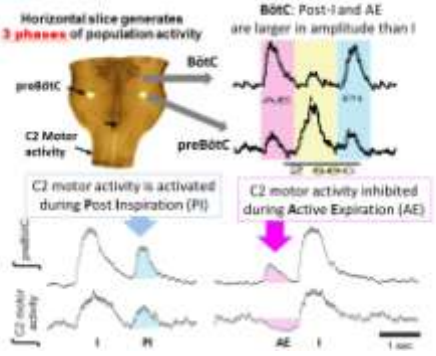
NIH Grant HL090554

NIH Grant HL107084

Title: Respiratory rhythm generation emerges from an excitatory column encompassing the preBötzinger and Bötzinger Complex

Authors: *T. ANDERSON[1,2], A. J. GARCIA, III[1], J.-M. RAMIREZ[1,3];
[1]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA; [2]Neurobio. and Behavior, [3]Neurosurg., Univ. of Washington, Seattle, WA

Abstracts: The three phases of mammalian respiration, inspiration (I), post-inspiration (PI), and active expiration (AE), are thought to be generated by interacting neuronal populations along the ventral respiratory column (VRC) that extend bilaterally along the ventral lateral medulla. Computational models based on *in vivo* and *in situ* studies propose that the three phases of breathing are generated by a ring of inhibitory neurons located in the Bötzinger complex and preBötzinger complex (preBötC). This ring requires excitation from the pons. Indeed transverse medullary slices containing only the preBötC fail to generate PI and AE and continue to generate only inspiration. Here we introduce two novel mouse (p5-p10) brainstem slice preparations: a transverse slice containing the BötC, and a horizontal slice that keeps the entire VRC intact bilaterally and maintains phrenic activity to define inspiratory motor output (Fig. 1). Using these *in vitro* approaches we demonstrate that the preBötC forms a contiguous excitatory rhythmogenic column with the BötC. A confined region within the BötC spontaneously generates a PI population rhythm, which can be stimulated with 2μM norepinephrine suggesting that under more intact conditions, noradrenergic input from the pons may excite the PI population. Similar to the preBötC, the BötC can burst rhythmically in an isolated transverse slice indicating that the BötC can function as an independent oscillator. We also demonstrate that 1) blocking glycinergic inhibition in the horizontal slice results in a decreased inspiratory burst duration, while 2) completely eliminating inhibition by the additional application of gabazine results in the synchronization of the two rhythms. Thus, we propose that inhibition is responsible for establishing phase relationships between the preBötC and the BötC, but not the underlying rhythmicity. We conclude that the preBötC is not the only excitatory kernel, but instead forms an excitatory column containing the preBötC and BötC and that similar rhythm generating mechanisms determine rhythm generation along this column.



Disclosures: **T. Anderson:** None. **J. Ramirez:** None. **A.J. Garcia:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.04/QQ23

Topic: E.04. Autonomic Regulation

Support: Alexander von Humboldt foundation

DFG HU797/7-1

DFG HU797/8-1

CNMPB

Title: Optogenetic activation of Glycinergic neurons of the rostral Ventral Respiratory Column inhibits inspiration and affects respiratory phase transition

Authors: *M. G. FORTUNA[1], A.-M. BISCHOFF[1], S. KÜGLER[2], S. HÜLSMANN[1,3];
[1]Neuro- und Sinnesphysiologie, [2]Neurology, Viral Vectors Lab., [3]Anesthesiol., Universitätsmedizin Göttingen, Goettingen, Germany

Abstracts: Neural circuitry controlling breathing in mammals is organized within brainstem compartments, which regulate its own activity by feedback loops, and extend from the pons to the lower medulla. The core network which generates the respiratory rhythm is distributed among adjacent, functional structures of the ventral respiratory column (VRC) in the medulla. We sought to investigate the role of a genetically defined population of Glycinergic neurons of this network, located in the Bötzinger complex (BötC) and the pre-Bötzinger complex (pre-BötC), by combining optogenetics with an *in situ* working heart-brainstem preparation (WHBP). Selective expressions of the Channelrhodopsin-2 (ChR2) in a anatomically defined population of glycinergic neurons was achieved by combining GlyT2-Cre mice (where Cre recombinase is expressed under glycinergic GlyT2 promoter) with Cre-inducible Adeno-Associated Virus (AAV-DIO) vectors. AAV6 virus was stereotactically injected into the rostral part of VRC of adult mice (Tg(Slc6a5-icre)121Veul). After at least 2 weeks of incubation, the effect of blue light stimulation on the inspiratory motor output was assessed in the WHBP by monitoring Phrenic Nerve Discharge (PND). We report here that selective, tonic (30-45s, 33Hz pulses, 50% duty cycle) stimulation of glycinergic neurons of the BötC and pre-BötC resulted in depression of inspiratory frequency of about 40%, independent of the level of baseline activity. The effect on the PND amplitude was much more variable with tendency for potentiation, depended on the level of baseline rhythm with the most pronounced effect on fast, low amplitude respiratory

activity. Burst stimulation (0.9s, 33Hz, at fixed interval) had the capacity to alter, reset and pace the rhythm. This work shows that glycinergic neurons located in the BötC /preBötC area of the VRC are important components of the circuitry controlling breathing; activity of these cells seems to be especially vital for the control of the duration of the expiratory period, transition from inspiration to expiration and the timing of the inspiratory output.

Disclosures: M.G. Fortuna: None. **A. Bischoff:** None. **S. Hülsmann:** None. **S. Kügler:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.05/QQ24

Topic: C.08. Ischemia

Support: Biotrofix

Ekam Imaging

Title: Recovering from isoflurane anesthesia: Evidence of reduced vascular reactivity and functional coupling using fMRI in awake rats

Authors: M. NEDELMAN[1], P. KULKARNI[2], *S. P. FINKLESTEIN[3], M. REN[3], M. DAVENPORT[3], C. F. FERRIS[2];
[1]Ekam Imaging, Boston, MA; [2]Northeastern University, Ctr. for Translational NeuroImaging, Boston, MA; [3]Biotrofix, Inc, Needham, MA

Abstracts: Non-clinical studies in juvenile animal models show that exposure to some anesthetics and sedatives is associated with memory and learning deficits and other neurodegenerative changes in the central nervous system. We examined changes in brain activity in response to breathing 5% CO2 or foot shock in sham operated and stroke rats looking at vascular reactivity and functional coupling at different times following insult. Studies were performed using BOLD imaging on awake animals at 7 Tesla on days 3, 7 and 14 post surgery. Stoke was induced by right medial cerebral arterial occlusion in a 90 min surgical procedure using 5% isoflurane anesthesia. The reduced BOLD signal change in response to foot shock was not unexpected given the severity of the stroke although it was noted that the diminished activity was bilateral and not just confined to the side of the stroke. Most surprising was an unexpected absence in vascular responsivity to CO2 challenge. Data from day 3 indicated both sham and

stroke animals were unresponsive to 5% CO2 challenge that persisted through day 7. On day 14 the changes in brain activity to response to 5% CO2 and foot shock appeared comparable for sham and stroke animals. Raising questions about unanticipated effects of prolonged isoflurane anesthesia during surgery and possible cognitive impairment.

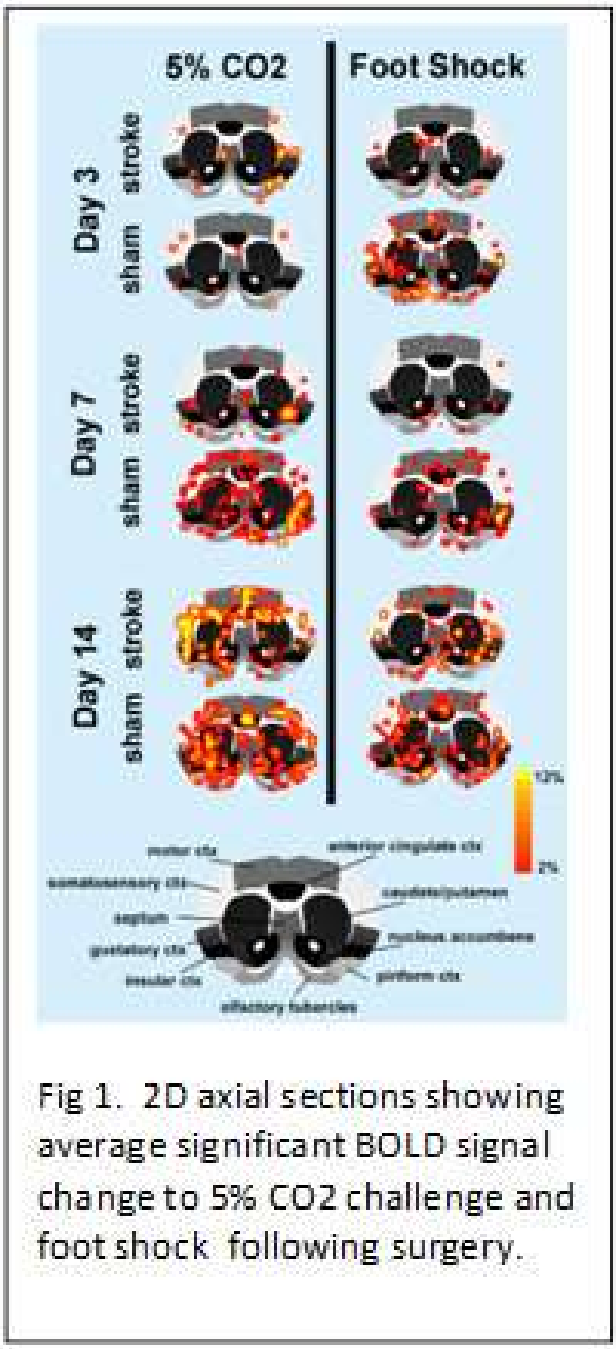


Fig 1. 2D axial sections showing average significant BOLD signal change to 5% CO2 challenge and foot shock following surgery.

Disclosures: **M. Nedelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **M. Davenport:** None. **M. Ren:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

Biotrofix. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging, Ainimal Imaging Research. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **S.P. Finklestein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biotrofix.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.06/QQ25

Topic: E.04. Autonomic Regulation

Support: SFB-TRR 58/B05

Title: The anticipation of respiratory threat impairs performance in a subsequent visual recognition memory task

Authors: *G. JURAVLE[1], P. REICHERTS[2], M. L. WEINSTEIN-RIECHMANN[1], M. J. WIESER[2], A. VON LEUPOLDT[3,1];
[1]Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Univ. of Würzburg, Würzburg, Germany; [3]Univ. of Leuven, Leuven, Belgium

Abstracts: It is well known that cognitive and emotional processes impact the anticipation and perception of bodily threat sensations, such as breathlessness. However, little is known about the reverse influence of breathlessness on cognitive and emotional processes. Here, we investigated how recognition memory (RM) is affected by the perception versus the anticipation of a respiratory threat (resistive-load-induced breathlessness, RLIB). For this, 35 participants were presented with 180 pictures of positive/neutral/negative content under conditions of either constant moderate RLIB, temporally unpredictable highly aversive RLIB anticipation, or an unloaded baseline. Participants were unaware of the subsequent RM test, to which they were subjected approximately 90 minutes later. For the RM test, participants were presented with 180 OLD and 180 NEW pictures of matched emotional content. For each of the pictures, participants indicated by means of button presses whether they have already seen the pictures before (a 'YES' response for the OLD photos) or not (a 'NO' response for the NEW photos). We analyzed RTs, percentages of correct responses, as well as measures of signal detection theory (d prime and criterion c). As expected, both percentages of correct responses and d prime data indicated

that participants' recollection was significantly better for the OLD emotional pictures, as compared to the OLD neutral ones. Furthermore, the same dependent measures indicated that participants remembered significantly fewer pictures that they have previously seen while anticipating the unpredictable respiratory threat, as compared to the conditions of constant RLIB and baseline, with no difference between the latter. Importantly, during the anticipatory condition participants were also significantly more conservative in giving a 'YES' response, as indicated by the criterion c data. Our results thus highlight that the anticipation, and not the perception, of respiratory threat leads to significant decrements in RM for picture stimuli, as well as to a conservative decisional criterion shift. Results such as these suggest that the brain automatizes its response to a perceived respiratory threat. On the other hand, this automatic process is absent for the anticipation of respiratory threat, thus suggesting that the available cognitive processing resources are diminished. These findings are relevant in the light of cognitive deficits in patient groups who frequently anticipate and/or perceive respiratory threat, such as patients with anxiety or severe respiratory disorders (e.g., COPD).

Disclosures: G. Juravle: None. **P. Reicherts:** None. **M.L. Weinstein-Riechmann:** None. **M.J. Wieser:** None. **A. von Leupoldt:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.07/QQ26

Topic: E.04. Autonomic Regulation

Support: Intramural Research Program of the NIH/NINDS

NIH Grant R01 NS057815

NIH Grant R01 NS069220

Title: The role of synaptic inhibition in respiratory rhythm and pattern generation in brainstem *in situ*

Authors: *N. KOSHIYA[1], H. KOIZUMI[1], R. ZHANG[1], B. P. MOSHER[1], M. F. TARIQ[1], V. MARCHENKO[2], I. RYBAK[2], J. C. SMITH[1];
[1]Cell. & Systems Neurobio. Section, NIH - NINDS, BETHESDA, MD; [2]Dept. of Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstracts: Inhibitory circuit interactions within the pre-Bötzinger (pre-BötC) and Bötzinger (BötC) complexes of the mammalian brainstem have been proposed to play an important role in generating respiratory rhythm and the normal three-phase respiratory pattern, but the exact role of inhibitory interactions in these processes has not been resolved. In this study we tested the role of synaptic inhibition by pharmacologically blocking endogenous GABAergic and glycinergic receptor-mediated inhibition within the active pre-BötC/BötC circuits of arterially perfused brainstem-spinal cord *in situ* preparations from Sprague-Dawley juvenile rats (3-5 wks old). Activities of phrenic and vagus nerves as well as extracellular unit and/or neural population activities in pre-BötC or BötC circuits were recorded and analyzed. The specific blockers of $GABA_A$ (gabazine, 25 μM) and glycine (strychnine, 25 μM) receptors were microinfused together bilaterally into the pre-BötC or BötC regions through glass micropipettes (inner diameter ~15 μm, at ~30 mmHg infusion pressure) for up to 2 min to determine perturbations of respiratory circuit activity. Microinfusion sites were marked and confirmed by *post hoc* histological analyses. Microinfusion of the inhibitory receptor blockers into the BötC or pre-BötC disrupted rhythm generation with site-specific patterns of respiratory activity perturbations. Block of inhibition in the BötC caused a reduction in the amplitude of inspiratory motor activity of all recorded nerves and bradypnea with accompanying prolongation of the expiratory phase (n=9/9) and in many cases cessation of respiratory motor output (apnea, n=5/9). Microinfusion of the inhibitory receptor antagonists into the pre-BötC always elicited progressive tachypnea and decline of inspiratory discharge amplitude (n=9/9), which in some cases led to loss of respiratory motor output. Before loss of inspiratory nerve activity and during recovery from the apnea, the normal three-phase respiratory pattern was disrupted with a loss of post-inspiratory activity in recorded vagus nerve motor output and/or BötC neural activities, and integrated phrenic inspiratory discharge was transformed from a normal ramping pattern to a low amplitude square-wave discharge pattern. These results agree with those obtained in rats *in vivo* (see Marchenko *et al*., this volume). We conclude that GABAergic and glycinergic receptor-mediated inhibition in pre-BötC and BötC circuits plays a major role in the generation of respiratory rhythm and the normal three-phase respiratory pattern in the brainstem respiratory network *in situ.*

Disclosures: **N. Koshiya:** None. **H. Koizumi:** None. **B.P. Mosher:** None. **M.F. Tariq:** None. **V. Marchenko:** None. **I. Rybak:** None. **J.C. Smith:** None. **R. Zhang:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.08/QQ27

Topic: E.04. Autonomic Regulation

Support: R01 NS057815

R01 NS069220

Intramural NIH/NINDS

Title: The role of synaptic inhibition in respiratory rhythm and pattern generation *in vivo*

Authors: *V. MARCHENKO[1], N. KOSHIYA[2], T. BEZDUDNAYA[1], H. KOIZUMI[2], R. ZHANG[2], I. A. RYBAK[1], J. C. SMITH[2];
[1]Dept Neurobiology/Anatomy, Drexel Univ. Col. of Med., Philadelphia, PA; [2]Cell. and Systems Neurobio. Section, NINDS, NIH, Bethesda, MD

Abstracts: A current model of the respiratory central pattern generator (Smith et al. 2007, 2013) suggests an important role of inhibitory circuit interactions within and between the pre-Bötzinger (pre-BötC) and Bötzinger (BötC) complexes in generating respiratory rhythm and the normal three-phase respiratory pattern. Janczewski et al. (2013) have challenged this suggestion, and the exact role of inhibitory interactions in respiratory rhythm and pattern generation *in vivo* remains unclear. In this study, we tested the role of synaptic inhibition within pre-BötC/BötC circuits for respiratory rhythm and pattern generation *in vivo* by imposing pharmacological blockade of endogenous GABAergic and glycinergic receptor-mediated inhibition within these regions. We used anaesthetized (1.75-2% isoflurane in O2), paralyzed, vagotomized, chemo-/baro-denervated, and artificially ventilated adult male Sprague-Dawley rats. Activities of hypoglossal, vagus, and phrenic nerves were recorded and analyzed. By a ventral surface approach, the specific blockers of GABAA (gabazine) and glycine (strychnine) receptors were bilaterally microinfused in mixed solutions (250 μM of each, 110 nl total volume infused) into the pre-BötC (n=9 rats) and BötC (n=12 rats). Microinfusion sites were marked with yellow-green beads and confirmed by post hoc histological analyses. Bilateral microinfusion (1-2 min.) of gabazine and strychnine into the BötC or preBötC disrupted rhythm generation with site-specific patterns of respiratory activity perturbations. Block of inhibition in the BötC caused either severe bradypnea (mean inspiratory frequency=25.32% of control, n=2/12 rats) or in most cases bradypnea followed by apnea (n = 10/12 rats), whereas microinfusion of the antagonists into the pre-BötC elicited a transient tachypnea (90.82±53% increase of inspiratory frequency, n=9 rats) with a progressive decline of inspiratory discharge amplitude in all recorded nerves and complete suppression of respiratory motor output in 6/9 rats. Prior to the loss of inspiratory nerve activity and during recovery, the normal three-phase respiratory pattern was also disrupted with a loss of post-inspiratory activity on the vagus nerve and change in pattern of integrated inspiratory discharge from a ramping pattern to a low amplitude square-wave pattern. These results agree with those obtained in perfused brainstem preparations *in situ* (see Koshiya et al., this volume). We conclude that GABAergic and glycinergic receptor-mediated inhibition within and between

pre-BötC and BötC circuits plays a major role in the generation of respiratory rhythm and the three-phase respiratory pattern *in vivo*.

Disclosures: V. Marchenko: None. **N. Koshiya:** None. **T. Bezdudnaya:** None. **H. Koizumi:** None. **R. Zhang:** None. **I.A. Rybak:** None. **J.C. Smith:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.09/QQ28

Topic: E.04. Autonomic Regulation

Support: CNPq

FAPESP

Title: Involvement of P2Y purinergic receptors in the nucleus of the solitary tract on the respiratory responses induced by hypoxia

Authors: *N. MARQUES, J. V. MENANI, P. M. DE PAULA;
Dept of Physiol. and Pathology, Dent. School, UNESP, Araraquara, Brazil

Abstracts: Cardiorespiratory responses to hypoxia are controlled by brainstem areas including the nucleus of the solitary tract (NTS). Bilateral injections of suramin (non-selective purinergic receptor antagonist) into the NTS reduced hypoxia-induced hyperventilation, whereas bilateral injections of PPADS (P2 purinergic receptor antagonist) into the NTS did not change cardiorespiratory responses to hypoxia. Nevertheless, the participation of P2Y purinergic receptors of the NTS on the cardiorespiratory responses induced by hypoxia is still unknown. Therefore, in the present study, we investigated the involvement of P2Y purinergic receptors of the NTS on cardiorespiratory responses induced by hypoxia in awake rats, using injections of MRS 2179 (selective P2Y purinergic receptor antagonist). Male Holtzman rats (290-310 g, n=4-6/group) with stainless steel cannulas implanted into the NTS were used. A polyethylene tubing (PE-10 connected to a PE-50) was inserted into abdominal aorta through femoral artery to record mean arterial pressure (MAP) and heart rate (HR) in awake rats. Respiratory frequency (fR), tidal volume (VT) and VE were recorded by whole-body plethysmography. VE, fR, VT, MAP and HR were recorded before and after bilateral injections of saline or MRS 2179 (1 nmol/100 nl) into the NTS during hypoxia (7% O2 for 35 min). Hypoxia reduced MAP (100 ± 3 mmHg,

vs. normoxia: 117 ± 4 mmHg, p <0.05) and HR (382 ± 14 bpm, vs. normoxia: 441 ± 15 bpm, p<0.05) and increased VE (992 ± 44 ml/min/kg, vs. normoxia: 488 ± 28 ml/min/kg, p<0.05). Bilateral injections of MRS 2179 into the NTS reduced VT during hypoxia (6.6 ± 0.4 mL/kg, vs. saline: 7.8 ± 0.2 mL/kg, p=0.028) and hypoxia-induced hyperventilation (804 ± 77 ml/min/kg, vs. saline: 992 ± 44 ml/min/kg, p=0.04), without changing tachypnea (122 ± 7cpm, vs. saline: 128 ± 4 cpm), MAP (99 ± 2 mmHg, vs. saline: 100 ± 3 mmHg) or HR (446 ± 23 bpm, vs. saline: 441 ± 15 bpm) during hypoxia. The present data show that P2Y purinergic receptors in the NTS are involved in the respiratory responses induced by hypoxia.

Disclosures: **N. Marques:** None. **J.V. Menani:** None. **P.M. de Paula:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.10/QQ29

Topic: E.04. Autonomic Regulation

Support: FAPESP

CNPq

Title: Medullary catecholaminergic (CA) neurons modulate hypoxic ventilatory response in neonatal rats (P7-8)

Authors: ***L. A. PATRONE**, V. BIANCARDI, K. C. BÍCEGO, L. H. GARGAGLIONI; SAO PAULO STATE UNIVERSITY, JABOTICABAL, Brazil

Abstracts: It is known that catecholaminergic (CA) neurons are involved in autonomic and respiratory regulation during low O_2 conditions in adult mammals. We evaluated the participation of medullary CA neurons of male and female neonatal rats (P7-8) in mediating the hypoxic ventilatory response (HVR) by specifically lesioning them with antidopamine beta-hydroxylase-saporin (DBH-SAP, 42ng / 100nL) injected into the 4th ventricle. We also quantified rates of O_2 consumption (VO_2) of control and lesioned neonates (P7-8) exposed to hypoxia. Minute ventilation (VE) of neonates was recorded by pressure-plethysmography from the body chamber during normoxia and hypoxia (10% O_2), and the VO_2 measurement by open flow respirometry. The mammalian HVR typically results in increased VE upon exposure to acute hypoxia. HVR was significantly reduced in male and female lesioned neonatal rats by

about 23 and 15%, respectively, (male- control group: 137.3±7.9 (% of baseline) vs. lesioned group: 105.3±2.4 (% of baseline), $p<0.01$; female- control group: 127.0±3.0 (% of baseline) vs. lesioned group: 108.6±1.7 (% of baseline) $p<0.02$). The VO_2 was decreased in the lesioned newborns, but only the lesioned male group was significantly lower (control group: 76.8±12.14 (% of baseline) vs. lesioned group: 45.3±13.3 (% of baseline) $p<0.03$). These results suggest that catecholaminergic neurons, specifically from medullary nuclei, exert an excitatory modulation of O_2 chemosensitivity in neonatal rats.

Disclosures: L.A. Patrone: None. **V. Biancardi:** None. **K.C. Bícego:** None. **L.H. Gargaglioni:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.11/QQ30

Topic: E.04. Autonomic Regulation

Support: NIH HL104101

NS073981

Sao Paulo Research Foundation (FAPESP)

Title: HCN channels contribute to serotonergic modulation of ventral surface chemosensitive neurons and respiratory activity

Authors: *V. E. HAWKINS[1], J. M. HAWRYLUK[1], A. C. TAKAKURA[2], T. S. MOREIRA[3], A. V. TZINGOUNIS[1], D. K. MULKEY[1];
[1]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Dept. of Pharmacol., Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Dept. of Physiol. and Biophysics, Univ. of Sao Paulo, Sao Paolo, Brazil

Abstracts: Chemosensitive neurons in the retrotrapezoid nucleus (RTN) provide a CO_2/H^+-dependent drive to breathe. They also function as an integration center for multiple brain regions involved in respiration, including serotonergic medullary raphe neurons. We recently showed that serotonergic modulation of RTN chemoreceptors involved inhibition of KCNQ channels and activation of an unknown inward current. HCN channels are the molecular correlate of the hyperpolarization-activated inward current (Ih) and have a high propensity for modulation by

neurotransmitters including serotonin. Therefore, we hypothesize that HCN channels contribute to serotonergic modulation of RTN chemosensitive neurons. To investigate this possibility, we characterized effects of serotonin on activity of RTN chemoreceptors *in vitro*, and on respiratory motor output of both anesthetized and awake rats, under control conditions and in the presence of a specific KCNQ channel blocker (XE991) and/or a specific HCN channel blocker (ZD7288). *In vivo*, phrenic nerve recordings show that unilateral RTN injection of XE991 alone blunted the ventilatory response to serotonin by ~50%, and XE991 plus ZD7288 together eliminated the serotonin responsive. In the brainstem slice preparation, cell-attached current-clamp recordings from chemosensitive RTN neurons show that bath application of XE991 decreased excitatory effects of serotonin by ~50%, and residual serotonin-sensitivity in XE991 was eliminated by blocking HCN channels with ZD7288 or CsCl, or by inhibition of adenylate cyclase with SQ22536. In the whole-cell voltage-clamp configuration (Ihold = -60 mV; TTX) chemosensitive RTN neurons exposed to serotonin showed a depolarizing shift in the voltage-dependent activation of Ih, as expected for cAMP-mediated activation of HCN channels. Further, a 5-HT_7 receptor agonist (5-carboxamidotryptamine; 5-CT) mimicked serotonin-mediated activation of RTN chemoreceptors and the 5-CT response was blocked by a selective 5-HT_7 receptor antagonist (SB258719). Together, these results suggest that serotonin modulates respiratory drive at the level of the RTN in part by activation of HCN channels.

Disclosures: V.E. Hawkins: None. **J.M. Hawryluk:** None. **A.V. Tzingounis:** None. **D.K. Mulkey:** None. **A.C. Takakura:** None. **T.S. Moreira:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.12/QQ31

Topic: E.04. Autonomic Regulation

Support: FAPESP (BEPE) 13/02350-9

NIH 5R01HL104101

Title: Muscarinic cholinergic mechanisms within the retrotrapezoid nucleus modulate breathing without changing CO2 chemosensitivity

Authors: *C. R. SOBRINHO[1], A. C. TAKAKURA[2], I. C. WENKER[3], T. S. MOREIRA[5], D. K. MULKEY[4];

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Abstracts: The retrotrapezoid nucleus (RTN) contains neurons that regulate breathing in response to changes in tissue CO_2/H^+ (central chemoreceptors). Previous evidence suggests that cholinergic transmission within the RTN contributes to the chemoreflex; however, cellular and molecular mechanisms underlying cholinergic-modulation of breathing are still unknown. Here, we used *in vivo* and *in vitro* experiments to evaluate effects of exogenous administration of acetylcholine (Ach) on baseline activity and CO_2-sensitivity of RTN chemoreceptors, and to dissect the signaling pathway by which Ach activates these neurons. *In vivo* non-anesthetized adult Wistar rats, unilateral injection of Ach into the RTN increased ventilation and MAP under control conditions but not after pre-treatment with the muscarinic antagonist atropine. *In vitro*, cell-attached recordings of membrane potential show that Ach directly activates CO_2/H+-sensitive RTN neurons, and this response could be substantially attenuated by various specific and nonspecific muscarinic antagonists (atropine, 4DAMP and pirezenpine). The effects of Ach on RTN chemoreceptor activity was also blunted by inhibition of IP3-receptors with 2APB and by a casein kinase 2 inhibitor (TBB), but not by a PKC inhibitor (SKF-96365) or a cation channel blocker (flufenamic acid). Finally, both *in vivo* and *in vitro* experiments show that the muscarinic antagonist does not change CO_2 responses. These results suggest that Ach stimulates RTN chemoreceptors by activation of M1 and/or M3-receptors and an IP3-mediated activation of CK2 without directly influence on chemosensitivity. The ion channel targets of Ach have yet to be determined.

Disclosures: C.R. Sobrinho: None. **A.C. Takakura:** None. **I.C. Wenker:** None. **T.S. Moreira:** None. **D.K. Mulkey:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.13/QQ32

Topic: B.04. Ion Channels

Support: NSF DMS 1122291

Title: Slow oscillations require a balance between the linear negative-slope conductance region of a regenerative inward current and persistent outward currents

Authors: *Y. GUAN[1], A. BOSE[2], J. GOLOWASCH[3], F. NADIM[3];
[1]Rutgers Univ., Newark, NJ; [2]New Jersey Inst. of Technol., Newark, NJ; [3]Rutgers Univ. and NJIT, Newark, NJ

Abstracts: Slow oscillations underlying neuronal bursting commonly involve a regenerative inward ionic current with a nonlinear inverted bell-shape IV curve. In the crab pyloric central pattern generator, multiple peptidergic modulatory inputs activate the regenerative inward current IMI in several pyloric neurons, which is critical for the generation of neuronal oscillations. Our recent work suggests that the contribution of such regenerative currents to the production of oscillations is limited to the region of the IV curve in which the current exhibits a linear negative-slope conductance (INL). When INL is introduced with dynamic clamp in the pyloric pacemaker PD neurons, it can recover oscillations, even when the neuron is isolated by TTX (Bose et al, 2014). However, it is unknown whether other pyloric neurons can produce oscillations in the presence of INL and, if not, what factors determine the ability of INL to produce them. We examined whether, in the presence of TTX, INL is sufficient for producing slow oscillations in synaptically-isolated pyloric neurons. We found that the PD neuron can produce INL-induced oscillations in a range of gNL (40-300 nS) and ENL (-15 to +15 rel. to Vrest) values. The oscillation frequency (1.1-1.8 Hz) in PD increased, and its amplitude (30-10 mV) decreased with |gNL|, but both frequency and amplitude had a non-monotonic dependence on ENL `(13/13 cases). In contrast to the PD neuron, even when gNL and ENL were varied in a large range, none of the follower pyloric neuron types PY (0/6), LP (1/8), IC (0/3), VD (0/3), LPG (0/3) could produce slow oscillations with INL. We explored what factors may oppose the expression of oscillations in LP neurons in the presence of INL. Our previous modeling work suggests that INL-induced oscillations depend on a balance between INL and the voltage-gated outward currents (Bose et al, 2014). We therefore compared the outward currents in the PD and LP neurons. We found that the LP neuron has a significantly larger high-threshold K current (IHTK: delayed rectifier + Ca-dep. K currents) than PD (20% larger at 0 mV), and that PD has a larger IA than LP (45% larger at 0 mV). However, the kinetics of IHTK and IA were statistically identical in PD and LP. We thus examined whether changing the levels of IHTK and IA would affect the ability of PD or LP to oscillate with INL. We found that LP can oscillate with INL by reducing IHTK using TEA (N=4). However, preliminary results using the blocker 4AP indicate that IA does not contribute to PD oscillation in INL (N=2). We conclude that slow oscillations in a neuron require a balance of the negative-slope conductance region of the regenerative inward current with the high threshold outward currents.

Disclosures: **Y. Guan:** None. **A. Bose:** None. **J. Golowasch:** None. **F. Nadim:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.14/QQ33

Topic: E.04. Autonomic Regulation

Title: Neonatal mortality in CPEB2 knockout mice reveals its physiological function in respiratory regulation

Authors: Y.-T. LAI[1,2], *Y.-S. HUANG[1];
[1]Academia Sinica/Institute of Biomed. Sci., Taipei, Taiwan; [2]Grad. Inst. of Microbiology, Natl. Taiwan Univ., Taipei, Taiwan

Abstracts: Cytolasmic polyadenylation element binding protein (CPEB2) is an RNA-binding protein and translational regulator. To understand the physiological function of CPEB2, we generated CPEB2 knockout (KO) mice and found most of the KO animals died within three days after birth. CPEB2 is highly expressed in the brainstem which controls vital life functions such as breathing and heartbeat. Using the ultrasound and whole body plethymography, we found the KO pups had normal heart rate but abnormal respiration with increased apnea. Nevertheless, the anatomical morphology of the respiratory center (i.e. pre-Botzinger complex and parafacial respiratory group) and the respiratory-like activity electro-recorded from C4 ventral roots *in vitro* are comparable between WT and KO littermates, indicating the apnea episodes in the KO neonates are unlikely caused by rhythm-generating failure. Immunohistochemical analyses only identified up-expressed choline acetyltransferase (ChAT) in the dorsal motor nucleus of vagus (DMNV) of the KO neonates. Many abnormalities have been found in the DMNV of SIDS (sudden infant death syndrome) infants. Because neurons in DMNV control bronchoconstriction via acetylcholine release, these SIDS infants are likely died of DMNV-related airway obstruction. Intriguingly, characterization of respiratory patterns recorded under hypercapnia also reveals the KO neonates have increased airway resistance. Therefore, the elevated cholinergic transmission-induced airway obstruction in the KO neonates likely accounts for their respiratory defect and mortality.

Disclosures: Y. Lai: None. **Y. Huang:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.15/QQ34

Topic: E.04. Autonomic Regulation

Support: FONDECYT 1130874

CONICYT DOCTORAL GRANT 21120591

DICYT-USACH

Title: Topical application of D-serine on raphe nuclei and ventral respiratory column increases fictive respiration in slices from mouse neonates

Authors: *S. BELTRAN-CASTILLO, I. LLONA, J. EUGENIN;
Biología, Univ. De Santiago De Chile, Santiago, Chile

Abstracts: We have reported that D-serine, an endogenous co-agonist for NMDAr in CNS, modulates fictive respiration in *in vitro* preparations from mouse neonates. Superfusion with D-serine increases the frequency of fictive respiration in a concentration-dependent way, reaching up to 150% of basal frequency in en bloc and in medullary slices. We have also showed that pre-incubation with D-aminoacid oxidase reduces slightly the effect of hypercarbia upon the respiratory rhythm, suggesting a role of D-serine as a mediator of respiratory response to hypercarbia. Now we have tested the effect of local application of D-serine on different brainstem nuclei to define possible sites of D-serine action. Spontaneous activity from the ventral respiratory column (VRC) was recorded with glass suction electrodes from medullary slices obtained from neonatal CF1 mice (P0-P4).Superfusion was done with artificial cerebrospinal fluid (aCSF) equilibrated with O2:CO2 = 95%: 5%, (pH 7.4, 29 ±1°C). D-serine or vehicle (aCSF) was applied locally into the brainstem nuclei using a borosilicate glass capillary (0.75 mm inside diameter and tip diameter of 3.75 µm) filled with D-serine 100 µM using pulse pressure generated with a pneumatic picopump (3 psi, 10 s). D-serine applied into raphe nuclei (RN) and VRC increased the frequency of fictive respiration up to 127% of basal frequency (n=5) and up to 131% (n=4), respectively. Microinjection with vehicle did not alter the basal frequency of fictive respiration. Our preliminary results indicate that D-serine can increase the frequency of fictive respiration acting directly on VRG or RN. Since both nuclei are chemosensitive, these results support the idea that D-serine may be a mediator of the respiratory response to hypercarbia

Disclosures: S. Beltran-Castillo: None. **I. Llona:** None. **J. Eugenin:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.16/QQ35

Topic: E.04. Autonomic Regulation

Support: Seattle Children's Research Institute

Title: Assessing the role for murine KCNQ5 in opioid suppression of respiratory drive: KCNQ5 genomic editing by CRISPR/Cas9

Authors: *A. D. WEI, P. WAKENIGHT, T. ZWINGMAN, K. J. MILLEN, J.-M. RAMIREZ; Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstracts: Reduced respiratory drive is a major cause of morbidity from opioid-based medications and drugs of abuse. Clinically, respiratory failure during post-operative surgical recovery can be a significant risk factor, limiting therapeutic options with existing opioid analgesics. Identifying molecular mechanisms that modulate opioid-mediated suppression of respiratory drive may provide novel targets for pharmaceutically augmenting respiratory drive and reversing opioid-mediated respiratory suppression (ORS), without compromising analgesic efficacy. We combined electrophysiological and genetic approaches to dissect the mechanism of ORS in mice. Contrary to expectation, we observed no significant contribution to respiratory suppression from opioid-mediated activation of GIRK potassium channels, nor from blocking large-conductance calcium-activated BK potassium channels. Instead, using selective pharmacology and electrophysiological recordings of fictive respiration from medullary slices containing the preBötzinger Complex (pBötC), we identified a subclass of non-canonical KCNQ potassium channels (KCNQ5), as a major target for modulating ORS by mu-opioid receptor activation. Genetic removal of CaV2.1 (CACNA1) which likely mimics mu-opioid-mediated suppression of P/Q calcium channel function compromised respiratory rhythm generation, consistent with a predominantly presynaptic locus for opioid-mediated suppression of central respiratory drive. We hypothesize that KCNQ5 serves to set basal neuronal excitability within pBötC respiratory circuits, and that blockers of KCNQ5 may override ORS due to opioid-mediated suppression of presynaptic function. To further test this hypothesis, we used CRISPR/Cas9 technology to target the murine KCNQ5 locus for genomic editing. Cas9/sgRNA constructs targeting exon 5 of KCNQ5 were transfected into Black Swiss/C57BL6 ES cells, and 19 clones were selected for molecular analysis. 89% (17/19) of these clones yielded deletions of the targeted exon detectable by T7 endonuclease assays. Sequence analysis of 7 selected clones revealed biallelic deletions in 6/7 clones ranging from 1 to 459 bps (mode=~20 bps), biased

towards the 5' side the targeted PAM site. Animals derived from these ES cells, with expected loss-of-function have been generated. We anticipate generating additional gain-of-function and GFP-fusion alleles of KCNQ5 by using similar CRISPR/Cas9 techniques. Analysis of these strains for opioid-mediated respiratory suppression will complement our pharmacological studies, and provide genetic evidence of our hypothesis of opioid suppression of pBötC circuit function.

Disclosures: **A.D. Wei:** None. **P. Wakenight:** None. **T. Zwingman:** None. **K.J. Millen:** None. **J. Ramirez:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.17/QQ36

Topic: E.04. Autonomic Regulation

Title: Opioidergic modulation of breathing revealed by naloxone application in the sagittaly sectioned rat hindbrain preparation

Authors: ***N. M. MELLEN**[1], B. GOURÉVITCH[2];
[1]Kosair Children's Hosp Res. Inst., Univ. Louisville, LOUISVILLE, KY; [2]UMR CNRS 8195, Université Paris-Sud, Orsay, France

Abstracts: Opioid receptors are broadly distributed along the respiratory column in ventrolateral medulla, and exogenous opioids have a profound effect on breathing. Less is known about how endongenous opioids interact with other neuropeptides and neurotransmitters to modulate breathing. Here, recordings were carried out from isolated, parasagittally sectioned medulla-spinal cord preparations (P0-P2, n=10) before and during bath administration of the opioid receptor antagonist naloxone (NAL; 10 μM). At the system level, NAL significantly shortened inspiratory duration and significantly increased inspiratory variability. In parallel with system-level recording, network activity was recorded optically using the medium affinity Ca2+ indicator fluo 8L (Kd=1.86 μM; AAT Bioquest), which permitted recording with single neuron resolution from local networks along the respiratory column at 45.5 Hz. Coupling between neuron pairs was inferred from changes in spike rate, estimated by deconvolving Ca2+ traces. A total of 309 neurons were recorded (control n=249, NAL n=201, match n= 142). Neurons were divided into 3 groups based on the effect of NAL on coupling relations: neurons whose (generally weak) coupling was unchanged by NAL (group 1); neurons with strong driver

coupling under control conditions that were transformed into weak followers under NAL (group 2); and neurons with weak follower coupling that were transformed into strong drivers under NAL (group 3). This consistent NAL-induced reconfiguration across preparations suggests that coupling relations between respiratory network constituents along the VRG are state-dependent, and are in part determined by endogenous opioid modulation.

Disclosures: **N.M. Mellen:** None. **B. Gourévitch:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.18/RR1

Topic: E.04. Autonomic Regulation

Support: FAPESP

CNPq

Fundunesp

Title: Control of the hypercapnic chemoreflex by Locus coeruleus noradrenergic neurons in female rats

Authors: ***M. B. DIAS**[1], D. C. DOURADO[2], J. ANSELMO-FRANCI[3], R. E. SZAWKA[4], K. C. BÍCEGO[2], L. H. GARGAGLIONI[2];
[1]Dept. of Physiol., UNESP, Botucatu, Brazil; [2]Dept. of Animal Morphology and Physiol., UNESP-FCAV, Jaboticabal, Brazil; [3]Dept. of Morphology, Physiol. and Pathology, Univ. of Sao Paulo, Ribeirao Preto, Brazil; [4]Dept. of Physiol. and Biophysics, Inst. of Biol. Sciences, Federal Univ. of Minas Gerais, Belo Horizonte, Brazil

Abstracts: The Locus coeruleus (LC) has been suggested as a CO2 chemoreceptor site. However, most of the studies involving the role of LC in hypercapnic ventilatory response have been performed in males. Ovarian steroids modulate the activity of LC neurons and females have a different respiratory response to CO2 comparing to males. Thus, we evaluated the c-Fos expression of LC noradrenergic neurons during normocapnia and hypercapnia in diestrus ovariectomized (OVX) and estradiol-treated ovariectomized (OVX+E2) female rats, and in intact orchidectomized (ORX), testosterone-treated orchidectomized (ORX+T) and estradiol-treated orchidectomized (ORX+E2) male rats. Double-staining was performed to show the presence of

tyrosine hydroxylase. In addition, we investigated the effect of noradrenergic LC neurons lesions in OVX and OVX+E2 females, by using 6-hydroxydopamine, in the hypercapnic chemoreflex. Hypercapnia (7%CO2) increased the double-staining in LC neurons in all groups comparing to air. In the OVX+E2 group there was attenuation in the c-FOs expression in normocapnia and hypercapnia. The hypercapnic ventilatory response was significantly decreased in 6-OHDA-lesioned rats compared with sham group (29.4% in OVX group and 28.7% in OVX+E2 group). Thus, we can conclude that noradrenergic neurons in the LC of female and male rats are activated by CO2 and play a role in the hypercapnic ventilatory response. Further, our results suggest that estradiol inhibits the activation of LC noradrenergic neurons during hypercapnia but does not change the hypercapnic ventilatory response probably due to compensatory mechanisms from other chemosensitive sites.

Disclosures: **M.B. Dias:** None. **D.C. Dourado:** None. **J. Anselmo-Franci:** None. **R.E. Szawka:** None. **K.C. Bícego:** None. **L.H. Gargaglioni:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.19/RR2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS 38632

Title: Gender differences in hypoxic acclimatization in cyclooxygenase-2 deficient mouse

Authors: ***K. XU**[1], X. SUN[2], C. P. TSIPIS[2], G. F. BENDERRO[2], J. C. LAMANNA[2];
[1]Dept Neurol., [2]Dept Physiol. & Biophysics, Case Western Reserve Univ., CLEVELAND, OH

Abstracts: Cyclooxygenase-2 (COX-2) is the inducible isoform of cyclooxygenase, the key rate-limiting enzyme in the prostaglandin biosynthesis pathway, which is known to be involved in many physiological and pathological processes including inflammation and angiogenesis. In this study we used COX-2 deficient mouse to investigate the role of COX-2 in acclimatization responses to prolonged hypobaric hypoxia. Age-matched wide-type (WT) and COX-2 knockout (KO) mice including both sexes were exposed to the equivalent of 8% O2 at sea level for up to 21 days in a hypobaric chamber at a pressure of 300 mmHg (0.4 ATM); littermate controls were kept normoxic in the same location to ensure identical ambient condition. Capillary density were determined in brains at 21 days of exposure; in separated groups of animals, blood and tissue

samples were collected at different time points of exposure for measurement of hematocrit and Western blot analysis for hypoxic inducible factor -1α (HIF-1α), vascular endothelial growth factor (VEGF) and erythropoietin (EPO). Results showed that there was no gender difference in hypoxic acclimatization in the WT mice; however, male COX-2 KO mice exhibited increased vulnerability to prolonged hypoxia; the female COX-2 KO mice had similar adaptive responses compared to the WT mice. At 21 days of exposure, the survival rate in the male KO group was 58% (8/14), which was significantly lower than that of the male WT group (100%, 21/21); the survival rate in the female KO group was similar to that of the WT mice (94%, 15/16 vs. 100%, 21/21). Compared to the WT and female COX-2 KO mice, during hypoxia, the erythropoietic response in kidney and polycythemic response were decreased in the male COX-2 KO mice. Cerebral vascular remodeling through angiogenesis is the major CNS acclimatization response to prolonged hypoxia. We observed that the baseline capillary density was similar in the WT and the COX-2 KO mice. The capillary density in brain cortex was increased about 20% after 21 days of hypoxia in the WT mice and female COX-2 KO mice; however, there was no hypoxia-induced angiogenesis in the male COX-2 KO mice. In addition, absence of COX-2 resulted in attenuated HIF-1α accumulation during hypoxia, which correlated a response deficit in downstream gene expressions of EPO and VEGF. Our data suggest that there is gender difference in hypoxic acclimatization in the COX-2 deficient mice, absence of an adaptive response in the male COX-2 deficient mice highlights the importance of COX-2 signaling pathway as a requirement for adaptive response to oxygen limiting environments in the males.

Disclosures: K. Xu: None. **X. Sun:** None. **C.P. Tsipis:** None. **G.F. Benderro:** None. **J.C. LaManna:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.20/RR3

Topic: E.04. Autonomic Regulation

Support: NIH, NICHD, NINDS

Title: Effects of isoflurane and caffeine on breathing in mice during the neonatal period

Authors: *C. A. MASSEY[1], Y. WU[1], K. R. CHIRCO[2], S. M. HARMAN[1], G. B. RICHERSON[1];

[1]Neurol., Univ. of Iowa Hosp. and Clinics, Iowa City, IA; [2]Biosci. Grad. Program, Univ. of Iowa, Iowa City, IA

Abstracts: Isoflurane is a commonly used anesthetic in both research and clinical settings. Isoflurane-induced breathing depression in infants, especially premature infants, has been well documented. In fact, anesthesia-induced apneas are a common problem in the neonatal intensive care unit and frequently require intubation. Previously, our laboratory has shown that isoflurane severely depresses CO_2 chemoreception due in part to inhibition of serotonin (5-HT) neurons. It is important to understand the underlying mechanisms for why this depression of baseline breathing is seen in neonates, but not adults. We used whole animal plethysmography to study the effects of isoflurane (1%) on breathing in mice at two different ages – postnatal day 1 (P1) and postnatal day 8 (P8). Experiments were performed in a chamber held at an ambient temperature of 30 °C. Relative minute ventilation (VE) was expressed in arbitrary units since body temperature was not measured from animals this young. We found that *Lmx1b*$^{f/f/p}$ mice, in which 5-HT neurons have been genetically deleted, at the age of P1 had decreased relative VE (0.85 ± 0.36; n=23) in 21% O_2/Balance N_2 compared to wild type (WT) littermates (2.33 ± 0.56; n=21). Isoflurane (1%) depressed relative VE in both WT (0.63 ± 0.12; n=21) and *Lmx1b*$^{f/f/p}$ (0.49 ± 0.10; n=23) mice. However, isoflurane had a more potent effect on WT mice as their relative VE decreased 71.97 ± 6.12% (n=21) compared to only 37.47 ± 15.34% in *Lmx1b*$^{f/f/p}$ mice (n=23). After washout of the drug, relative VE in WT mice increased but did not return to baseline (1.24 ± 0.46; n=21). *Lmx1b*$^{f/f/p}$ mice did not recover from isoflurane (0.60 ± 0.19; n=23). We then tested the same mice one week later at P8, and found that isoflurane had less of an effect on baseline breathing in both WT and *Lmx1b*$^{f/f/p}$ mice than it did at P1. In WT mice at P8, relative VE was reduced in isoflurane by 38.62 ± 18.26% compared to 71.97 ± 6.12% at P1. Furthermore, after washout the relative VE of these mice returned to baseline at P8. These data indicate that isoflurane induces hypoventilation at baseline in the early neonatal period, but this effect decreases as mice age. Furthermore, these results suggest that 5-HT neurons play a role in isoflurane-induced hypoventilation. Caffeine is used to treat neonatal apnea and hypoventilation, so we are now investigating whether it has similar effects in mice. Preliminary data (n=2) indicate that neonatal exposure to caffeine increases breathing and may counteract isoflurane-induced hypoventilation.

Disclosures: C.A. Massey: None. **Y. Wu:** None. **K.R. Chirco:** None. **S.M. Harman:** None. **G.B. Richerson:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.21/RR4

Topic: E.04. Autonomic Regulation

Support: RGC Grant CUHK479213

Title: Impact of chronic intermittent hypoxia on hippocampal neuronal function: An *in vivo* recording study

Authors: ***L. XU**[1], Q. LI[1], Y. KE[2], W. YUNG[2];
[1]The Chinese Univ. of Hong Kong, Hong Kong, China; [2]The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstracts: Chronic intermittent hypoxia (IH) occurs in obstructive sleep apnea (OSA), a very common sleep and breathing disorders that is associated with central nervous system dysfunction, including learning and memory impairment. Despite the obvious importance, the question of exactly what happens to neuronal activities in different brain regions during intermittent hypoxia has never been addressed. We envision that long-term recording of the firing activities of neurons and brain rhythms *in vivo* during and after the IH will provide direct information to address this question and thus considerable insight into the cause of cognitive dysfunctions in OSA. Based on a well-established OSA model in which the rats were subject to daily 8-hr cycling of oxygen between 21% and 10% every 90s, the firing activities of neurons in the CA1 region of the hippocampus were followed for 14 days by chronically implanted multi-electrode arrays. We found that both CA1 principal neurons and interneurons tended to increase their firing activities during the first week of IH treatment, particularly apparent at the late hours (at 8 h) but not early hours (at 5 min and 3 h) of daily IH paradigm. The hyper-excitability, however, was followed by gradual suppression of firing in the second week. At Day 14, the firing rates of principal neurons and interneurons were typically less than 60% and 25%, respectively of those at Day 1. These profiles paralleled the increase in long-term potentiation magnitude at Day 2 and its suppression in the second week of IH treatment measured from acutely prepared hippocampal slices. Partial recovery of the neuronal activities was found after 1-week recovery in normoxia but confined to principal neurons only. Interestingly, analysis of the field potential revealed that the hippocampal theta rhythm (4-8 Hz) was diminished by chronic IH with no sign of recovery 1 week after the IH treatment. Together, these data suggest that hippocampal neurons respond to short-term hypoxia treatment by boosting their activities. However, long term IH impairs the firing activities, suppresses synaptic plasticity and related theta rhythm, which is more likely contributed by impaired functions of interneurons than pyramidal neurons. These results provide the cellular function correlates of the memory deficit observed in OSA subjects.

Disclosures: **L. Xu:** None. **Q. Li:** None. **Y. Ke:** None. **W. Yung:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.22/RR5

Topic: E.04. Autonomic Regulation

Support: FONDECYT #1090375

FONDECYT #1130874

DICYT

CONICYT fellows Training Program for Advanced Human Capital

Title: Perinatal exposure to fluoxetine diminishes the respiratory response to hypercarbia *in vivo* and *in vitro* in mouse neonates

Authors: *K. A. BRAVO, J. EUGENÍN, I. LLONA;
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Abstracts: Serotonin (5HT) is known to play a principal role during development of SNC in mice (Gaspar et al., 2003, Nat. Rev. Neurosci. 4: 1003). We have reported that ventilatory response to hypercarbia at P8 (postnatal age) is reduced in mouse neonates exposed to fluoxetine (specific 5HT reuptake inhibitor) during pregnancy and early postnatal age (Bravo et al., 8th FENS Forum Neuroscience, 2012). Serotoninergic neurons of raphe nucleus are chemosensitive i.e. stimulated by hypercapnia *in vivo* and by acidosis *in vitro*. In the present work we investigated the effect of fluoxetine treatment on a) the CO2-induced activation of raphe neurons *in vivo* and b) the changes in fictive respiration induced by acidosis using the medullary slice preparation. Three experimental groups were used: neonates born from CF-1 dams to which were implanted with osmotic minipumps delivering fluoxetine (7mg Kg-1 day-1) or saline from the fifth day of gestation up to day 14 postnatal A third group consisted of neonates born from dams not receiving any minipump. We used c-Fos immunocytochemistry to assess neuronal activation by hypercapnia (20 minutes of inhalation air enriched with 10% CO2). Fictive respiratory activity was recorded from the ventral respiratory group in medullary slices at P8. Acidosis was obtained by switching the artificial cerebrospinal fluid equilibration from 5 to 10% CO2 (pH 7.4 to 7.2). Fluoxetine decreased the number of c-Fos positive neurons induced by hypercapnia in the raphe and tractus solitarius nuclei, but not in the hypoglossal nucleus. In slices from P8

neonates, fluoxetine reduced the increase in the frequency of fictive respiration induced by acidosis. In contrast, in sham operated and intact mice no detrimental effects were observed. Our results suggest that fluoxetine treatment during perinatal period alter central chemosensory responses of raphe nucleus during the postnatal life.

Disclosures: K.A. Bravo: None. **J. Eugenín:** None. **I. Llona:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.23/RR6

Topic: E.04. Autonomic Regulation

Support: Supported by National Institutes of Health R01 HL-113251

Title: Altered insular resting-state functional connectivity in patients with obstructive sleep apnea

Authors: *B. PARK[1], N. TOMA[2], P. M. MACEY[3,4], M. A. WOO[3], F. L. YAN-GO[5], R. M. HARPER[2,4], R. KUMAR[1,4,6];
[1]Anesthesiol., [2]Neurobio., [3]Sch. of Nursing, [4]Brain Res. Inst., [5]Neurol., [6]Radiological Sci., Univ. of California at Los Angeles, Los Angeles, CA

Abstracts: Obstructive sleep apnea (OSA) is accompanied by brain tissue injury and dysfunction in regions that regulate autonomic and motor actions. Brain injury appears in various sites, including insular cortices, which may play important roles in serving these deficient functions through altered functional connections with other brain regions; however, integrity of insular functional connectivity in OSA remains unclear. Our aim was to assess the resting-state functional connectivity (FC) of insular cortices to other brain areas in newly-diagnosed, treatment-naïve OSA compared to control subjects. We acquired resting-state functional MRI data from 47 OSA (age, 46.9±9.0 years; Females, 11; BMI, 30.4±5.2 kg/m2; AHI, 31.8±21.1 events/hr), and 59 control subjects (46.18±8.13 years; 18; 24.8±3.8kg/m2), using a 3.0 Tesla MRI scanner. Data were processed using SPM8 with standard procedures, canonical nuisance signals were removed by regression, and data band-pass filtered. Using left and right insula seed regions, we calculated individual correlation maps between each seed area and whole-brain voxels, and converted those maps into z-scored maps with Fisher's r-to-z transformation. We compared z-scored maps voxel-by-voxel between OSA and control subjects using analysis of

covariance (covariates; age and gender; $P<0.05$; cluster-corrected). The left insula showed decreased FC in OSA with the left inferior occipital, caudate, anterior cingulate cortex, orbitofrontal cortex, and rolandic operculum, and both left and right cerebellum and supplementary motor area. However, increased FC appeared with the left postcentral gyrus, left middle occipital gyrus, right hippocampus, right putamen, right supramarginal, and left and right inferior frontal regions. The right insula in OSA showed decreased FC with the left and right calcarine and cerebellum, left caudate, left thalamus, anterior cingulate cortex, and orbitofrontal gyrus, and increased FC in the right putamen, left and right postcentral gyrus, and posterior cingulate cortex. These findings suggest that recently-diagnosed treatment-naïve OSA subjects show complex aberrant FC between the insular cortices and several other brain regions regulating autonomic and motor actions. The altered FC may affect both parasympathetic and sympathetic interactions, as well as sensorimotor integration, all of which are affected in OSA. The functional changes likely result from the prominent structural changes with the condition in these regions. Supported by National Institutes of Health R01 HL-113251.

Disclosures: B. Park: None. **N. Toma:** None. **P.M. Macey:** None. **M.A. Woo:** None. **F.L. Yan-Go:** None. **R.M. Harper:** None. **R. Kumar:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.24/RR7

Topic: E.04. Autonomic Regulation

Support: Supported by National Institutes of Health R01 HL-113251.

Title: Association of axonal and myelin changes with obstructive sleep apnea severity

Authors: *R. KUMAR[1,2,3], S. K. YADAV[1], B. PARK[1], J. A. PALOMARES[1], M. A. WOO[4], D. W. KANG[5], R. M. HARPER[6,3];
[1]Anesthesiol., [2]Radiological Sci., [3]Brain Res. Inst., [4]Sch. of Nursing, [5]Med., [6]Neurobio., Univ. of California at Los Angeles, Los Angeles, CA

Abstracts: Obstructive sleep apnea (OSA) patients show axonal and myelin changes in several brain sites that regulate autonomic, cognitive, and neuropsychologic functions, all of which are deficient in the condition. Brain axonal and myelin changes may vary with disease severity; however, the extent of such associations is unknown, and we examined those relationships using

the apnea-hypopnea-index (AHI), as an index of disease severity. We collected diffusion tensor imaging (DTI) data (two series; 64 diffusion directions and 7 b0 images) from 17 OSA subjects (age, 49.8±9.5 years; 4, females; AHI, 40.8±23.4 events/hour; body mass index, 29.4±8.9 kg/m2), using a 3.0 Tesla MRI scanner. Using each DTI series, we calculated axial diffusivity (reflecting axonal changes) and radial diffusivity (indicating myelin alterations) maps; both maps were realigned, averaged, normalized to common space, and smoothed. The smoothed axial and radial diffusivity maps were used to examine relationships between regional axial and radial values and AHI scores using linear regression analyses with ANCOVA (covariates; age and gender; uncorrected threshold; $p = 0.005$). Multiple brain sites with axial and radial diffusion changes showed negative and positive relations with AHI scores, indicating acute and chronic axonal and myelin changes, respectively. Brain regions with negative associations between axial diffusivity and AHI scores appeared in the putamen, internal capsule, anterior and posterior thalamus, middle cerebellar peduncle, cerebellar cortex, and occipital cortex, and positive relations emerged in the prefrontal, medial frontal, and ventral prefrontal cortices, extending to white matter, insular cortices, posterior cingulum bundle, caudal pons, ventral temporal cortices and white matter, and parietal and occipital cortices. Brain sites with negative relations between radial diffusivity and AHI scores appeared in the putamen, occipital cortex, and cerebellar peduncles and cerebellar cortices, and positive association emerged in the insular cortices, ventral medial prefrontal cortex, dorsal temporal cortex, posterior cingulum bundle, caudal pons, and frontal and parietal cortices. Both positive and negative correlations appeared between axial and radial diffusivity and AHI scores, indicating acute and chronic axonal and myelin changes, in various brain sites that regulate autonomic, cognitive, and neuropsychologic functions. The findings indicate that disease severity plays a significant role in axonal and myelin changes in OSA. Supported by National Institutes of Health R01 HL-113251.

Disclosures: **R. Kumar:** None. **S.K. Yadav:** None. **B. Park:** None. **J.A. Palomares:** None. **M.A. Woo:** None. **D.W. Kang:** None. **R.M. Harper:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.25/RR8

Topic: E.04. Autonomic Regulation

Support: Supported by National Institutes of Health R01 HL-113251.

Title: Alterations in caudate nuclei resting-state functional connectivity in obstructive sleep apnea patients

Authors: *R. M. HARPER[1,2], T. IBRAHIM[1], B. PARK[3], P. M. MACEY[4,2], M. A. WOO[4], F. L. YAN-GO[5], R. K. HARPER[1], R. KUMAR[3,6,2];
[1]Neurobio., [2]Brain Res. Inst., [3]Anesthesiol., [4]Sch. of Nursing, [5]Neurol., [6]Radiological Sci., Univ. of California at Los Angeles, Los Angeles, CA

Abstracts: Obstructive sleep apnea (OSA) patients show cognitive, autonomic and neuropsychologic deficits, likely resulting from brain injury which appears in the caudate nuclei, among other sites. However, the integrity of functional connections from the caudate nuclei to other brain sites in OSA subjects is unknown. We aimed to examine the resting-state functional connectivity from both left and right caudate nuclei to other brain areas in untreated, newly-diagnosed OSA over control subjects. We performed, using a 3.0 Tesla MRI scanner, resting-state functional MRI in 47 OSA (age, 46.9±9.0 years; Females, 11) and 59 controls (46.18±8.13 years; Females, 18). Functional MRI data were processed using SPM8 with standard procedures and MATLAB-based custom software. Using left and right caudate seed areas, we derived individual correlation maps between each seed site and whole-brain voxels, and converted those data into z-scored maps with Fisher's r-to-z transformation. We compared z-scored maps voxel-by-voxel between OSA and control groups using analysis of covariance, with age and gender included as covariates ($P<0.05$, cluster-corrected). The left caudate showed decreased functional connectivity in OSA with the right superior frontal gyrus and right superior parietal gyrus, while the right caudate showed decreased functional connectivity with the left medial superior frontal gyrus. However, increased functional connectivity with the right caudate appeared in the left insula, left superior temporal gyrus, left precuneus, right temporal pole, and right postcentral gyrus, and the left caudate showed increased functional connectivity in the left parietal cortex. Untreated, recently-diagnosed OSA subjects have altered functional connectivity between the caudate nuclei and various other brain areas that regulate cognitive function, with connectivity in the condition enhanced to some regions but reduced to others, possibly reflecting altered perseverative and decision-making behaviors, among other neuropsychological deficits in the syndrome. Supported by National Institutes of Health R01 HL-113251.

Disclosures: **R.M. Harper:** None. **T. Ibrahim:** None. **B. Park:** None. **P.M. Macey:** None. **M.A. Woo:** None. **F.L. Yan-Go:** None. **R.K. Harper:** None. **R. Kumar:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.26/RR9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1K08NS083736-01

Title: Nicotinamide mononucleotide adenylyltransferases in a mouse model of term equivalent birth asphyxia

Authors: *R. GALINDO, M. GREENBERG, D. M. HOLTZMAN;
Neurol., Washington Univ. In St. Louis, Saint Louis, MO

Abstracts: Neonatal hypoxia-ischemia (H-I) is an important and often devastating neurological disorder and it is the major cause of newborn death. Despite its high prevalence, there are only limited therapies available for its prevention and treatment. Furthermore, we still know little about the neurobiological processes that regulate neuronal cell fate in the injured and healthy developing brain. The family of nicotinamide adenine dinucleotide (NAD+) metabolizing enzymes known as Nicotinamide Mononucleotide Adenylyltransferases (NMNATs) have been shown to be importantly involved in the survival of axons following peripheral nerve injury. Utilizing quantitative PCR methods, we found that term equivalent neonatal cerebral hypoxia and/or ischemia results in a time-dependent 1.5 to 7 fold up regulation in the endogenous expression of the three NMNAT isoforms during the first 72 hours after injury in the mouse cerebral cortex and hippocampus, two brain regions significantly affected after neonatal H-I. Injury-associated NMNAT induction is seen as early as 8 hours post H-I and returns to baseline levels by 7 days after injury. Utilizing immunohistochemical methods we confirm that the observed increases in NMNAT mRNA directly translate into temporally-associated increases in NMNAT1 immunoreactivity. These data suggest that cerebral injury in the immature brain triggers endogenous mechanisms that enhance NMNAT expression during the acute phase of neonatal H-I. Given the putative role of these molecules in neuroprotection, we asked whether the endogenous NMNAT up regulation seen after injury is related to the selected survival or death of the affected neurons. To begin answering this question and to further understand the role of endogenous and exogenous NMNATs in immature neuronal survival, we developed and characterized an *in vitro* and *in vivo* NMNAT knockdown and NMNAT overexpression model utilizing serotype 8 Adeno-associated viral (AAV) vectors containing small hairpin RNAs targeting or each of the three NMNAT genes. We find that intraventricular injection of AAV8-shNMNAT vectors at postnatal day 0 results in effective gene knockdown and wide spread and stable infection throughout the brain. Utilizing this method, we then investigate whether NMNAT2 knockdown, the NMNAT isoform most highly expressed in neurons, results in increases in the amount of neuronal degeneration and apoptosis following neonatal H-I. Understanding the function of NMNATs in neuronal survival may offer new potential targets for

the effective treatment and prevention of the immediate and long-term neurological consequences that affect neonates exposed perinatal asphyxia.

Disclosures: **R. Galindo:** None. **M. Greenberg:** None. **D.M. Holtzman:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.27/RR10

Topic: E.04. Autonomic Regulation

Support: KAKENHI 25430012, 24500473

Title: TRPA1 agonist, cinnamaldehyde induced long-lasting facilitation of respiratory rhythm in the brainstem-spinal cord preparation isolated from newborn rat and in the *in situ* perfused-preparation from juvenile rat

Authors: ***H. ONIMARU**[1], S. LIN[1], M. TANI[1], I. YAZAWA[2], K. IKEDA[3], K. KAWAKAMI[4]; [1]Dept Physiol, [2]Dept Anat., Showa Univ. Sch. of Med., Tokyo, Japan; [3]Div. Biol., Hyogo Col. Med., Nishinomiya, Hyogo, Japan; [4]Div. Biol., Jichi Med. Univ., Shimotsuke, Tochigi, Japan

Abstracts: It is not well understood whether chemicals that are known as transient receptor potential (TRP) channel agonists or antagonists exert any effects on the medullary respiratory center. In the present study, we examined effects of TRPA1 agonist (cinnamaldehyde or ally isothiocyanate) on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat (P0-P3) and in the *in situ* perfused- preparation from juvenile rat (P11-13). The experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Showa University. The preparations were superfused by modified Krebs solution at 25-26oC, and inspiratory C4 ventral root (or phrenic nerve) activity was monitored. In the newborn rat *in vitro* preparation, cinnamaldehyde (0.5 mM) induced typically biphasic responses in C4 rate; initial short increase (0.5-2 min) and subsequent decrease followed by gradual recovery of the rhythm during 15 min bath application. After washed out, the rate of respiratory rhythm further increased and has been kept higher than that of control (200% of control) for more than 2 hrs (i.e. long-term facilitation). Ally isothiocyanate induced effects similar to cinnamaldehyde. The long-lasting facilitation of respiratory rhythm was partially antagonized by TRPA1 antagonist, HC-030031 (10 μM). We have obtained similar results of a long-lasting facilitation in the *in situ* perfused- preparation from P11-13 rats. On the basis of

results from cutting experiments of the medulla and whole-cell recordings from pre-inspiratory neurons in the parafacial respiratory group (pFRG), we suggest that the rostral medulla including the pFRG was important in the induction of long-lasting facilitation. The histochemical analysis demonstrated a wide distribution of TRPA1 channel positive cells in the reticular formation of medulla including the pFRG, with strong expression in the motor neuron pools. Similar distribution was also confirmed by *in situ* hybridization of mRNA for TRPA1 channel protein. Our findings suggest that activation of TRPA1 channels could induce a long-lasting facilitation of the respiratory rhythm.

Disclosures: H. Onimaru: None. **S. Lin:** None. **M. Tani:** None. **I. Yazawa:** None. **K. Ikeda:** None. **K. Kawakami:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.28/RR11

Topic: E.04. Autonomic Regulation

Support: 1R01HL104127-01

Title: Morphometric properties of Dbx1 pre-Bötzinger complex (preBötC) neurons that contribute to respiratory rhythm and pattern generation in neonatal mice slice preparations *in vitro*

Authors: *V. AKINS, C. DEL NEGRO;
Applied Sci., Col. of William and Mary, Williamsburg, VA

Abstracts: The inspiratory phase of the respiratory rhythm is generated by the preBötzinger complex (preBötC), a bilaterally distributed site located in the ventral medullary brainstem. Putatively rhythmogenic neurons in the preBötC are derived from a single genetic line, whose precursors express homeodomain transcription factor Dbx1. We performed electrophysiological experiments in slice preparations that retain the preBötC and generate spontaneous inspiratory motor output, which revealed a suite of intrinsic properties that could contribute to rhythm generation but did not provide a complete understanding of the role of Dbx1 neurons in respiratory rhythm generation. An analysis of the morphological features of Dbx1 neurons, independent of their intrinsic membrane properties, provided further insight into how they communicate and contribute to network behavior. Dbx1 neurons have smooth un-branched

dendrites that remain largely within the plane of the soma (~37 μm). Most Dbx1 neurons showed commissural axon projections, but some also showed premotor-like projections to the XII motor nucleus. These morphological features facilitate signal transduction, provide additional evidence supporting bilateral synchronization the preBötC through Dbx1 neurons, and demonstrate that Dbx1 preBötC neuron connectivity includes recurrent interconnections, as well as premotor projections. These findings through morphometric analyses show that Dbx1 preBötC neurons influence respiratory rhythm and pattern.

Disclosures: V. Akins: None. **C. Del Negro:** None.

Poster

643. Respiratory Neurobiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 643.29/RR12

Topic: E.04. Autonomic Regulation

Support: R01HL109025

Title: Multielectrode recording of brainstem neurons: Swallow control of the respiratory neural network

Authors: H.-W. TSAI[1], G. ZHOU[2], K. F. MORRIS[3], C. GESTREAU[3], S. C. NUDING[3], L. S. SEGERS[3], B. G. LINDSEY[3], *P. W. DAVENPORT[4];
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Abstracts: The pharyngeal phase of swallow requires a modulation of respiratory patterns to inhibit inspiration (i.e. swallow apnea). We hypothesized that swallow and respiration have shared components in the brainstem and generate specific swallow patterns by the reconfiguration of the respiratory neural network. We stimulated the superior laryngeal nerve (SLN) or applied fluid to the pharynx of paralyzed, decerebrate cats to elicit swallow. We used multiple electrodes placed into the nucleus of solitary tract (NTS) and ventral respiratory column (VRC) to simultaneously record spike trains during fictive swallow and breathing. SLN elicited swallow recruited non-respiratory modulated (NRM) neurons in the NTS while fluid elicited different groups of NRM swallow NTS neurons. However, expiratory (E) neurons in the VRC responded similarly to both SLN and fluid stimuli. Although most E neurons in the VRC were

inhibited by SLN and fluid stimuli, a few were facilitated by both perturbations. The results suggest that SLN afferents are processed by NTS neurons that are different from NTS neurons processing fluid elicited swallow. Once swallow is elicited, VRC E neurons have the same swallow related pattern reconfiguration. The results also suggest that NTS neurons differentiate between swallow stimuli and participate in the control of VRC respiratory neural network modulation to produce swallow apnea.

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Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.01/RR13

Topic: E.05. Stress and the Brain

Support: Department of Defense #10494473; Craig H Neilsen Foundation

Emory University Scholars Program in Interdisciplinary Neuroscience Research

Title: Operantly conditioned slow breathing in the rat modifies baseline respiration and engages behavioral changes consistent with the relaxation response

Authors: *D. NOBLE[1], M. L. MCKINNON[1], T. I. NEBLETT[1], W. N. GOOLSBY[2], S. HOCHMAN[1];
[1]Physiol., [2]Cell Biol., Emory Univ., Atlanta, GA

Abstracts: In humans, exercises involving slowed respiratory rate/deep breathing (SRR) have been found to counter autonomic sympathetic bias and engage the relaxation response. This state of deep rest reduces responses to stressors, including in individuals with various degrees of autonomic dysfunction. We sought to develop an animal model of the SRR-induced relaxation response to better understand the neurophysiology of stress reduction. We previously reported preliminary operant conditioning studies of SRR in a small cohort of animals. Here, we successfully replicate pilot work and show that animals conditioned to slow their breathing display a maintained reduction in respiratory rate following the final session of training, as well as reduced behavioral stress responses. We used electric field sensors (Plessey Semiconductors) to non-invasively monitor respiratory rate and motor activity in response to stress. For

conditioning, respiratory rate was continuously monitored during 20 two-hour sessions using whole body plethysmography, with feedback provided via a customized interface in LabVIEW. Experimental (SRR) animals, but not yoked controls, were able to turn off aversive visual stimulation (intermittent bright light) by slowing their breathing. Only SRR rats displayed an increasing incidence of breaths below the target respiratory rate over training, with average respiratory rate decreasing by 20 breaths/minute. Following training, baseline respiratory rate was recorded in the absence of visual reinforcement for one hour to monitor the persistence of observed changes into the post-training period, while subsequent testing addressed the impact of conditioned slow breathing on stress reactivity: i) anxiety-like behavior in an open field, and ii) respiratory rate and overall activity levels in confined, stress-inducing restraint chambers. We observed that the reduction in respiratory rate during training was maintained in SRR animals, and corresponded to increased periods of calm inactivity under pseudo-restraint and increased exploration in an open field (4.5 vs. 2.25 center-field visits). In conclusion, rats could be trained to reduce their respiratory rate, rate reductions were maintained following training, and additional behavioral changes seen following conditioning were consistent with the development of an animal model of the relaxation response focused on respiration as a triggering stimulus.

Disclosures: D. Noble: None. **W.N. Goolsby:** None. **M.L. McKinnon:** None. **T.I. Neblett:** None. **S. Hochman:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.02/RR14

Topic: E.05. Stress and the Brain

Title: ADRA2B deletion variant selectively predicts stress-induced enhancement of long-term memory in females

Authors: *P. R. ZOLADZ[1], A. E. KALCHIK[1], C. E. CADLE[1], D. M. PETERS[1], M. M. HOFFMAN[1], R. L. AUFDENKAMPE[1], S. M. LYLE[1], C. M. BROWN[1], A. R. SCHARF[1], A. M. DAILEY[1], N. E. WOLTERS[2], J. N. TALBOT[3], B. R. RORABAUGH[2];
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Abstracts: Clarifying the mechanisms that underlie stress-induced alterations of learning and memory may lend important insight into susceptibility factors governing the development of stress-related psychological disorders, such as post-traumatic stress disorder (PTSD). Previous work has shown that carriers of the ADRA2B Glu^{301}-Glu^{303} deletion variant exhibit enhanced emotional memory, greater amygdala responses to emotional stimuli and greater intrusiveness of traumatic memories. We speculated that carriers of this deletion variant might also be more vulnerable to stress-induced enhancements of long-term memory, which would implicate the variant as a possible susceptibility factor for traumatic memory formation. Participants submerged their hand in ice cold (stress) or warm (no stress) water for 3 min. Immediately afterwards, they studied a list of 42 words varying in emotional valence and arousal and then completed an immediate free recall test. Twenty-four hours later, participants' memory for the word list was examined via free recall and recognition assessments. Stressed participants exhibiting greater heart rate responses to the stressor had enhanced recall on the 24-hr assessment. More importantly, stressed female ADRA2B deletion carriers, particularly those exhibiting greater heart rate responses to the stressor, demonstrated greater recognition memory than all other groups. These findings support our hypothesis that the ADRA2B deletion variant is associated with increased susceptibility to stress-induced enhancements of learning. Furthermore, they extend this speculation by revealing that females are selectively influenced by the genetic variant, which could lend insight into sex-dependent susceptibility to traumatic memory formation and PTSD.

Disclosures: **P.R. Zoladz:** None. **A.E. Kalchik:** None. **M.M. Hoffman:** None. **R.L. Aufdenkampe:** None. **S.M. Lyle:** None. **D.M. Peters:** None. **C.M. Brown:** None. **C.E. Cadle:** None. **A.R. Scharf:** None. **A.M. Dailey:** None. **N.E. Wolters:** None. **J.N. Talbot:** None. **B.R. Rorabaugh:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.03/RR15

Topic: E.05. Stress and the Brain

Support: NSERC

Title: Selective deficits in object recognition memory are part of the depressive phenotype produced by repeated corticosterone in rats

Authors: *K. BRYMER[1], E. Y. FENTON[2], J. SIMPSON[1], J. G. HOWLAND[3], L. E. KALYNCHUK[4];
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Abstracts: Exposure to life stressors frequently precedes the onset of depression in human patients. Accordingly, many preclinical rodent models of depression make use of chronic exposure to stress or glucocorticoids to induce a depressive phenotype. We and others have found that repeated corticosterone injections induce a depressive phenotype in rodents, characterized by increased immobility in a forced swim test (FST), decreased sucrose preference, and decreased sexual behavior. However, little is known about the effects of corticosterone on object recognition memory and sensorimotor gating. In this experiment, we examined the effect of repeated corticosterone treatment on three object recognition memory tests, prepulse inhibition (PPI), and FST behavior. Rats received either 21 days of daily corticosterone injections (40 mg/kg) or vehicle injections, with behavioral testing commencing on day 22. Corticosterone-treated rats weighed significantly less than vehicle-treated rats by the end of the study and they also showed increased immobility time in the FST, thus replicating previous findings. Interestingly, corticosterone had differential effects on the three object recognition tasks used in this experiment. That is, the corticosterone-treated rats showed deficits in object-location recognition and object-in-place recognition, but not novel object-recognition. They also displayed less prepulse facilitation than vehicle-treated rats. These results suggest that impaired object recognition memory is part of the depressive phenotype seen in corticosterone-treated rats. They also suggest that corticosterone affects the hippocampus-prefrontal cortex circuit to a greater degree than the perirhinal cortex, as it is known that object-location and object-in-place recognition tasks are hippocampal-prefrontal dependent tasks whereas novel-object recognition is a perirhinal-cortex dependent task. These dissociations are consistent with previous work showing that the hippocampus and prefrontal cortex are particularly susceptible to the deleterious effects of chronic stress.

Disclosures: K. Brymer: None. **E.Y. Fenton:** None. **J. Simpson:** None. **J.G. Howland:** None. **L.E. Kalynchuk:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.04/RR16

Topic: E.05. Stress and the Brain

Title: A matter of time: Post stress enhancement of rule based category learning performance

Authors: *S. B. HUTCHINSON[1], L. HAWTHORNE[1], L. SZYMULA[1], S. K. MCCOY[1], S. W. ELL[1,2];
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Abstracts: Stressful situations result in the activation of multiple physiological responses. Recent research suggests that the time varying nature of these physiological responses has important implications for cognitive function, particularly processes dependent upon prefrontal cortical function. Presently we consider the temporal impact of this response in relation to rule-based categorization - a task thought to depend on working memory and cognitive control processes. Rule-based category learning performance was tested after completion of a social-evaluative stressor (modified version of the Trier Social Stress Test) at varying time delays relative to cessation of the stressor (no delay, short delay, and long delay conditions) or after a no stress, comparison condition. As expected, participants in the three stress conditions, but not the no stress condition, were physiologically and psychologically stressed. Participants in the long delay condition performed better on the rule-based category learning task than participants in the no delay, short delay, and no stress conditions. These data are consistent with a literature suggesting that cognitive processes dependent upon working memory may be enhanced after physiological recovery from acute stress.

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Poster

644. Stress and Cognition

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Program#/Poster: 644.05/RR17

Topic: C.01. Brain Wellness

Support: Alberta Innovates - Health Solutions (AI-HS; FZ and GM)

Preterm Birth and Healthy Outcomes funded by the AI-HS Interdisciplinary Team Grant #200700595 (GM)

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Title: Shipping stress in rats permanently alters stress response, gestational duration and epigenetic signatures

Authors: *F. C. ZUCCHI[1,2], Y. YAO[2,3], I. KOVALCHUK[3], G. METZ[2];
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Abstracts: Transport of laboratory animals between facilities can cause shipping stress which may significantly affect experimental outcomes. It may represent a traumatic event with potentially long-term consequences for hypothalamic-pituitary-adrenal (HPA) axis function, behavioural and health outcomes. Thus, shipment of animals may represent a significant confound in the comparison of results between laboratories. Here we investigated if shipment of laboratory rats between facilities has lasting effects on basal HPA axis activity, stress response, and standard measurements of simple and complex behaviours. We hypothesized that the shipment of laboratory rats would result in permanent changes in stress responsiveness, behaviour, gestational length and potentially heritable epigenetic alterations of gene expression, which are potentially heritable to subsequent generations. Stress responses and behavioural phenotypes were measured in adult male and female Long-Evans rats shipped from an external breeder (EB; Charles River, ON) and given one month of acclimatization in the new housing facility. Outcomes in EB rats were compared with age-matched Long-Evans rats raised in a local breeding colony (LB). Animals were trained and tested in skilled reaching and assessed in standard open field task as measurements of behavioural performance and emotional state. A subset of rats was assigned to one week of daily sessions of 20 min restraint to investigate their response to acute stress. Another group of rats underwent timed pregnancy to determine gestational length as an indicator of physiological integrity. Furthermore, global DNA methylation status of prefrontal cortex and hippocampus was measured by cytosine extension assay as an indication of epigenetic status associated with the shipping experience. Naïve male and female EB rats displayed significant alterations in skilled and non-skilled motor behavior when compared to LB rats. EB female rats showed shorter gestational lengths and greater risk of poor birth outcomes. Notably, EB rats were resistant to restraint stress effects. Furthermore, shipping experience in EB rats was associated with significant epigenetic modifcations, including hippocampus global hypermethylation and prefrontal cortex global hypomethylation. Shipment experience may represent a significant stressor to laboratory rats that interferes with physiological mechanisms and epigenetic regulation. Long-term alteration of HPA axis response, behaviour and poor pregnancy outcomes associated with shipping stress may significantly challenge attempts to standardize and compare models and techniques between laboratories.

Disclosures: **F.C. Zucchi:** None. **Y. Yao:** None. **G. Metz:** None. **I. Kovalchuk:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.06/RR18

Topic: E.05. Stress and the Brain

Support: NIH Grant MH092438

Title: Corticotropin releasing factor (CRF) impairs sustained attention in male and female rats

Authors: ***Y. KAWASUMI**, R. COLE, G. VAN BUSKIRK, V. PARIKH, D. BANGASSER; Dept. of Psychology, Temple Univ., Philadelphia, PA

Abstracts: Stress can disrupt a variety of cognitive processes, including attention. Moreover, patients with stress-related psychiatric disorders, such as depression, often report difficulty sustaining attention. Despite these well-documented effects, the neurobiological basis for stress regulation of sustained attention systems remains underexplored. During a stressful event, corticotropin releasing factor (CRF) is released centrally to modulate cognitive and behavioral stress responses. Previous research identified sex differences in the CRF1 receptor that increase neuronal sensitivity to CRF in female compared to male rats. The present study was designed to examine whether CRF alters sustained attention and if so, whether there are sex differences in this effect. To this end, male and female Sprague-Dawley rats were trained on an operant Sustained Attention Task (SAT) in which they had to discriminate visual signals from non-signaled events. After attaining criterion (70% correct responses on signal and non-signal trials), one of three doses of CRF (100ng, 500ng, and 1μg) or vehicle (artificial cerebral spinal fluid) were administered intracerebroventricularly 20-min prior to the task onset. The doses were administered in a counterbalanced fashion using a within-subjects design (successive infusions were separated by at least a week). In both male and female rats, CRF significantly reduced average response accuracies and vigilance index (a measure of overall attentional performance) in a dose-dependent fashion, with the highest dose of CRF causing the largest deficit. Interestingly, the ability to sustain vigilance throughout the session declined in female rats at the 500 ng dose, while the performance of male rats remained stable across the session at this dose. Although the number of omissions increased with the CRF dose in both males and females, female rats omitted more trials, presumably reflecting a lower motivation to perform under stressful conditions than males. Together, the results reveal that intracranial CRF administration

disrupts sustained attention in both male and female rats. However, on some measures, attention deficits are greater in females than in males, an effect that may be linked to sex differences in CRF receptors. Clinically, these findings suggest that CRF antagonists represent a viable therapeutic option to treat attentional deficits that characterize certain stress-related psychiatric disorders.

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Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.07/RR19

Topic: E.05. Stress and the Brain

Title: Post-learning stress facilitates long-term memory consolidation and differentially influences emotional memory in females depending on stage of menstrual cycle

Authors: *C. E. CADLE[1], A. E. KALCHIK[1], D. M. PETERS[1], C. M. BROWN[1], A. R. SCHARF[1], A. M. DAILEY[1], M. B. EARLEY[1], C. L. KNIPPEN[1], E. D. SCHOLL[1], B. R. RORABAUGH[2], P. R. ZOLADZ[1];
[1]Psychology, Sociology, & Criminal Justice, [2]Pharmaceut. & Biomed. Sci., Ohio Northern Univ., Ada, OH

Abstracts: Stress-induced alterations of learning and memory underlie the formation of traumatic memories and, thus, one of the most debilitating and costly psychological disorders that society faces, post-traumatic stress disorder (PTSD). However, the effects of stress on learning and memory are complex and still poorly understood. One relatively consistent finding in this area has been that post-learning stress enhances long-term memory; however, recent work has challenged this view with contradictory findings. Therefore, we examined the influence of post-learning stress on 24-hr declarative memory. Fifty-two participants learned a list of words varying in emotional valence and arousal and were then given an immediate free recall test. Participants then submerged their dominant hand in a bath of ice cold (stress) or warm (no stress) water for 3 min. Twenty-four hours later, participants returned to the laboratory and completed free recall and recognition assessments. Results indicated that stress enhanced participants' long-term free recall, while having no effect on recognition memory. Also, females in the follicular phase of the menstrual cycle recalled more arousing than non-arousing words following stress

exposure. These findings corroborate a significant portion of the stress-memory literature by revealing that stress enhances consolidation. They also suggest that post-learning stress exerts effects on memory that depend on female hormone levels.

Disclosures: **C.E. Cadle:** None. **A.E. Kalchik:** None. **D.M. Peters:** None. **C.M. Brown:** None. **A.R. Scharf:** None. **A.M. Dailey:** None. **M.B. Earley:** None. **C.L. Knippen:** None. **E.D. Scholl:** None. **B.R. Rorabaugh:** None. **P.R. Zoladz:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

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Program#/Poster: 644.08/RR20

Topic: E.05. Stress and the Brain

Support: F32MH102983

Title: Functional significance of mobile genetic elements

Authors: *T. A. BEDROSIAN, C. QUAYLE, F. H. GAGE;
Salk Inst. for Biol. Studies, La Jolla, CA

Abstracts: Environmental experience causes profound changes in brain structure and function. One mechanism contributing to these changes may be the mobilization of active retrotransposons in the genome. For example, during neuronal differentiation LINE-1 retrotransposons can insert into genes, leading to changes in gene expression and putatively neuronal function. We have developed an approach to investigate the effects of experience on active retrotransposable elements, with the hypothesis that changes in the frequency or location of genomic insertions may alter neuronal function and behavioral phenotypes. First, we designed primers targeted to active retrotransposon families in the mouse genome. Then we developed an assay using droplet digital PCR as a highly sensitive method of detection for mobile elements. Using contrasting environmental experiences, including chronic stress and environmental enrichment, we are measuring the frequency of insertions in various brain regions using our droplet digital PCR approach. In addition, we are developing a targeted sequencing method to identify where new insertions occur within the genome. Furthermore, to determine the effect of retrotransposition on neuronal and behavioral phenotypes, we have developed a pharmacological approach to attenuate retrotransposon activity in mice. Studies are being conducted to examine neuronal and behavioral phenotypes under conditions of limited retrotransposition. Retroelements were once

considered ‘junk DNA’ but accumulating evidence suggests they can actively reshape the genome. These experiments will contribute to our understanding of the functional relevance of retrotransposons to behavioral and neuronal phenotypes.

Disclosures: T.A. Bedrosian: None. **C. Quayle:** None. **F.H. Gage:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.09/RR21

Topic: E.05. Stress and the Brain

Support: PROMEP-SEP 2012 To FJH

Title: Chronic stress effects on spatial learning and hippocampal citogenesis of senile male rats

Authors: T. MORALES-SALCEDO[1], G. YAÑEZ-DELGADILLO[1], P. HERNANDEZ-CARRILLO[1], G. CHIPRES-TINAJERO[1], F. JÁUREGUI-HUERTA[1,2], Y. RUVALCABA-DELGADILLO[2], J. GARCÍA-ESTRADA[2], *M. S. LUQUIN DE ANDA[1];
[1]Univ. Guadalajara, Guadalajara, Mexico; [2]Neurosci., Inst. Mexicano del Seguro Social, Guadalajara, Mexico

Abstracts: The hippocampal formation and its cognitive functions have been the most explored targets for the deleterious effects of stress. The hippocampus is considered the main structure in the study of brain aging. Proliferative changes have also been proposed at the basis of volumetric changes seen in chronically stressed subjects. It is no clear however whether proliferative changes may affect senile subjects suffering stressing conditions. There is also controversy about the direction of changes produced by stress over spatial learning in aged subjects. The aim of this study was then to evaluate the effects of chronic exposure to stress on spatial learning and hippocampal proliferation on senile male rats. A group of aged male rats (18 month old) were exposed for 15 days to a chronic variate stress model. At the end of the exposure, the rats were assessed on the MWM paradigm. All the subjects were also injected with the cellular proliferation marker 5'bromodeoxiuridine(BrdU), and their brains were processed for immunohistochemical analysis. Coronal sections were immunolabeled with anti--BrdU antibodies to identify newborn cells in dentate gyrus (DG), cornu amonis areas CA1 and CA3. We found an improving effect of stress over spatial learning abilities of senile rats as showed by MWM execution. We also found region specific changes on the proliferative rate of hippocampal

cells. These findings sustain the idea that senile subjects may be affected by chronic stress in ways different from young or mature subjects.

Disclosures: **T. Morales-Salcedo:** None. **M.S. Luquin de Anda:** None. **G. Yañez-Delgadillo:** None. **G. Chipres-Tinajero:** None. **P. Hernandez-Carrillo:** None. **F. Jáuregui-Huerta:** None. **J. García-Estrada:** None. **Y. Ruvalcaba-Delgadillo:** None.

Poster

644. Stress and Cognition

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Program#/Poster: 644.10/RR22

Topic: E.05. Stress and the Brain

Support: NIH Grant RISE GM60665

Title: The relationship between oxytocin and cortisol during acute psychosocial stress in a non-clinical undergraduate sample

Authors: *K. MONDE[1], M. PANERO[2], J. KIM[3], D. SIMEON[4], V. LUINE[1];
[1]CUNY Hunter Col., New York, NY; [2]Boston Col., Boston, MA; [3]Michigan State Univ., East Lansing, MI; [4]Mount Sinai Sch. of Med., New York, NY

Abstracts: Increased levels of stress and chronic stress is known to negatively affect both physical and mental health. Cortisol, a steroid hormone, is released as part of the hypothalamic pituitary-adrenal axis of humans and is associated with both physical and emotional stress. Oxytocin (OT), a neurohypophysial hormone, is also related to stress and emotionality. Studies have indicated that cortisol administration facilitates the production of OT, and OT administration attenuates the stress response. Additionally, two studies have demonstrated a positive relationship between basal levels of endogenous OT and Cortisol (Taylor et al., 2006; Altemus et al., 2001), and one study found an inverse relationship between OT and cortisol levels during psychosocial stress (Pierrehumbert et al., 2010). In the current study, 55 undergraduates underwent the Stressful Event Speech task (SES) in which they described a stressful interpersonal event. Salivary OT and cortisol levels were assessed before, directly following, and after a 20 minute recovery period. Cortisol increased in response to the SES and returned to baseline post-recovery. OT decreased during recovery. We also found a positive relationship between post-SES cortisol levels and OT decrease during recovery ($r=.445$, $p<.05$) and a positive correlation between cortisol stress reactivity (post-SES cortisol - basal cortisol

levels) total OT decrease at the level of a trend ($r = .364$, $p = .06$). Thus, higher cortisol levels were associated with more decrease in OT. The temporal relationship between increased cortisol and decreased oxytocin demonstrates not only the responsiveness of OT system to stress, but that under stress, heightened cortisol may trigger changes in OT system function that lead to decreases in endogenous OT concentrations. Associations between OT and emotion suggest that decreased OT levels may aid individuals in feeling less stressed.

Disclosures: **K. Monde:** None. **M. Panero:** None. **J. Kim:** None. **V. Luine:** None. **D. Simeon:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

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Topic: E.05. Stress and the Brain

Support: RDECOM W15P7T-12-C-5015

Title: Odor-induced stress effects on first responder medical decision-making

Authors: ***B. D. WINSLOW**, N. NGUYEN, E. GOODRICH, S. DUFF, V. LUGO, D. JONES; Design Interactive, Inc., Oviedo, FL

Abstracts: Exposure to stress has the potential to significantly affect human cognitive performance processes such as reaction time, decision making, and memory. Odorant exposure can cause initiation of a stress response, activating the sympathetic division of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal cortex axis (HPA), resulting in neurotransmitter and corticosteroid release and subsequent downstream physiological effects. The coding of pleasant and negative odors is computed in distinct brain areas, including the orbitofrontal cortex and anterior cingulate, respectively. Building resilience - the human ability to adapt in the face of tragedy, trauma, adversity, hardship, and ongoing stressors - to odors is beneficial to first responders who are presented with novel odors during the treatment of trauma, and are expected to maintain high cognitive performance. Here we report a model of human resilience to odor by measuring and classifying HPA and ANS activation from physiological sensors during odor exposure. Novice participants were trained in medical triage, and performed either in the presence or absence of medically relevant odors. The rate of learning to perform triage classifications and retention of that knowledge were the primary measures of cognitive

function. Measurements of HPA and ANS activity including heart rate variability, respiratory sinus arrhythmia, and electrodermal activity were quantified using ECG, respiration, and electrodermal sensors. Fine motor performance was assessed following odor exposure using the Purdue Pegboard Test. Medically-relevant odors were presented during training and retention evaluation via a custom scent-delivery system. Odor exposure significantly increased the time/trials needed to attain proficiency in triage task performance, and significantly increased sympathetic and corticosteroid activity in early triage trials, but approximated the performance and physiology of the control group after several trials. Retention was assessed two weeks later. In addition, the generalizability of the odor-induced stress response was assessed using a second medically-relevant odor. An analysis of the physiological and behavioral data showed evidence for the cross-odor transfer of resilience to a novel odor after learning in the presence of odor. Strategies aimed at increasing resilience through pre-exposure and providing stress management techniques prior to task performance reduced sympathetic activity and increased performance in the odor condition, suggesting that the integration of stress training and odor exposure may improve first responder performance.

Disclosures: B.D. Winslow: None. **N. Nguyen:** None. **E. Goodrich:** None. **S. Duff:** None. **V. Lugo:** None. **D. Jones:** None.

Poster

644. Stress and Cognition

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Program#/Poster: 644.12/RR24

Topic: E.05. Stress and the Brain

Support: Basic Science Research Program through the National Research Foundation of Korea(NRF), funded by the Ministry of Education, Science, and Technology (No. 2011-0005029)

Title: Bidirectional effects of stress on decision-making and its neural correlates in a dynamic environment

Authors: *J. CHEY[1,2], H. PARK[1], D. LEE[3], N. DAW[4];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Neurol., Columbia Univ. Med. Sch., New York City, NY; [3]Neurobio., Yale Univ. Med. Sch., New Haven, CT; [4]Psychology, New York Univ., New York City, NY

Abstracts: Although too much stress impairs performance in a variety of tasks, it can also improve cognitive performance when a certain level of arousal is beneficial, as often referred to as the Yerkes-Dodson law (inverted U). In addition, previous studies have reported that stress increases habits while decreasing goal-directed behaviors. However, whether and how such bidirectional effects of stress manifest as a potentially shifting balance between habit versus goal-directed systems has not been investigated. In this study, we examined the dose-dependent effects of stress on model-free and model-based reinforcement learning at both behavioral and neural levels, using computational models and functional magnetic resonance imaging. Participants were randomly assigned to one of the three conditions, no-stress, single-stress-treatment, and double-stress-treatment. Two types of psychological stress protocol were employed in the study, which was immediately followed by a two-stage Markov decision-making task in which the reward probabilities for the choices in the second stage underwent reversals without notice. Analogous to the Yerkes-Dodson hypothesis, goal-directed behaviors increased in a single-stress-treatment condition but decreased in a double-stress-treatment condition. Similarly, BOLD activity in the frontal and parietal cortex was higher during decision making in the single-stress-treatment condition than in the no-stress and the double-stress-treatment conditions. These results suggest that stress can play a critical role in arbitrating the two controllers (habit and goal-directed) of decision-making in opposite directions depending on the level of stress currently experienced by the decision-maker, and further confirm the role of prefrontal cortex and superior parietal cortex in goal-directed actions.

Disclosures: J. Chey: None. **H. Park:** None. **D. Lee:** None. **N. Daw:** None.

Poster

644. Stress and Cognition

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Topic: E.05. Stress and the Brain

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (No. 2011-0005029)

Title: Multiple effects of stress on decision making in a changing environment

Authors: *P. HEYEON[1], D. LEE[2], J. CHEY[3];
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Neurobio., Yale university, New Haven, CT; [3]Dept. of Psychology, Seoul Natl. Universiy, Seoul, Korea, Republic of

Abstracts: Previous computational and lesion studies suggested that behaviors might be controlled by at least two partially separate neuroanatomical systems. Habit or model-free reinforcement learning incrementally shapes behaviors by trial and error, whereas goal-directed behaviors or model-based reinforcement learning rely on the internal model of the decision maker's environment and motivation. Here, we examined how stress influences human decision-making in a changing environment by testing whether it biases the contribution of model-free and model-based reinforcement learning processes and alters the rate of incorporating new information from the environment. Participants were randomly assigned to stress and control conditions, and performed a two-stage Markov decision-making task in which the reward probabilities underwent reversals without notice. We found that stress increased the contributions of model-free reinforcement learning while diminishing the influence of model-based reinforcement learning. It also decreased the rate at which the decision making strategy is updated by new experience. These results have implications for the underlying neural mechanism mediating the effects of stress on the formation of maladaptive habits, such as addictive behavior, as well as dysfunctional behaviors associated with stress-related disorders.

Disclosures: P. Heyeon: None. **D. Lee:** None. **J. Chey:** None.

Poster

644. Stress and Cognition

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Program#/Poster: 644.14/RR26

Topic: E.05. Stress and the Brain

Title: Rescuing inhibitory tone following chronic stress reverses stress-induced hippocampal-dependent deficits on object placement

Authors: *J. B. ORTIZ, S. TAYLOR, P. PAODE, C. D. CONRAD;
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Abstracts: Chronic stress leads to hippocampal-dependent spatial learning and memory deficits and the simplification of hippocampal CA3 dendrites. Past studies report that chronic stress skews the inhibitory (gamma-aminobutyric acid, GABA) and excitatory (glutamate) hippocampal neurotransmitter systems toward hyperexcitability. Correcting the inhibitory tone

through pharmacological manipulations to reduce hyperexcitability or to enhance inhibition can prevent stress-induced CA3 dendritic retraction. Consequently, we hypothesized that the dysregulated inhibitory tone following chronic stress might also contribute to the deficits in spatial learning and memory. Since blocking the GABAergic system or facilitating the glutamatergic system can potentiate hippocampal-dependent spatial learning and memory in non-stressed animals, we predicted that higher doses of a GABAA antagonist or glutamatergic agonist would be needed to improve spatial memory in chronically stressed rats than would be needed in nonstressed controls. Young male Sprague-Dawley rats were chronically stressed (STR, wire mesh restraint, 6h/d/21d) or not (CON). The day after chronic stress ended, all rats were injected i.p., with either Bicuculline (BIC, 0.0 mg/kg, 0.25 mg/kg, 0.50 mg/kg, a GABAA antagonist) or D-Cycloserine (DCS, 0.0 mg/kg, 3 mg/kg, or 15 mg/kg, a partial NMDA receptor agonist). Thirty min later, rats were tested for spatial memory using a 2-trial object placement task designed to be challenging to animals without pharmacological treatment. During training, rats were allowed to explore two identical objects in an open field. After a 3-hour inter-trial interval, one object was moved to a new location and time spent exploring both objects was recorded. BIC produced dose-dependent effects; BIC facilitated spatial memory in CON rats at both 0.25 and 0.50 mg/kg doses, whereas BIC facilitated spatial memory in STR at the highest dose only (0.50 mg/kg). DCS also led to dose-dependent effects with both doses (3, 15 mg/kg) facilitating spatial memory in CON rats, but only the 3 mg/kg dose improving spatial memory in STR rats. Consequently, STR rats required a higher dose of a GABAA antagonist to rescue spatial memory than was needed in CON rats. In contrast, the amount of DCS needed to rescue spatial memory in STR rats may follow an inverted U-shaped function. Thus, dose-dependently rescuing the inhibitory tone through GABAA antagonism reverses the impairing effects of chronic stress on spatial ability, whereas rescuing the inhibitory tone via the NMDA receptor is more complex than observed for the GABAergic system.

Disclosures: **J.B. Ortiz:** None. **S. Taylor:** None. **P. Paode:** None. **C.D. Conrad:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.15/RR27

Topic: E.05. Stress and the Brain

Support: CNPq

FAPERJ

Title: D-serine prevents acute stress-induced cognitive deficits in mice

Authors: G. D. GUERCIO[1], L. E. BEVICTORI[1], C. M. MADEIRA[1], C. VARGAS-LOPES[1], J. D'ÁVILA[2], V. F. CARVALHO[2], *R. A. PANIZZUTTI[1];
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Abstracts: Increasing evidence indicates that acute stress can disrupt cognitive functions mediated by the N-methyl-D-aspartate receptor (NMDAR), although the mechanisms are not fully understood. D-serine is the major endogenous co-agonist of the NMDAR, and is produced in the brain exclusively by serine racemase. Importantly, serine racemase is inhibited by phosphorylation on serine residues, resulting in lower D-serine levels. D-serine is crucial to long term potentiation induction and it also contributes to learning and memory. Also, D-serine is involved in sensorimotor gating, a filtering mechanism that is thought to prevent sensory overload. However, regulation of D-serine by acute stress remains to be investigated. Here we examined whether the D-serine pathway is regulated by acute stress in C57BL/6 male mice. To this end, we euthanized the mice after the stress protocol and dissected the prefrontal cortex and the hippocampus. We used high performance liquid chromatography to study D-serine levels and we performed an immunopreciptation followed by immunoblotting of the brain homogenates to study serine racemase phosphorylation. Interestingly, acute restraint stress decreased D-serine levels ($p<0.01$, $t=3.071$) and increased serine racemase phosphorylation on serine residues ($p<0.0001$, $t=5.393$) in the prefrontal cortex. In the hippocampus, neither D-serine levels nor serine racemase phosphorylation were altered by acute restraint stress. To study whether D-serine could prevent the effect of stress on memory, we performed an object recognition task. We tested the effect of acute restraint stress immediately after memory acquisition, during the memory consolidation phase. Recognition memory was impaired by acute restraint stress, which was prevented by peripheral administration of D-serine (1g/kg) 30 minutes before acquisition ($p<0.05$) or immediately after acquisition, before the stress procedure ($p<0.05$). However, D-serine had no effect on the memory impairment caused by stress when given one hour before memory retrieval. To investigate the impact of stress on sensorimotor gating, we measured the prepulse inhibition of the startle response (PPI). Acute restraint stress impaired PPI ($p<0.01$), which was prevented by D-serine administration 20 minutes before stress ($p<0.01$). Taken together, our results show for the first time the interplay between acute stress and D-serine. We demonstrated acute restrain stress lowered normal levels of D-serine, and that exogenous D-serine injections were efficient at restoring normal cognitive performance.

Disclosures: G.D. Guercio: None. **R.A. Panizzutti:** None. **L.E. Bevictori:** None. **C. Vargas-Lopes:** None. **C.M. Madeira:** None. **J. D'Ávila:** None. **V.F. Carvalho:** None.

Poster

644. Stress and Cognition

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Program#/Poster: 644.16/RR28

Topic: E.05. Stress and the Brain

Support: NIH 144PRJ23LC

NIH 144PRJ77NW

NIH T32-GM0077507

Title: Corticotropin-releasing factor acts within the prefrontal cortex to impair cognitive function

Authors: *S. HUPALO[1,2], R. C. SPENCER[2], C. W. BERRIDGE[2];
[1]Neurosci. Training Program, [2]Psychology, Univ. of Wisconsin - Madison, Madison, WI

Abstracts: The prefrontal cortex (PFC) regulates a variety of 'executive' cognitive processes critical for goal-directed behavior, particularly under distracting and/or ambiguous conditions. Stress impairs PFC-dependent cognitive function in humans, primates, and rodents. Stress-related impairment in PFC-dependent function is posited to contribute to a variety of psychopathologies. Yet, our understanding of the neural mechanisms responsible for stress-related impairment in PFC function is currently limited. The majority of research on this topic has focused on the actions of PFC catecholamines, demonstrating an important role of dopamine and norepinephrine in the cognition-impairing effects of stress. However, additional stress-related neurotransmitters are present in the PFC, including corticotropin-releasing factor (CRF). To date, the degree to which CRF acts within the PFC to modulate PFC-dependent cognition is not known. Given the prominent role of CRF in stress, we hypothesized that CRF signaling in the PFC elicits a stress-like impairment in PFC-dependent cognition. To test this hypothesis, we examined the effects of bilateral infusions of varying doses of CRF (25, 50, 100, 250 ng/hemisphere) into the medial PFC of rats on performance in a delayed response task of spatial working memory (T-maze). We observed that CRF exerts a regionally-specific and dose-dependent impairment in working memory. Specifically, CRF infusion into the dorsocaudal region of the medial PFC elicited robust impairment in working memory performance. In contrast, CRF infusion into the ventrocaudal or rostral PFC (both ventral and dorsal subfields) had no impact on performance. This is in contrast to what is seen with catecholamines, which modulate working memory via actions in the rostral medial PFC. These results provide the first evidence that CRF signaling in the PFC modulates higher cognitive function in a stress-like manner and suggest that CRF signaling within the PFC may contribute to stress-related

dysregulation of cognition. Ongoing studies are investigating the degree to which endogenous CRF signaling in the PFC modulates working memory in the absence and presence of stress. Finally, these findings provide further evidence for a topographic organization of medial PFC function that exists across both rostrocaudal and dorsoventral axes. This research lends new insight into the neurobiology of PFC-dependent function and may have relevance for treating psychopathologies associated with PFC dysfunction, including stress-related disorders.

Disclosures: S. Hupalo: None. **R.C. Spencer:** None. **C.W. Berridge:** None.

Poster

644. Stress and Cognition

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Program#/Poster: 644.17/RR29

Topic: E.05. Stress and the Brain

Support: Wellcome Trust Program Grant to Raymond J. Dolan

MRC PhD Funding to Archy O. de Berker

European Research Council, ERC, 260424 to Sven Bestmann

Title: Computations of uncertainty predict acute stress responses in humans

Authors: *A. O. DE BERKER[1,2], R. RUTLEDGE[2], R. J. DOLAN[2], S. BESTMANN[1]; [1]Sobell Dept. for Motor Neurosci., Univ. Col. London, London, United Kingdom; [2]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstracts: Indirect evidence suggests that uncertainty is an important contributor to stress, with one striking suggestion being that predictable events should not be described as stressful (Koolhaas et al., 2011). Building on this idea, we formalised stress as an adaptive reaction to uncertainty about threat. In this framework, stress responses function to reduce uncertainty by focusing attention and improving mnemonic function, with uncertainty providing a key parameter by which the amplitude of stress responses are controlled. We therefore hypothesised that physiological and subjective stress responses in human participants would scale with the current level of uncertainty about threat. To test this, we used a learning paradigm in which participants earned money by learning a probabilistic mapping between stimuli and shocks. On each trial, subjects were presented with one of two stimuli and asked to predict whether they were about to receive a shock. For each correct prediction, they earned a small amount of money.

Shocks were delivered irrespective of predictive accuracy, so that all participants received the same number of shocks (160). Crucially, the contingencies between stimuli and shocks shifted unpredictably over time, introducing uncertainty and requiring subjects to constantly update their estimates of threat for each stimulus. We dynamically assessed subjective stress using a visual analogue scale presented every 4-5 trials, and continuously monitored heart rate, breathing rate, and skin conductance. We used a computational model of learning under uncertainty (Hierarchical Gaussian Filter; Mathys et al., 2011) to characterise expectations, uncertainty, and prediction errors experienced by each subject throughout the course of the experiment. We used these quantities to construct a predictive model of task-elicited stress responses. We found that surprising events, including the surprising omission of a shock, are strong contributors to emotional and physiological stress responses, and that individuals' emotional stress dynamics are predictive of their physiological sensitivity to shock and uncertainty. This study provides empirical support for a theory of stress as an adaptive response to uncertainty.

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Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.18/RR30

Topic: E.05. Stress and the Brain

Title: Arousal modulates short-term emotional memory in women on hormonal contraception

Authors: *S. E. NIELSEN, S. J. BARBER, M. MATHER;
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Abstracts: To date, few studies have investigated whether sex hormones and arousal interact to modulate short-term memory. Thus, the present study tested whether arousal at encoding differentially modulates short-term memory for emotional and neutral images in naturally cycling women (NC women) and women on hormonal contraception (HC women). Based on arousal-biased-competition theory (Mather & Sutherland, 2011), we predicted that arousal at encoding would enhance short-term memory for negative images (i.e., high priority images) and suppress memory for neutral images (i.e., low priority images). Given that long-term emotional memory is modulated by contraceptive status (Nielsen, et al., 2011), we also tested whether the effects of arousal on short-term memory also depended on contraceptive status. This study had a

2 (Arousal vs. No arousal at encoding) X 2 (NC vs. HC women) X 3 (Negative vs. Positive vs. Neutral pictures) design. To induce arousal at encoding, at the beginning of the experiment some women were administered an isometric handgrip protocol, which has reliably increased endogenous norepinephrine levels in previous studies. For the control condition, the handgrip task was replaced with a relaxed water bottle hold. Immediately after this, all participants saw a slideshow containing negative, positive, and neutral pictures. Later, all women completed a free recall test for the images. We used eye-tracking technology to assess pupil diameter changes (index of arousal) in response to the handgrip v. control tasks, and we collected salivary samples to assess changes in salivary alpha-amylase (a biomarker for norepinephrine) and levels of 17β-estradiol and progesterone. We tested whether increases in arousal would enhance the tendency to preferentially recall the negative rather than the neutral images, and whether this would vary as a function of contraceptive status. A preliminary ANOVA revealed a significant three-way interaction between picture valence (negative v. neutral), arousal condition (handgrip v. control) and contraceptive status (HC v. NC women). Follow-up analyses suggest that in HC women only, arousal enhanced short-term memory for negative images while suppressing short-term memory for neutral images. NC women exhibited no differences in short-term recall of negative or neutral images, regardless of arousal condition. These preliminary results suggest that physiological arousal at encoding modulates short-term emotional memory differently depending on contraceptive status. In women, sex hormones and contraceptive status appear to modulate both immediate and long-term memory consolidation processes for emotional stimuli.

Disclosures: **S.E. Nielsen:** None. **S.J. Barber:** None. **M. Mather:** None.

Poster

644. Stress and Cognition

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 644.19/RR31

Topic: E.05. Stress and the Brain

Title: L. reuteri induced anxiety behavior and fear related memory change in C57BL/6J mice

Authors: ***M. J. EIMERBRINK**[1], J. D. WHITE[1], M. J. CHUMLEY[2], G. W. BOEHM[1]; [1]Psychology, [2]Biol., Texas Christian Univ., Fort Worth, TX

Abstracts: This research utilized the bacteria Lactobacillus reuteri to induce behavioral changes in C57BL/6J. Specifically, we were interested in the how chronic treatment with L. reuteri can alter anxiety behaviors and fear related memory. To assess anxiety, we utilized an elevated zero

maze and an open field test. Behavioral data from both measures support the hypothesis that treatment with L. reuteri can effectively reduce anxiety related behaviors. To assess fear related memory, we used both contextual and delay conditioning paradigms. Again, we found evidence to support fear related memory alterations such that L. reuteri treated animals display less freezing behavior during testing compared to control animals. To explore potential biological mediators of behavior, we analyzed both corticosterone levels 30 minutes after behavior data collection and GABA receptor expression in the hippocampus and amygdala. Additionally, we evaluated whether L. reuteri can protect against exaggerated anxiety behaviors induced by fear conditioning.

Disclosures: **M.J. Eimerbrink:** None. **J.D. White:** None. **M.J. Chumley:** None. **G.W. Boehm:** None.

Poster

644. Stress and Cognition

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Program#/Poster: 644.20/RR32

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: R01MH094358

Title: Blood chromatin as a protein biosensor: Morphometrics, cognition and behavior

Authors: B. M. FEINER, K. A. CHASE, *R. P. SHARMA;
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Abstracts: Introduction: Histone modifications in peripheral mononuclear blood cells (PBMCs) from living patients can be conceptualized as analogous to blood glycated hemoglobin (HbA1c) levels in diabetic patients in that both serve as a proxy for the degree of their respective modifications in the entire body. Across the genome, epigenetic regulation of chromatin can be seen as an organizing principle by which central and peripheral compartments are interlinked by virtue of commensurable gene network entrainment. Chromatin deposition along these networks is imposed by the totality of epigenetic influences, including significant contributions from biochemicals that readily traverse the blood-brain barrier. Utilizing PBMCs allows us to generate a hypothesis connecting the epigenetic influence on gene networks and clinical outcomes of aberrant histone modification levels in real-time. Method: 30 subjects were recruited from the community at the University of Illinois at Chicago, Illinois. Clinical measurements included

morphometric measurements (waist circumference and BMI), the MATRICS cognitive battery, and the Heinrichs-Carpenter Quality of Life Scale (QLS). Acid extraction of PBMC extracts was performed and protein levels were quantified by the Bradford assay (n=30). Three histone modifications were measured via Elisa assays from Active Motif (H3K9acetyl-#53114, H3K9me2-#53109, H3K4me3-53109, Total H3-#53110). All histone modifications were first normalized to an internal standard curve, followed by a plate-control standard, and finally to the total histone H3 protein. Results: a) Increases in H3K9me2 levels were correlated with increases in attention and vigilance (MATRICS; $\rho=0.46$; $p<0.03$), decreases in both intelligence (WAIS-III score: $\rho=-0.42$; $p<0.039$) and sociosexual relationship functioning (QLS; $\rho=-0.46$; $p<0.01$). In addition, H3K9me2 levels were increased in overweight participants, as measured by both waist circumference ($\rho=0.44$; $p<0.016$) and BMI ($\rho=0.35$; $p<0.002$), and in participants with a history of hyperlipidemia ($t=2.76$; $p<0.01$). H3K9acetyl modifications were correlated with increases in a sense of purpose (QLS; $\rho=0.47$; $p<0.014$). H3K4me3 modifications were significant decreased in overweight participants, ($t=-2.12$; $p<0.035$), but were increased in participants with a history of diabetes ($t=3.98$; $p<0.001$). Discussion: Real time blood levels of chromatin modifications in living patients have the capacity to integrate all ambient epigenetic inputs. In this preliminary study, we can report that specific modifications are enriched in certain physiological and cognitive phenotypes.

Disclosures: B.M. Feiner: None. **K.A. Chase:** None. **R.P. Sharma:** None.

Poster

644. Stress and Cognition

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Topic: E.05. Stress and the Brain

Support: T32MH017168-30

DA09082

Title: Decreased sensitivity of female rat locus coeruleus neurons to μ-opiate agonists: Electrophysiological, protein and behavioral studies

Authors: *H. M. GUAJARDO[1,2], A. HO[2], X.-Y. ZHANG[3], R. VALENTINO[3,2];
[1]Neurosci. Grad. Group, [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Stress Neurobio., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstracts: Females are more likely than males to have depression and anxiety disorders. It has been hypothesized that this sex difference results from differences in stress response systems. The locus coeruleus (LC)-norepinephrine (NE) system, is a major stress response system that is thought to be dysregulated in stress-related psychiatric disorders. During stress, the LC-NE system is activated by corticotropin releasing factor (CRF), a primary mediator of the stress response. At the same time, endogenous opioid neuropeptides provide an inhibitory influence on LC activity that restrains CRF activation and facilitates a return to baseline activity when the stressor ends. An imbalance in the opposing regulation of the LC-NE system by CRF and opioids could influence stress-sensitivity and enhance stress vulnerability. Sex differences could be expressed through differences in sensitivity to either CRF or to endogenous opioids. The goals of this study were to determine whether there are sex-differences in 1) LC neuronal responses to μ-opiate receptor (MOR) agonists; 2) LC-MOR expression levels; or 3) cognitive/behavioral correlates of MOR activation in the LC. We generated dose response curves for the MOR-agonist, DAMGO, on LC discharge rates. Extracellular single-unit LC activity was recorded in anesthetized male and female Sprague Dawley rats using double barrel micropipettes and DAMGO was simultaneously microinfused. Notably, the highest dose [10 pg] completely suppressed LC firing in males (n=7) but not in females (n=7). A two way repeated measures ANOVA, revealed sex differences in the response to DAMGO such that females were less sensitive ($F(1, 12) = 15.281$, $p<0.002$). Western blot analysis revealed decreased MOR levels in LC punches from female (n=11) compared to male (n=11) rats ($t(20) = 2.142$, $p< 0.05$). Finally, the ability of DAMGO in the LC to affect behavior in an attentional set-shifting task (AST), an animal model of cognitive flexibility were assessed. In male rats DAMGO (3 pg, intra-LC) increased the number of errors in performance of a strategy shifting task compared to vehicle control ($F(2, 23) = 3.6$, $p<0.05$, n=7-9). Preliminary data in females (n=3-5/group) suggest that unlike males, DAMGO does not impair strategy shifting behavior in females. Together, the data suggest that LC neurons of females are less sensitive to MOR agonists as a result of decreased MOR in the LC. Given the protective effects of MOR activation in the LC during stress, this could be a mechanism underlying enhanced stress sensitivity of females.

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Poster

644. Stress and Cognition

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Program#/Poster: 644.22/RR34

Topic: E.05. Stress and the Brain

Support: NSERC

CIHR

Title: Is enhanced retrieval of fear memories part of the depressive phenotype produced by repeated corticosterone treatment in rats?

Authors: *L. E. KALYNCHUK[1], W. N. MARKS[1], E. Y. FENTON[1], N. M. FOURNIER[2], B. D. KULYK[1];
[1]Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]Yale, New Haven, CT

Abstracts: Repeated exposure to corticosterone (CORT) is often used as a preclinical model of depression in rats as it produces robust increases in depressive-like behaviour. However, although a bias towards maladaptive thinking patterns and emotional responses is a cardinal symptom of depression, these symptoms have been rarely considered in preclinical models. One way to investigate maladaptive thinking is through the use of fear conditioning paradigms. Typically fear conditioning tasks are used to model post traumatic stress disorder and anxiety in rodents. However, fear conditioning tasks also demonstrate more general alterations in emotion-associated memories, a symptom of depression in humans. We propose that fear conditioning also be used as a measure of depressive-like behaviour in rodents. In a series of experiments, we systematically investigated the effects of repeated injections of CORT on two variations of fear conditioning, delay fear conditioning and trace fear conditioning, as well as whether the antidepressant fluoxetine reverses changes in fear-related behaviour produced by CORT. In the first experiment, male Long-Evans rats received either vehicle or 40 mg/kg of CORT injections for 21 consecutive days followed by forced swim testing to demonstrate a depressive phenotype using traditional measures of depressive-like behaviour. Results revealed a significant increase in helplessness behaviour in CORT rats during the forced swim test. In the second experiment, rats received similar treatment as experiment one with the addition of a 5mg/kg CORT group to examine dose dependent effects of CORT on learning and memory of delay conditioned cues. We found that 40mg/kg of CORT significantly enhances freezing behaviour during retrieval of both contextual and tone cued fear, whereas only tone cues are responsive to the effects of 5mg/kg of CORT. A third experiment examined the effects of repeated injections of 40 mg/kg of CORT on trace conditioning. Interestingly, we found that CORT enhanced freezing behaviour both during acquisition and retrieval of trace conditioning. In a final experiment, we provide preliminary evidence that fluoxetine administered concurrently with 40mgk/kg CORT significantly reduces freezing during retrieval of tone cues. Collectively, these experiments demonstrate that enhanced freezing during retrieval of conditioned fear cues is reliably produced by repeated exposure to CORT, effects that are partially reversed by fluoxetine. Overall, this suggests that fear conditioning is a potential tool for exploring the circuits that mediate pathological alterations in emotion-associated memory associated with depression.

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Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.01/RR35

Topic: E.08. Biological Rhythms and Sleep

Title: Effect of a metformin-derived small molecule on circadian clock phase-resetting

Authors: *H. S. ROW[1], S. KIM[3], K. KIM[2];
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Abstracts: Circadian rhythm governs daily physiology and behavior in mammals. Disruption of circadian clock molecule(s) has been known to cause metabolic disorders such as obesity and diabetes. Metformin is the first line of antidiabetic drug in the biguanide class. We investigated the effect of a metformin derivative (designated as HL156A) on circadian clock regulation. HL156A phosphorylated 5'-AMP activated kinase (AMPK), a key energy sensor at much lower dosage as compared to Metformin. HL156A shortened pulse period and dampened amplitude of Period2 oscillation in a dose-related manner in the mouse embryonic fibroblast cells cultured *in vitro*. In a jet lag *in vivo* model, administration of HL156A enhanced duration of re-entrainment after 6-h phase advance in LD cycle, while it did not affect the free running locomotor activity in a constant dark condition. HL156A degraded circadian clock proteins such as PER2 and CRY1 in the presence of cyclohexamide, much faster than that of metformin, indicating that Metformin-drived small molecule may affect phase resetting. Taken together, it appears that a novel metformin derivative, HL156A may hold a potential as a chronomodulator to treat desynchronous syndrome such as jet lag.

Disclosures: **H.S. Row:** None. **S. Kim:** None. **K. Kim:** None.

Poster

645. Circadian Clock

Location: Halls A-C

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Topic: E.08. Biological Rhythms and Sleep

Support: This study was supported by Research Grant Program at Escuela de Medicina, Universidad Anáhuac Mayab given to E.M.-R.

Title: Expression of MAPK in hypotalamus and pons on the circadian fluctuation on rats

Authors: *R. JIMENEZ-MORENO[1], S. MIJANGOS-MORENO[2], A. POOT-AKE[2], A. MANJARREZ-MARTÍN[3], E. PACHECO-PANTOJA[3], P. AQUINO-HERNÁNDEZ2[3], M. SALAS-CRISÓSTOMO[2], A. TEJEDA-PADRÓN[2], E. MURILLO-RODRIGUEZ[2];
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Abstracts: Mitogen-activated protein kinase (MAPK) is a protein involved in several neurobiological functions, including sleep modulation. However, no evidence is available regarding the circadian fluctuation of this protein in sleep-related brain areas such as hypothalamus (HYP) or pons (PONS). Thus, the aim of the experiment was to describe the circadian pattern of MAP-K in HYP and PONS. For this purpose, male Wistar rats were sacrificed by decapitation at different times during 24h: Zeitgeber hour 4 [ZT1], ZT8, ZT12, ZT16, ZT20, ZT24. Brain samples were collected for MAP-K analysis using Western blot techniques. It was found that MAP-K expression was higher in HYP and PONS during the lights-on period, especially at ZT4, whereas a diminution was observed across time, specifically during the lights-off period (ZT16). Importantly, we found that MAP-K expression was higher in PONS compared to HYP in the time points studied across time points. Preliminary data suggest that MAP-K expression is under a circadian influence and changes in its expression depends upon the lights/dark period. Further studies are needed to elucidate the mechanism of action of circadian fluctuation of MAP-K in sleep-related brain areas such as HYP and PONS.

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Poster

645. Circadian Clock

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Program#/Poster: 645.03/RR37

Topic: E.08. Biological Rhythms and Sleep

Title: Identification and characterization of a novel agonist modulating circadian nuclear receptor, REV-ERBα

Authors: *J. J. LEE[1], Y. SUH[2], K. KIM[3];
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Abstracts: Rev-erba (NR1D1) belongs to the nuclear receptor superfamily of transcription factors which regulate a wide range of biological processes including circadian rhythm and metabolism. We attempted to identify small synthetic molecules that modulate Rev-erba by cell-based screening with 1200 drug-like compounds based on 2x RORE (Rev-erba/Ror response element)-mediated transcriptional activity. One of compounds (designated as KK-S6) repressed RORE- but not mutant RORE-mediated transcriptional activity. KK-S6 compound dramatically altered amplitude, but not period of circadian oscillation of BMal1 in a dose-related manner. We also evaluated Rev-erba dependency in mouse embryonic fibroblast cells from Rev-erba KO mouse. In Hepg2 cells, KK-S6 repressed endogenous expression levels of Rev-erba-regulated genes including hBMal1, hPlasminogen-activator inhibitor (type 1) and hCitrate synthase, which values are comparable to GSK4112, a known Rev-erba agonist as a reference. Further optimization and subsequent functional studies may lead to development of potential therapeutic agents of Rev-erba involved in circadian rhythm and metabolism. ***Key words: Circadian clock, Rev-erba, synthetic compound, RORE***

Disclosures: J.J. Lee: None. **Y. Suh:** None. **K. Kim:** None.

Poster

645. Circadian Clock

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Topic: F.04. Neuroethology

Support: UdelaR DT

PEDECIBA

UNIV. CLAUDE BERNARD

Title: An integrative study of the circadian rhythmicity of electric behavior: From the field to the dish

Authors: *A. SILVA[1], A. MIGLIARO[1], P. MARCHAL[2,3];
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Abstracts: Keeping an adequate timing among physiological and behavioral processes is a key point for survival, hence, endogenous biological rhythms are synchronized with external cues, mainly photoperiod, which vary in circadian and circa annual fashion. Gymnotus omarorum, a nocturnal South American weakly electric fish, displays electric behaviors consisting on modulations of the electric organ discharge (EOD). The EOD is driven by a medullary pacemaker nucleus (PN) whose activity is modulated by environmental, sensory, and social cues. The basal rate of the EOD is an indicator of social hierarchy, and shows a transient small-amplitude melatonin-dependent nocturnal rise (NR) in coincidence with general arousal and the increase in locomotor activity. With the aim of characterizing the NR as a circadian rhythm, we continuously recorded the EOD and locomotor activity of isolated non-sexually mature adults (n=6) for 10 days at constant water temperature and conductivity (22°C, 150µS/cm), both with a light-dark cycle 12:12 and in constant darkness. A robust rhythm of EOD rate and locomotor activity was confirmed in the light-dark regime, which did not persist in all the individuals during the dark-dark regime. However, the characterization of circadian rhythms in wild animals using exclusively laboratory settings is currently questioned as it might limit the detection of behavioral patterns that only emerge in nature. We therefore recorded circadian changes in EOD basal rate in the wild for 72 h (isolated individuals in restricted areas, Laguna del Sauce, Maldonado Uruguay, 34°51'S, 55°07'W, n=6); and EOD basal rate and sheltering behavior in semi-natural conditions (400L outdoor tanks with equally-sized EOD-recording shelters, n=10). To test if the main circadian messenger, melatonin, is directly involved in the modulation of EOD rate, we are currently testing its effect on the spontaneous activity of the PN recorded from brainstem slices.

Disclosures: **A. Silva:** None. **A. Migliaro:** None. **P. Marchal:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.05/RR39

Topic: E.08. Biological Rhythms and Sleep

Title: LD-stress alters circadian expressions of GAD in mice

Authors: *A. MUTO, K. OSADA, T. WATANABE, A. TAGUCHI, T. HAGA, Y. OGAWA, M. NAKANO, Y. SASUGA, N. YAMAGUCHI;
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Abstracts: Amino acids [glutamate and γ-aminobutyric acid (GABA)] are the most abundant neurotransmitters and are largely responsible for the excitation/inhibition balance in the brain. These studies have suggested that GABA levels may be decreased in animal models of depression, and clinical studies reported low plasma and CSF GABA levels in depression. Living matter are known to have 24 h circadian rhythm. It is considered as one factor of major depressive disorder (MDD), this circadian rhythm is the disturbance because a lot of depressed patients is sleeplessness. A circadian rhythm is subject to influence of light. Preclinical study had suggest that 3.5-h light and 3.5-h dark (LD-stress mice) increased depression-like behaviors by forced swimming test and sucrose preference (Tara A et al, Nature, 2012). Administration of the antidepressant drugs to LD-stress mice improved depression-like behaviors, then LD-stress mice was considered to model animal of depression. We used this animal models, determined the mRNA clock gene and Gad1, Gad2 from hypothalamus and salivary glands cells, and investigated how circadian oscillation of clock gene and Gad1, Gad2 expression changed in the LD-stress mice. In addition, we examined the effect of fluoxetine. The hypothalamus contain the primary mammalian circadian clock that regulates rhythmic physiology and behavior. GABA is an important neurotransmitter in the hypothalamus cells. This study investigated whether the circadian rhythm to GABA existed in the mouse hypothalamus and salivary glands cells by real-time PCR. Total RNA was extracted using a protocol combining the Oragene RNA Self-Collection Kit (DNA Genotek) and the RNeasy Micro Kit (QIAGEN). To quantify the amount of mRNA in hypothalamus, we performed real-time PCR by using TaqMan Fast Universal PCR Master Mix (life technologies).

Disclosures: **A. Muto:** None. **K. Osada:** None. **T. Watanabe:** None. **A. Taguchi:** None. **T. Haga:** None. **Y. Ogawa:** None. **M. Nakano:** None. **Y. Sasuga:** None. **N. Yamaguchi:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.06/RR40

Topic: E.08. Biological Rhythms and Sleep

Support: J.P. Scott Center Center for Neuroscience, Mind & Behavior

Title: Circadian rhythms in differentiating adult neural stem cells from the dentate gyrus of the hippocampus

Authors: *A. MALIK, R. J. JAMASBI, M. E. GEUSZ;
Biological Sciences, Bowling Green State University, Bowling Green, OH

Abstracts: Embryonic stem cells appear to lack circadian rhythms. It is unclear whether neural stem cells contain an endogenous clock and express circadian rhythms. Our previous work indicated that neural stem cells from the mouse subventricular zone lack a circadian rhythm until they differentiate. Reports have shown *mPer2* expression in the dentate gyrus but did not detect a circadian rhythm in the activity of this circadian clock gene. Studies have shown a daily rhythm in hippocampal adult neurogenesis. It is possible that circadian control of neurogenesis produces nascent neurons at a time of day when fine discrimination of sensory information is most critical. To determine whether hippocampal adult neural stem progenitor cells can generate circadian rhythms, neural stem cell cultures (neurospheres) were prepared from the dentate gyrus of 4 to 5-month-old transgenic mice expressing firefly luciferase under control by the *mPer1* promoter. Presence of stem cell markers nestin and musashi-1 was confirmed by immunocytochemistry. Neurospheres were imaged after they were treated with forskolin to synchronize any circadian pacemaker cells present and then placed in medium that induces differentiation into neurons and glial cells (10% fetal bovine serum) or medium that maintains the stem cell state (containing fibroblast growth factor-2 and epidermal growth factor). Only the neurospheres in serum medium produced circadian rhythms, suggesting that differentiation allows circadian timing to be detected. Average period for the first and second cycles was 23.71 hours (±2.16 S.D., n=7). Because circadian rhythms were present before the neurospheres differentiated completely, it appears that transit-amplifying cells derived from the stem cells contain a circadian clock before they form mature neurons.

Disclosures: A. Malik: None. **R.J. Jamasbi:** None. **M.E. Geusz:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.07/RR41

Topic: E.08. Biological Rhythms and Sleep

Support: IOS-1021957

Title: Circadian and subregional gene expression in the suprachiasmatic nucleus

Authors: *J. L. LENSIE[1], E. M. MINTZ[1,2];
[1]Biol. Sci., [2]Biomed. Sci., Kent State Univ., Kent, OH

Abstracts: The suprachiasmatic nucleus (SCN) of the hypothalamus acts as an endogenous circadian clock. Individual neurons of the SCN are capable of acting as independent circadian oscillators, but the SCN exhibits a specific structural organization, with subregions that can be defined by a variety of criteria, including neuropeptide expression and cellular responses to stimuli. Individual subsets of cells can show different rhythms of gene expression. Individual genes have been identified that are differentially expressed in three specific SCN subregions. In mice, these include 1) The ventral SCN, characterized by expression of vasoactive intestinal polypeptide (VIP), 2) the central SCN, characterized by expression of gastrin-releasing peptide (GRP), and 3) the dorsomedial SCN, characterized by expression of arginine vasopressin (AVP). There is some overlap among all of these regions. In this study we hypothesized that these 3 regions were not only defined by their neuropeptide expression, but other circadian and non-circadian gene expression, thus revealing previously unknown details of the organization of the neuronal components of the SCN. Thirty-seven adult, male, C57BL/6J mice were housed in constant dark for two weeks in cages equipped with running wheels to assess circadian time (CT). Animals were sacrificed at 2-hr time points across the circadian cycle, and brains removed and flash frozen. Cells from SCN subregions were extracted using laser capture microdissection. RNA was purified from the extracts, amplified and labeled, and hybridized to Affymetrix mouse ST 1.0 whole genome expression arrays. The most pronounced region-specific differences in expression occurred in genes for known SCN neuropeptides, with few other genes showing large regional variations with the exception of a set of genes presumably expression in oligodendrocytes in the ventral SCN relating to myelin production. Regional and circadian expression of clock and neuropeptide genes was confirmed by qPCR analysis of a separate set of samples. We have identified numerous transcripts that show very robust circadian cycling across all three subregions. Known genes among these all have important roles in the circadian clock or circadian output. Some genes have not been identified as important in the clock, but represent good targets for further investigation. These data suggest that functional variation across SCN subregions are largely a function of neuropeptide expression, spatial location, and network connectivity, and not to fundamental differences in neuronal function.

Disclosures: **J.L. Lensie:** None. **E.M. Mintz:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.08/RR42

Topic: E.08. Biological Rhythms and Sleep

Support: Taiwan NSF 102-2321-B-002-081

Title: Light effects on circadian clock modulation of metabolism

Authors: ***Y.-F. ZOU**, C.-C. LEE, S.-K. CHEN;
Life Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstracts: Abstract: The disrupted circadian rhythm is related to higher risks of getting metabolic disorders such as insulin resistance, obesity and hyperlipidemia, which could further develop severe diseases related to cardiovascular, gastrointestinal and metabolic function, including type II diabetes. It was revealed in previous research that light signals would influence non-image forming physiological functions through intrinsically photosensitive retina ganglion cells (ipRGCs). Althought recent studies have shown that circadian rhythm is involved in modulation of physiological regeneration and storage of energy , whether the light signal can manipulate key pathways of the metabolism remains unknown. Thus, we want to exame how light signal transduction from the retina affects the metabolic status. We housed several ipRGC mutant mouse lines, inclulding *melanopsin* knockout (Cre/Cre) strain and Brn3b positive ipRGCs eliminated strain (3bDTA/+), under dim light at night and evaluated the metabolic conditions of these mice. Our preliminary data showed that the body weight gain, final body weight, as well as the composition of the intestinal microbiota of these mice were altered in different light-dark conditions. It suggests that light signals regulates the mammalian metabolic status through ipRGCs integration without shifting the central clock at SCN.

Disclosures: **Y. Zou:** None. **S. Chen:** None. **C. Lee:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.09/RR43

Topic: E.08. Biological Rhythms and Sleep

Title: Alteration of circadian clock in Afh mutants results in specific DNA methylation changes

Authors: F. TINARELLI[1], E. IVANOVA[2], G. KELSEY[2], *V. TUCCI[1];
[1]Neurosci. and Brain Technologies, Inst. Italiano Di Tecnologia (IIT), Genova, Italy;
[2]Epigenetics Programme, The Babraham Inst., Cambridge, United Kingdom

Abstracts: Independent molecular clocks are integrated across various cell types, enabling the synchronization of circadian rhythms within an organism. These molecular regulatory processes include transcriptional and translational positive/negative feedback loops of core clock genes. To date, very little is known about the role of specific epigenetic mechanisms regulating the circadian clock. In this study we address specific questions about the interplay between circadian clock alterations and DNA methylation. Traditionally, DNA methylation has been considered a permanent silencing mechanism for gene expression. However, a few recent studies have shown dynamic methylation changes in the brain which depend on environmental variations and neuronal activity. Here we have conducted a CpG island (CGIs) methylation genome-wide screen in the mouse After-hours (Afh) mutant, expressing an extreme lengthening of the circadian period, and wild-type littermate controls. We have used a reduced representation bisulphite sequencing (RRBS) approach and we have identified significant changes of DNA methylation in a small number of specific targets. Preliminary results indicate that 20 CGIs regions were differentially methylated between Afh homozygous mutants and wild-type controls in the suprachiasmatic nucleus (SCN) of the hypothalamus. A cutoff of 5 sequencing reads for each CpG site was used to identify significant methylation changes between mutants and controls. We have observed a range of changes between Afh mutants and controls, which oscillate from 5% to 20% according to specific genes. Among the targets we have identified we report here NKx2-2 (homeobox protein Nkx-2.2), Brd4 (bromodomain-containing protein 4), Kcnh3 (potassium voltage-gated channel subfamily H member 3), Opn4 (opsin 4) and Sfn (stratifin). Moreover, 15 regions presented a hypermethylation in the mutants SCN compared to the wild type, which suggest a possible involvement of Afh mutation in hypermethylation of specific CpGs intragenic sequences. Overall, our study revealed that a genetically determined alteration of the circadian clock (due to the Afh mutation) can alter the DNA methylation profile of genes involved in different functions such as cell signaling, synaptic plasticity and transcriptional activity.

Disclosures: **F. Tinarelli:** None. **E. Ivanova:** None. **G. Kelsey:** None. **V. Tucci:** None.

Poster

645. Circadian Clock

Location: Halls A-C

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Topic: E.08. Biological Rhythms and Sleep

Support: DFG Grants GSC 226/1, GSC 226/2 to A.T.

Volkswagen Foundation to H.O.

Title: Metabolic feedback to the hypothalamic circadian clock by adiponectin

Authors: *A. TSANG[1,2,3], H. OSTER[1,3];
[1]Med. Dept. I, Univ. of Luebeck, Luebeck, Germany; [2]The Göttingen Grad. Sch. for Neurosciences, Biophysics, and Mol. Biosci., Univ. of Goettingen, Goettingen, Germany; [3]Circadian Rhythm Group, Max Planck Inst. for Biophysical Chem., Goettingen, Germany

Abstracts: There is a growing body of evidence suggesting an extensive crosstalk between circadian clocks and energy metabolism. Peripheral metabolic state can feed back on central clock function, but the mechanism of this link is still poorly understood. In this study, we engineered a hypothalamic neuronal cell line to stably express a circadian reporter and used it as a model to screen for metabolic signals that are capable of resetting neuronal clocks. With this methodology, we identified an adipokine hormone, adiponectin, as a potential modulator of hypothalamic circadian circuits. Adiponectin deficient mice show dampened 24-h feeding rhythms associated with altered diurnal profiles of clock and appetite-regulating gene expression in the mediobasal hypothalamus. Further analysis revealed that adiponectin acts via adiponectin receptor 1 (ADIPOR1) and PGC1a-mediated activation of the core clock gene Bmal1 to reset the molecular oscillations of hypothalamic neurons. Together, these data reveal a novel metabolic feedback mechanism to the central circadian clock.

Disclosures: A. Tsang: None. **H. Oster:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.11/RR45

Topic: E.08. Biological Rhythms and Sleep

Title: Rhythmic control of mrna stability is essential for circadian amplitude of mouse period3 mrna

Authors: *J. CHOI[1], S.-H. KIM[2];
[1]Life science, [2]school of interdisciplinary bioscience and bioengineering, POSTECH, POHANG, Korea, Republic of

Abstracts: The daily oscillations observed in most living organisms are endogenously generated with a period of 24 hours, and their fundamental structure is an autoregulatory transcription-translation feedback loop. In addition to transcriptional and post-translational regulation, mechanisms of untranslated region (UTR)-mediated post-transcriptional regulation (i.e., mRNA degradation and internal ribosomal entry site (IRES)-mediated translation) have been suggested to fine tune clock gene expression. Mouse Period3 (mPer3) is one of the Period paralogs and its functions are important in peripheral clocks and a sleep physiology. mPer3 mRNA displays a circadian oscillation and a circadian phase-dependent stability, while the stability regulators still remain unknown. In this study, we identify three proteins - heterogeneous nucleus ribonucleoprotein (hnRNP) K, polypyrimidine tract-binding protein (PTB), and hnRNP D - that associate with the cis-acting element suggested from the previous study. Two of these proteins contribute to mRNA stability. Both experiments and mathematical modeling describe their roles in the cytoplasm for mRNA stability regulation and circadian amplitude formation. Moreover, our mathematical model suggests a mechanism of how post-transcriptional mPer3 mRNA stability modulation provides the flexibility of oscillation amplitude, as well as the robustness of the period and the phase, for its circadian expression.

Disclosures: J. Choi: None. **S. Kim:** None.

Poster

645. Circadian Clock

Location: Halls A-C

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Program#/Poster: 645.12/RR46

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant 2R25GM061151

NSF Grant 1026560

Title: Honey bees exhibit shift work in foraging and fanning behavior

Authors: *M. A. GIANNONI GUZMAN, T. GIRAY, J. L. AGOSTO-RIVERA;
Dept. of Biol., Univ. of Puerto Rico Rio Piedras Campus, San Juan, PR

Abstracts: Although shift work is an essential component of human society, for example in areas such as healthcare and security, it is associated with circadian misalignment and increased susceptibility to a number of diseases. Studying these processes in humans has proven difficult and limited by lack of experimental manipulations. Honey bees for a long time have been used as a model for social interactions, learning and memory and addiction among many others. A recent study investigating if pollen foragers captured during the morning were genetically different from those captured in the afternoon, found that there are paternal lineages that are only observed either in the morning or the afternoon suggesting that that shift work may occur in pollen foragers. Based on these findings, we hypothesized that honey bee foragers exhibit shift work in their foraging tasks. To test this hypothesis we number tagged 1-day-old workers and made direct behavioral observations of entry, exit, pollen load and fanning behavior for 14 days. Our results revealed that about 18% of foragers only made trips during the morning, while approximately 26% only foraged in the afternoon and the remaining bees foraged during both morning and afternoon. By evaluating the foraging pattern of individuals throughout the observation period we found that some individuals change shifts as they age, while others remain in the same shift (morning or afternoon). Analysis of the foraging trips of individual foragers revealed that individuals with no shift work perform more foraging trips. In addition, we looked at fanning behavior at the entrance of the colony and found that fanning behavior is also performed in shifts. Understanding the underlying mechanisms of shift work in honey bees will open the door for a better understanding of how individual differences in chronotype result in increased disease susceptibility.

Disclosures: M.A. Giannoni Guzman: None. **T. Giray:** None. **J.L. Agosto-Rivera:** None.

Poster

645. Circadian Clock

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 645.13/RR47

Topic: E.08. Biological Rhythms and Sleep

Support: NIH NINDS

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Caltech Biology Division Postdoctoral Fellowship

Title: Application of wavelet analysis to zebrafish circadian and behavioral data

Authors: *E. A. MOSSER, C. N. CHIU, D. A. PROBER;
California Inst. Technol., PASADENA, CA

Abstracts: A commonly used method for analyzing circadian physiological and behavioral oscillations is sinusoidal curve fitting. This approach allows for the characterization of circadian oscillatory parameters such as period, phase, and amplitude but assumes a constant rhythm in which these parameters do not vary over time. However, conditions that alter circadian oscillations can have effects that change over time. Wavelet analysis of biological rhythms provides a means to capture time-varying changes in circadian oscillations that more standard methods cannot obtain. An advantage to using larval zebrafish for circadian studies is that both molecular clock oscillations and circadian locomotor behavior can be monitored in the same organism. We have applied wavelet transforms to *in vivo* zebrafish molecular and behavioral circadian oscillations to examine the kinetics of rhythms in fish treated with known small molecule circadian clock effectors. We have found that several compounds known to lengthen circadian periods via different molecular targets yielded distinct kinetics. For example, the period lengthening effect of a casein kinase 1 (CK1) inhibitor decreased over the time course of the experiment, while the period lengthening effect of a cyclin-dependent kinase (CDK) inhibitor increased over the time course of the experiment. We have also compared the kinetics of CK1 inhibitor induced period lengthening of molecular rhythms to the kinetics of the period lengthening of behavioral rhythms and found that while the period lengthening effects on the molecular clock decreased over the time course of the experiment, the period lengthening effects on behavioral rhythms remained constant over time. These results highlight the potential of using wavelet analysis to capture dynamic changes in rhythms. We expect this approach will lead to a deeper understanding of circadian clock regulation and function in physiology and behavior.

Disclosures: **E.A. Mosser:** None. **C.N. Chiu:** None. **D.A. Prober:** None.

Poster

645. Circadian Clock

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Program#/Poster: 645.14/RR48

Topic: E.08. Biological Rhythms and Sleep

Support: MRC Core Grant (Nolan)

MRC Core Grant (Hastings)

Title: Short-circuit: A circadian mutant in a novel suprachiasmatic nucleus transcription factor

Authors: ***M. J. PARSONS**[1], M. BRANCACCIO[2], L. MAYWOOD[2], J. K. EDWARDS[1], M. SIMON[1], S. SETHI[1], J. E. CHESHAM[2], A.-M. MALLON[1], M. HASTINGS[2], P. M. NOLAN[1]; [1]MRC Harwell, Harwell, Oxfordshire, United Kingdom; [2]Div. of Neurobio., MRC Lab. of Mol. Biol., Cambridge, United Kingdom

Abstracts: Short circuit (Sci) is a semi-dominant homozygous lethal mutant found in an N-ethyl-N-nitrosourea (ENU) mutagenesis screen for circadian phenotypes. Heterozygotes (Sci/wt) have a shortened tau in constant darkness τDD=23.0. The gene containing the Sci mutation, zinc finger homeobox 3 (Zfhx3), encodes for a highly pleiotropic transcription factor that has been implicated in neuronal differentiation and development. Moreover, the gene is highly expressed in the adult suprachiasmatic nucleus (SCN). We found that mutant ZFHX3 results in less effective transcriptional activation of a conserved promoter motif *in vitro* compared to wildtype protein. The same motif is activated in a circadian fashion in *ex vivo* SCN cultures. We also found a diminished activation in *ex vivo* SCN of heterozygotes, in line with the *in vitro* findings. Together these data suggest that Zfhx3's role as a transcription factor may underlie the circadian phenotype. In order to investigate transcriptional changes in the SCN of Sci/wt animals, we conducted RNA sequencing experiments using RNA from the SCN of both Sci/wt and wt/wt animals. We found 27 high confidence differentially expressed genes ($q < 0.05$). Clustering and enrichment analysis revealed a functional gene module in which the heterozygotes have decreased SCN specific expression of a number of neuropeptides and neuropeptide receptors (including Vip, Vipr2, Grp and Avp) that have been shown to be important for normal SCN firing. We have now verified the decreased expression of a number of these genes using RT-PCR and immunofluorescence. Interestingly, many of these contain the conserved motif within their promoters. Using luciferase specific reporter gene assays we found that mutant ZFHX3 is less effective at transcriptional activation. In summary, this suggests that the decreased ability of mutant ZFHX3 to transcriptionally activate the novel circadian motif in the promoters of genes may underlie its circadian phenotype and further suggests a wider role for Zfhx3 in circadian transcriptional regulation.

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Poster

645. Circadian Clock

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Topic: E.08. Biological Rhythms and Sleep

Support: JSPS, MEXT; KAKENHI

JST; SICP, CREST

HFSP

Takeda Science Foundation

Mitsui Life Social Welfare Foundation

Title: A novel protein, CHRONO, functions as a core component of the mammalian circadian clock

Authors: *F. HATANAKA[1,2], A. GORIKI[1,2], J. MYUNG[1,2], J. K. KIM[3,4], K. FUJIMOTO[2], Y. KATO[2], A. MATSUBARA[2], D. FORGER[3], T. TAKUMI[1,2];
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Abstracts: Circadian rhythms are controlled by a system of negative and positive genetic feedback loops composed of clock genes. Although many genes have been implicated in these feedback loops, it is unclear whether our current list of clock genes is exhaustive. We have recently identified Chrono as a robustly cycling transcript through genome-wide profiling of BMAL1 binding on the E-box. Here, we explore the role of Chrono in cellular timekeeping. Remarkably, endogenous CHRONO occupancy around E-boxes shows a circadian oscillation antiphasic to BMAL1. Overexpression of Chrono leads to suppression of BMAL1-CLOCK activity in a histone deacetylase (HDAC) -dependent manner. *In vivo* loss-of-function studies of Chrono including Avp neuron-specific knockout (KO) mice display a longer circadian period of

locomotor activity. Chrono KO also alters the expression of core clock genes and impairs the response of the circadian clock to stress. CHRONO forms a complex with the glucocorticoid receptor and mediates glucocorticoid response. Our comprehensive study spotlights a previously unrecognized clock component of an unsuspected negative circadian feedback loop that is independent of another negative regulator, Cry2, and that integrates behavioral stress and epigenetic control for efficient metabolic integration of the clock.

Disclosures: **F. Hatanaka:** None. **A. Goriki:** None. **J. Myung:** None. **J.K. Kim:** None. **K. Fujimoto:** None. **Y. Kato:** None. **A. Matsubara:** None. **D. Forger:** None. **T. Takumi:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.01/RR50

Topic: F.01. Human Cognition and Behavior

Title: Continuous theta-burst TMS to the right inferior frontal gyrus increases local EEG theta power and memory for contextual and non-contextual objects

Authors: *W. LEGON[1], R. MORAN[2,3,1];
[2]Electrical and Computer Engin., [1]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [3]Dept. of Psychiatry and Behavioral Med., Virginia Tech. Sch. of Med., Roanoke, VA

Abstracts: Previous research has demonstrated significant changes in inferior frontal gyrus (IFG) in response to change in contextual information provided during episodic memory encoding. Here, we tested the effect of delivering low intensity (20% max. stimulator output) continuous theta burst stimulation (cTBS) to the right IFG upon broad spectrum (4 - 30 Hz) EEG dynamics and recall behavior. Participants were randomly assigned to either the real cTBS group or the sham stimulation group. Stimulation was performed 5 minutes prior to the encoding task. 64 channel EEG was collected while participants performed a simple two-choice visual encoding task. Two household items were presented on a screen with a question below asking if either of the objects is 1) colored Red (low contextual information) or 2) belongs in a Kitchen (high contextual information). A total of 28 pairs of objects were shown, repeated 10 times. A one hour retention period followed and participants were then tested whether they had seen particular pairs of objects. Behavior was quantified as response time to either Red or Kitchen questions during encoding as well as percent items pairs recalled correctly after the retention period. EEG epochs around visual stimulus onset (-380 - 1345 msec) were convolved with a 3 cycle complex

Morlet wavelet at 4Hz increasing linearly with frequency up to 30Hz. Spectra were grouped into established frequency bins including theta (4 - 8 Hz), alpha (9-13 Hz) and beta (14 - 30 Hz) bands. Preliminary behavioral analysis indicates participants responded slower to Kitchen questions compared to Red questions - stimulation had no effect upon response rate. There was a main effect for stimulation where cTBS retention rates were higher for both Kitchen and Red trials though no interaction as Red trial recall was higher compared to Kitchen trials for both cTBS and Sham stimulation. This second main effect of context is commensurate with our previous fMRI study and challenges the notion that "deep" encoding strategies are more efficacious. EEG data indicate cTBS to increase the power of theta frequency in right frontal areas beginning at ~100 msec after stimulus continuing to ~ 1000 msec for both Red and Kitchen trials. cTBS induced a non-spatially specific increase in alpha power for both Red and Kitchen trials. Beta power displayed non-specific power increases starting at ~ 350 msec for Kitchen trials to cTBS stimulation and no differences for sham stimulation. These data indicate that cTBS to the right inferior frontal cortex specifically increases local theta power in IFG and that this may be associated with an increase in general memory retention for objects regardless of contextual framework.

Disclosures: W. Legon: None. **R. Moran:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

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UAM/Santander Inter-University Cooperation Project

José Castillejo mobility grant (Ministerio de Educación, Cultura y Deporte, Programa Nacional de movilidad de Recursos Humanos)

Title: Memory representations of context guides visual search in an immersive virtual environment

Authors: *C.-L. LI[1], M. P. AIVAR[2], D. M. KIT[3], M. H. TONG[4], M. M. HAYHOE[4];
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Abstracts: Despite limited attentional resources, humans reliably allocate gaze to appropriate regions in the visual environment to guide daily behavior. It is likely that this ability depends on the existence of memory representations or learned priors that govern attentional allocation. With simple stimulus displays it has been demonstrated that familiar contexts aid visual search. It has also been demonstrated that the preview of an image of a natural scene facilitates subsequent search. However, experiments attempting to characterize the role of memory in gaze allocation almost all use two-dimensional (2D) images, and it is not clear how the results from 2D studies generalize to behaviors in immersive, interactive three-dimensional (3D) environments, given the drastically different nature of the stimulus exposure, both spatially and temporally. In this study, we developed an immersive virtual environment to determine (i) to what extent does pre-exposure to the global scene context and target location benefit subsequent visual search, and (ii) what aspect of local context guides visual search. Eye movements of human subjects were recorded while they were immersed in a virtual apartment using a head-mounted display with a large field-of-view. The apartment was composed of two rooms; one room was explored for 1 minute before the search trials while the other was not. Early search targets were geometric objects, while later targets were realistic apartment objects that were part of the context during early searches. The search time and number of fixations spent to locate targets were indicators of search efficiency. We found that 1 minute of pre-exposure to one of the rooms led to a small benefit during early searches of geometric targets, whether the targets were present during exploration or not. Search time and number of fixations drop rapidly during the first five search trials in the environment, even though each trial was for a different target, suggesting that there is a significant benefit from learning the context through active experience. There was an added benefit on the second search trial for a given object. Targets were located rapidly after only one previous search trial, suggesting that search is driven by memory for target location, as well as memory for the context itself. Interestingly, search time was not dependent on the number of previous incidental fixations on the object. Together the results provide the insight into the time course of developing memory representations in an immersive 3D environment, and demonstrate the importance of memory for object location, memory for context, and task relevance in visual search in natural immersive environments.

Disclosures: C. Li: None. **M.P. Aivar:** A. Employment/Salary (full or part-time):; Universidad Autónoma de Madrid. **D.M. Kit:** A. Employment/Salary (full or part-time):; University of Bath. **M.H. Tong:** A. Employment/Salary (full or part-time):; The University of Texas at Austin. **M.M. Hayhoe:** A. Employment/Salary (full or part-time):; The University of Texas at Austin.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.03/SS2

Topic: F.01. Human Cognition and Behavior

Support: UNAM DGAPA PAPIIT ID300312.

Title: The effects of the amount of binding information on the recognition and retrieval of episodic memory

Authors: *F. E. TORRES TREJO, S. CANSINO;
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Abstracts: The aim of the present study was to examine the effects of binding two and three items on recognition and retrieval. Thirty participants were asked at encoding to determine whether the two- and three-item stimuli comprised natural, artificial or both types of common objects. During recognition, the participants indicated whether the stimuli were equal to those presented at encoding, were modified through the exchange of one of the two-item stimuli for one of the three-item stimuli or represented a new stimulus. The correctly identified modified item pairs and triads were included in a subsequent cued-recall task in which participants verbally reported the missing item. Recognition diminished as the number of bound items increased for both old and modified stimuli; whereas the retrieval of the missing item was unaffected by the number of bound items. The results suggest that both familiarity and recollection processes took place in the recognition task and both were influenced by the number of items. Bindings based on recollection allowed the retrieval of the individual elements independent of the complexity of the episodic event.

Disclosures: F.E. Torres Trejo: None. **S. Cansino:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

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Program#/Poster: 646.04/SS3

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 AG034613

NIH R01 MH085828

Graduate Assistance in Areas of National Need (GAANN) Fellowship

Title: Memory for sequences of events shows bilateral hippocampal and medial prefrontal cortical activity in humans

Authors: *V. K. BOUCQUEY[1,2], T. A. ALLEN[1,2], N. J. FORTIN[1,2], C. E. L. STARK[1,2]; [1]Neurobio. and Behavior, [2]Ctr. for the Neurobio. of Learning and Memory, Univ. of California, Irvine, CA

Abstracts: Memory for the sequence of events is a critical component of episodic memory. However, the neurobiological substrates of this type of memory are not well understood, especially in humans. To address this, we acquired BOLD fMRI data in humans while preforming our cross-species sequence memory task. Importantly, this task tests memory for non-spatial sequences of events and shows cognitive parallels in rats and humans (Allen et al., 2014), allowing for multidisciplinary research. Subjects were first presented with four different sequences of six distinct kaleidoscopic images in succession (e.g., Sequence "ABCDEF"). Subsequently, BOLD fMRI was acquired while subjects were presented images with all items in sequence (e.g., "ABCDEF"), or with one item out of sequence (e.g., "ABC**F**EF"). Subjects initiated image presentations by pressing and holding down a button. Images disappeared when the button was released, or after the decision threshold (1sec) was reached. If an image was "in sequence" the subjects were instructed to hold down the button for 1sec, if the image was "out of sequence" they were instructed to release the button before 1sec. Successful sequence memory was demonstrated if subjects responded accurately to both "in sequence" and "out of sequence" items. All subjects completed 240 probe sequences (1440 images). Each subject accurately indicated items as "in sequence" and "out of sequence" at levels significantly greater than chance. We then completed a whole-brain analysis contrasting "in sequence" with "out of sequence" presentations, corrected for multiple comparisons at a brain-wise $\alpha<0.05$. We found that the sequence task showed strong bilateral activation of the hippocampus and the medial prefrontal cortex (dorsal and ventral). These results are consistent with previous research in rats that used a temporary inactivation approach to show the cross-species sequence task relies on the hippocampus and medial prefrontal cortex. Further, single-unit recordings in rats from the CA1 region of the hippocampus and prelimbic region of the medial prefrontal cortex show a dynamic and complementary series of changing representations that can solve the sequence task. The current results extend this work to humans and strongly suggest homologous underlying neural substrates in memory for sequences of events. These results encourage the use of the cross-

species sequence memory task in understanding the neural underpinnings of this aspect of episodic memory as it normally functions, as it changes with healthy aging in which episodic memories decline, and in disorders such as Alzheimer's disease where the declines in episodic memories are more deleterious.

Disclosures: V.K. Boucquey: None. **T.A. Allen:** None. **N.J. Fortin:** None. **C.E.L. Stark:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

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Program#/Poster: 646.05/SS4

Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP DGE1148900

NSF MRI 1229597

The John Templeton Foundation

Title: Manipulating mental context in a memory task using real-time fMRI

Authors: *M. T. DEBETTENCOURT[1], N. B. TURK-BROWNE[1,2], K. A. NORMAN[1,2]; [1]Princeton Neurosci. Inst., Princeton, NJ; [2]Dept. of Psychology, Princeton, NJ

Abstracts: Context has a powerful effect on memory: we are much more likely to recall information when the external environment and our internal state match what was present when that information was encoded. Recent developments in fMRI acquisition and analysis make it possible to identify brain states in real-time, potentially allowing us to detect when particular contexts are active in the brain. Here we not only detect such contexts, but also provide this information as neurofeedback to participants, to manipulate their current context. Specifically, we hypothesized that inducing the same context in a participant during study and test by providing neurofeedback would boost recall of the studied information, relative to a condition in which a different context was induced. We defined two arbitrary contexts by training a classifier to distinguish between multivoxel patterns associated with different perceptual categories. Then, participants were trained to modulate the degree to which one of these contexts was active. Finally, we incorporated this learned control of context into a standard memory retrieval paradigm. During the study phase, participants were induced into a particular context with

neurofeedback, and when they succeeded in activating the target context to a predetermined threshold, the presentation of the study item was triggered. During the test phase, participants were again given neurofeedback to induce a context that, across runs, either matched or mismatched the study-phase context, and then were instructed to recall the study items. Initial results suggest that participants were able to successfully activate the desired context during study and retrieval, and that the ability to activate contexts improved with practice and concurrent neurofeedback. Moreover, we compared activity patterns at retrieval for items that were encoded in different contexts and observed that the study-phase context was reactivated at retrieval. This provides preliminary evidence of contextual reinstatement in this paradigm. Other analyses (e.g., looking at how context match affects recall behavior) are ongoing. Using real-time fMRI to induce contexts provides a way to conduct more causal manipulations of how contextual reinstatement and context change impact memory. More broadly, this approach could become a powerful way to probe theories of memory retrieval.

Disclosures: **M.T. deBettencourt:** None. **N.B. Turk-Browne:** None. **K.A. Norman:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.06/SS5

Topic: F.01. Human Cognition and Behavior

Title: An ERP study of memory differences between musicians versus non-musicians

Authors: ***J. SCHAEFFER**, R. MEAHL, H. PARK;
Psychology, Univ. of Texas At Arlington, Arlington, TX

Abstracts: Previous research has suggested that music training is related to improvements in working memory performance and verbal processing. However, little research has been conducted to investigate the relationship between music training and long-term memory beyond working memory. It is also unknown whether the effect of music training on memory is content-specific (e.g., verbal vs. nonverbal). In this study, we employed event-related potentials (ERPs) to investigate both behavioral and neural differences in memory between professional musicians and non-musicians using both words and pictures. Behaviorally, musicians outperformed non-musicians on working memory tasks for both words and pictures; however, musicians showed a memorial advantage on long-term memory only with pictures. These differences were associated with electrophysiological activity of increased discriminability to target words and pictures on

working memory tasks and enhanced sensitivity to studied pictures on long-term memory tasks. ERPs differed between musicians versus non-musicians in mid-frontal (300-500ms) and parietal old/new (400-800ms) regions. These findings extend previous studies of music training and indicate the influence of long-term music training on memory with both behavioral and ERP measures.

Disclosures: J. Schaeffer: None. **R. Meahl:** None. **H. Park:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

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Program#/Poster: 646.07/SS6

Topic: F.01. Human Cognition and Behavior

Support: MECD Postdoctoral Grant EX2009-1029

Title: Cerebral bases of re-learning: An fMRI study on picture-word association learning

Authors: *P. E. ROMAN[1,2], S. A. KOTZ[2,3];
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Abstracts: Convergent evidence shows that learning is supported by two systems with different cerebral correlates (Knowlton et al. 1996). The first system allows acquisition of declarative knowledge during initial stages and is supported by structures in the medial temporal lobe, and attention-related regions such as lateral prefrontal and medial frontal cortex (Chein et al., 2005). A second system is the procedural system that works independently of executive control and involves the basal ganglia. While there is agreement with regards to the neural bases of declarative memory across domains, neuroimaging and neuropsychological research suggest that procedural learning engages different brain regions dependent on task (Poldrack, 2000). In the present study, we put forward the concept of "re-learning" to account for learning situations that cannot be fully explained by traditional learning distinctions. We investigated the neural bases of re-learning in the acquisition of a second language. We assume that re-learning is based on the re-arrangement of associative connections within an established network and, most likely, a change in representations that belong to the native language of second language learners. To address this question, we presented picture-words pairs to be learnt. We manipulated Number of Repetitions and Relatedness of picture and words. Stimuli included high, low, and non-related

pairs. Using mixed-design fMRI and rs-fMRI, we replicated previous results of the language-acquisition literature that show early activation of the IFG (Ye et al. 2011) and activation of regions involved in executive control in non-related trials such as the ACC, SMA (Wible et al. 2006) bilaterally. However, we failed to find greater activation as repetitions increased. Comparison of pre- and post-learning resting state showed connectivity changes associated with a conflict score (as measured with the multi-source interference task), but not with the learning slope. A mechanism similar to that found in other language-acquisition studies might be involved at least in early learning phases. Further, the resting state connectivity analyses together with increased activation in ACC and SMA, bilaterally, suggest that executive control is a crucial mechanism involved in re-learning. As these areas did not show any increase during repetitions, further research is needed to fully describe this phenomenon. By means of this, we can reach a deeper understanding of re-learning processes in specific populations and use it to improve its training. Additionally, future studies will have to focus on the consequences of re-learning on previously acquired representations.

Disclosures: P.E. Roman: None. **S.A. Kotz:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

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Program#/Poster: 646.08/SS7

Topic: F.01. Human Cognition and Behavior

Support: R01-NS078396-01

Title: Intracranial evidence of parietal cortex involvement in the encoding of episodic memories

Authors: *A. GONZALEZ-BARBOSA[1,2], J. HUTCHINSON[6], K. F. LAROCQUE[3,2], J. CHEN[6], B. L. FOSTER[4], V. RANGARAJAN[4], J. PARVIZI[4,5,2], A. D. WAGNER[3,5,2]; [1]Electrical Engin., [2]Stanford Human Intracranial Cognitive Electrophysiology Program, [3]Psychology, [4]Neurol. and Neurolog. Sci., [5]Neurosciences Program, Stanford Univ., Stanford, CA; [6]Psychology, Princeton Univ., Princeton, NJ

Abstracts: Functional MRI has shown that activity in lateral posterior parietal cortex during the encoding of episodic memories predicts later memory outcomes. In particular, superior parietal lobe (SPL) shows greater activity during events that are subsequently remember vs. forgotten, which is thought to reflect the role of top-down attention at encoding. In this experiment, we

used electrocorticography (ECoG) to investigate the temporal dynamics of SPL activity during encoding. Four epilepsy patients, with grids of electrodes covering the left intraparietal sulcus (IPS, #elec=29) and the SPL (#elec=30), performed multiple encoding-retrieval blocks. At encoding, subjects viewed words (20 per block, 2-4 blocks), deciding whether each was abstract or concrete. During retrieval, 20 new words were randomly intermixed with the studied words, and subjects indicated whether they recognized each word as 'old' or 'new'. As a proxy for subsequent memory strength we used the response time for correct trials at retrieval to label the encoding trials as 'subsequently-remembered fast-response' (strongMem), and 'subsequently-remembered slow-response' (weakMem). Given the relationship between fMRI BOLD signal and high-gamma power, we examined the relationship between high frequency power (80-180Hz) at encoding in individual electrodes and subsequent memory strength. Left SPL electrodes showed greater power for strongMem than weakMem from 400-to-600ms, and from 1200ms-to-1400ms post-word presentation. By contrast, left IPS electrodes showed greater power for weakMem than strongMem at a similar late temporal window (1100-1400ms). Together, these results suggest that greater neural activity in SPL during event encoding contributes to the building of strong memories for the event.

Disclosures: **A. Gonzalez-Barbosa:** None. **J. Hutchinson:** None. **K.F. LaRocque:** None. **J. Chen:** None. **B.L. Foster:** None. **V. Rangarajan:** None. **J. Parvizi:** None. **A.D. Wagner:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.09/SS8

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Funded PhD Student

Title: Dopamine improves overnight consolidation of episodic memory: Recall, not recognition

Authors: ***J. P. GROGAN**[1], R. BOGACZ[2], D. TSIVOS[3], A. WHONE[3], E. COULTHARD[1,3]; [1]Univ. of Bristol, Bristol, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom; [3]North Bristol Trust, NHS, Bristol, United Kingdom

Abstracts: Previous work has established an important role of dopamine in memory, however it is not known to what extent its effects are due to encoding, consolidation or retrieval in humans.

This study aimed to separate the effects of dopamine on learning and testing on an episodic memory test. Fifteen Parkinson's Disease (PD) patients and 13 healthy age-matched controls completed the Hopkins Verbal Learning Test, which has 3 immediate recall trials, and 30 minute and 24 hour delayed recall and recognition tasks. PD patients were tested on and off their dopaminergic medication for each of the two days, giving four conditions tested in a within subjects design (on-on, on-off, off-on, off-off). Controls were tested once. PD patients show large impairments on every aspect of this task, with lower recall accuracy on the immediate and delayed recall trials, and a decrease over the delays that controls do not show. Dopaminergic medication during learning had a significant detrimental effect on 30 minute and 24 hour delayed recall with lower word retention when patients were on medication on day 1. Dopamine on day 2, however, had a positive effect with patients having higher 24 hour retention when on medication at time of testing. As retrieval takes place at both 30 minute and 24 hour recalls but the positive effect of dopamine is only seen after the longer delay, this suggests a role of dopamine in consolidation. Patients are also impaired on the recognition task, compared to controls, and again show a decrease in recognition discrimination accuracy on the delayed tasks which the controls do not show, which was unaffected by medication state. However, day 1 dopaminergic state did affect response bias (from signal detection analysis) on the day 2 recognition test. Patients that were on medication on day 1 had a more negative response bias on day 2, meaning more "yes" responses to the question "Was this word on the list you heard yesterday?" Patients that were off medication on day 1 had the opposite effect, with a more positive response bias on day 2, so more "no" responses. This suggests that although dopamine does not affect recognition accuracy, dopamine levels at the time of learning (or soon thereafter) do affect the amount of evidence needed for a word to be accepted as a memory when tested 24 hours later. In conclusion, PD patients were impaired on delayed recall and recognition compared to controls, and dopamine overnight and at testing 24 hours after learning improves recall, but without improving recognition of those words. This suggests that consolidation of episodic memories for later recall and recognition use different mechanisms, only the former of which is dopamine dependent.

Disclosures: J.P. Grogan: None. **R. Bogacz:** None. **D. Tsivos:** None. **A. Whone:** None. **E. Coulthard:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

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Program#/Poster: 646.10/SS9

Topic: F.01. Human Cognition and Behavior

Support: CIHR

Title: Naturalistic and laboratory encoding contexts dissociate subjective and objective measures of episodic memory in older adults

Authors: *N. DIAMOND[1,2], B. LEVINE[1,2];
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Abstracts: Introduction Laboratory-based studies of episodic memory (EM), which are typically highly controlled but use relatively artificial stimuli, are assumed to tap into the same core processes that support memory for past experiences from outside the laboratory. However, compared to lab-based measures of EM, autobiographical episodes involve a greater range of factors that affect encoding and subsequent recollection. Accordingly, there is evidence for important differences in the neural substrates of EM across lab-based and naturalistic paradigms. We addressed this disparity by probing memory for a rich and interactive yet homogeneous and controlled event, using aging as a model for EM decline. We also manipulated event properties by testing memory for the same content encoded in a naturalistic (real-life walking tour) vs. laboratory (photo-slideshow of same tour) context in order to investigate how aging interacts with the encoding context to produce differences in EM quality and accuracy. **Methods** Younger and older healthy adults were randomly assigned to the naturalistic tour (NT) or the lab tour (LT). Participants in both conditions listened to the same audio tour guide that controlled item sequence and viewing time. After a 48 hour delay, all participants completed the same novel recognition memory test comprising 86 true/false statements and a subjective rating (remember/know/guess) for each statement. **Results** Overall, the NT condition evoked greater recognition accuracy than the LT condition, $f(1,36) = 8.966$, $p = .005$, but this difference was only significant in the younger group. The NT condition elicited significantly more remember responses overall $f(1,36) = 7.279$, $p=.011$). In the older group only, we observed a significant interaction between event condition and subjective rating ($f(2,44) = 6.575$, $p = .003$) with NT eliciting more remember responses and fewer guesses than LT despite no accompanying advantage in objective recognition accuracy. **Conclusions** These results suggest that subjective re-experiencing and high-fidelity retrieval of event details are behaviorally dissociable. The richer spatiotemporal context of the naturalistic encoding context elicited greater subjective recollection in older adults despite no significant difference in objective recognition memory accuracy across conditions. Age-related brain changes may affect retrieval of fine-grained details while sparing the felt vividness of mnemonic representations. We were able to detect this finding by designing a naturalistic experimental event approximating the complexity and scale of autobiographical episodes and the control and homogeneity of the laboratory.

Disclosures: **N. Diamond:** None. **B. Levine:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.11/SS10

Topic: F.01. Human Cognition and Behavior

Title: Transcranial direct current stimulation improves audioverbal memory in patients with stroke

Authors: *T. KAZUTA[1], R. OSU[2], K. TAKEDA[3], S. TANAKA[4], A. OISHI[1], K. KONDO[1], M. LIU[5];
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Abstracts: Objectives: We investigated whether anodal transcranial direct current stimulation (tDCS) over the left temporoparietal area improved the audioverbal memory in patients with stroke. **Mehods**: 12 patients (mean age 71.8 yrs) with mild to moderate cognitive deficits after a stroke participated in this single-masked, crossover, and sham-controlled experiment. All participants performed the Rey Auditory Verbal Learning Test (RAVLT). In the RAVLT, we presented the participants aurally a list of 15 common words one-by-one (encoding procedure). After the presentation of the 15 words, the participants were asked to repeat as many words as they could (recall procedure). This encoding-recall procedure was repeated five times. Each participant underwent two tDCS conditions: 10 min anodal and 15 s sham stimulation over the left temporoparietal cortex within 7 days of a washout period. The order of the two conditions was randomly assigned for all participants. The stimulation was applied from the second encoding procedure until the end of the session. We measured the number of recalled words in each recall procedure (1-5). The results were analyzed with two-tailed paired-samples t tests for within-subjects using the Bonferroni correction for multiple comparisons. **Results**: The mean number of recalled words was larger in anodal compared with that in sham conditions on the second, third and fifth procedure. Especially, the mean number of recalled words positioned at the beginning of the list was larger in anodal stimulation than in sham control (primacy effect). In addition, anodal tDCS increased the amount of recalled words on the fifth procedure compared with the first procedure, and the improvement was greater than that with the sham, suggesting improvement in verbal learning ability. **Conclusion**: Our results imply that anodal tDCS over the left temporoparietal cortex induced short-term modulation and the primary effect

in learning aurally presented words in patients after a stroke. tDCS could be a promising tool for improving memory performance.

Disclosures: **T. Kazuta:** None. **R. Osu:** None. **K. Takeda:** None. **S. Tanaka:** None. **A. Oishi:** None. **K. Kondo:** None. **M. Liu:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.12/SS11

Topic: F.01. Human Cognition and Behavior

Title: Contributions from memory competition on intentional forgetting

Authors: *J. A. LEWIS-PEACOCK[1,2], K. PLACEK[1];
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Abstracts: Forgetting is often viewed as a failure of memory; however, forgetting can be understood as an adaptive process that prunes unneeded memories to increase our cognitive efficiency. How do we forget? According to the nonmonotonic plasticity hypothesis (Newman & Norman, 2010), neural competition leads to forgetting, such that moderate activation of a neural representation will lead to weakening of that representation. This hypothesis has been used to explain incidental forgetting in a variety of behavioral paradigms (e.g., in paired-associate retrieval, visual statistical learning, and working memory switching; e.g., Norman, Turk-Browne, Lewis-Peacock, and colleagues). Does competition also underlie the deliberate scrubbing of memories? Intentional forgetting of information has been described an active, effortful cognitive process (Fawcett & Taylor, 2008). We hypothesized that neural competition between lingering memory items contributes to the cognitive effort observed during intentional forgetting. Specifically, we predicted that lingering activation of a to-be-forgotten item will thrust that item into a losing competition with other, more strongly activated to-be-remembered items, thus leading to weakening of that item. To test this prediction, we designed a fMRI study to assess whether the degree of activation of memory items that are cued for deletion correlates with subsequent forgetting of those items. We used an item-method directed forgetting procedure: on each trial, participants were shown a single picture, followed by a brief delay period. They were asked to make a subcategory judgment about each picture and to focus on remembering the picture during the subsequent memory delay. On a random one-third of the trials, however, a cue during the delay period indicated that the preceding item should instead be forgotten. At the end

of the experiment, participants performed recognition judgments on the studied pictures with novel pictures as foils. Behavioral piloting replicated the standard directed-forgetting effect: items cued to be forgotten were less well remembered. Preliminary fMRI data collection has begun, and we are performing multi-voxel pattern analysis on brain data from the learning phase of the experiment and correlating these activation levels with subsequent memory performance on an item-by-item basis. We hypothesize that an item will more likely be forgotten if, following the forget instruction, its neural representation is moderately active relative to the preceding to-be-remembered items. Results of these analyses will be discussed in the context of the neural mechanisms underlying deliberate forgetting.

Disclosures: J.A. Lewis-Peacock: None. **K. Placek:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.13/SS12

Topic: F.01. Human Cognition and Behavior

Support: NSERC

NSERC Create Award given to student

Title: Long term memory influences the deployment of auditory attention as revealed by neuromagnetic recordings

Authors: *J. ZIMMERMANN[1], M. MOSCOVITCH[2], C. ALAIN[2];
[1]Rotman Res. Inst., Oakville, ON, Canada; [2]Rotman Res. Inst., Toronto, ON, Canada

Abstracts: Numerous studies have shown that long term memory (LTM) can influence the deployment of visuo-spatial attention (Patai et al., 2012). Here, we tested whether LTM can also bias the deployment of auditory attention. Participants completed a learning and a test phase, which were separated by a one hour retention interval. During the learning phase, participants were presented with 2.5 second audio clips (e.g. bar sound scene, birds chirping, etc.). Half of the audio clips, referred to as valid scenes, contained a brief (200 ms) and slightly louder pure tone target. The target was presented to the left or right ear at 2000 ms after sound sound clip onset. The pairing between an audio clip and a target location was kept constant during the learning and test phases. The other half of the stimuli did not include a target (neutral scenes).

During the learning phase, participants were asked to press a button indicating the location of the target. Immediately following the learning phase, a memory test was administered to determine for which audio clips the association between the clips and the target location was formed. During the memory task, participants heard the audio clips from the learning phase (but with no pure tone in any of the clips), and were asked to indicate where they remember the tone being presented with a keypress response (right side, left side, or none). Results of the memory test indicated that participants formed memory contingencies between audio clips and location of embedded target for a large proportion of trials (M = .71, SD = .15), significantly more than the proportion expected by chance (M = .33). Following an hour retention interval, event related fields were measured using magnetoencephalography during the subsequent testing phase, where participants were cued with valid or neutral sound scenes from the learning phase (i.e., all previously heard), and pressed a button indicating the location of a pure tone target, which appeared either at a previously learnt target location (for valid scenes) or at an unlearned location (neutral scenes). Participants were faster and more accurate in judging the location of a target sound when they had previously learned the target location within that scene (valid scenes) than when no contextual memory existed (neutral scenes). This gain in performance was accompanied by specific changes in neuroelectric activity associated with allocation of attention to expected target locations, which differed from responses to unlearned target locations. The study shows that auditory LTM facilitates the deployment of attention toward the location of an incoming target.

Disclosures: J. Zimmermann: None. **M. Moscovitch:** None. **C. Alain:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

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Program#/Poster: 646.14/SS13

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant T32 AG20506

NIH Grant R00-NS069788

Title: Dominant elements are powerful memory cues for entire episodes

Authors: *D. J. BRIDGE[1], J. L. VOSS[2];
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Abstracts: Episodic memories are composed of various elements encountered within a given spatial and temporal context. Out of the many elements, are any more likely than others to later serve as the strongest cues for retrieval of the entire episode? We have previously demonstrated that the hippocampus selectively binds dominant memory elements with relatively novel contextual information. Building on these findings, we hypothesized that dominant elements of an event would provide the strongest cues for retrieving the associated elements, because dominant features may be preferentially bound to the other information. In contrast, we predicted that nondominant elements would provide relatively weak cues for episodic retrieval. To test this hypothesis, we collected EEG and eye-movement data while subjects completed a spatial association task. During the study phase, subjects studied three objects in unique spatial locations on a grid. In order to manipulate element dominance, we prompted subjects to either actively recall one object's associated location or passively move one object back to its associated position on a grid. Thus, for each trial, one object and its location (i.e., an element) was made relatively dominant, while the other two objects remained equally non-dominant. During the test phase, subjects were cued with one location on the grid: either the dominant object or a nondominant object, and were required to recall the identity and location of another nondominant element. Memory of nondominant elements was enhanced when given a dominant cue relative to a nondominant cue. These results suggest that dominant event elements serve as powerful retrieval cues, potentially due to their ability to preferentially support pattern completion for the various elements comprising an episode. ERP and eye-movement correlates of these effects of element dominance will also be discussed.

Disclosures: D.J. Bridge: None. **J.L. Voss:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.15/SS14

Topic: F.01. Human Cognition and Behavior

Support: T32-MH067564

P50-MH094263

Title: Different modes of contextual association learning modulate the neural correlates memory

Authors: *D. R. O'YOUNG, J. L. VOSS;
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Abstracts: Associative learning occurs in specific contexts that govern the appropriate behavioral expression of memory (e.g. remembering to dress formally for a wedding, but not the gym). Human and animal research implicates differential involvement of hippocampus/prefrontal cortex versus striatum during associative learning, with the former involved in relatively flexible, rule-based memory and the latter in inflexible, habit-based memory. We studied the effects of contextual association learning under conditions designed to promote flexible versus habitual expression of memory. Trials were presented in one of four quadrants (e.g. contexts). Given a Cue object, subjects indicated if a subsequent Target object was associated or not (e.g. yes or no). Cue-Target associations were varied by context such that Targets could be classified as Associated, Conflicting (e.g. associated in other contexts), or Non-associated. Each subject performed the task during two sessions, including either: (i) repetitive, predictable trial sequences to promote learning associations in series and habitual responding, or (ii) pseudo-randomized trial sequences to promote learning associations in parallel rule-based responding. Learning was based on feedback to subject responses. A subsequent test phase, which did not contain feedback, was used to examine memory of the learned associations. Accuracy was high and did not vary by session (N=10), indicating successful learning and expression of memory across conditions. ERP findings during the test phase indicate enhanced late positive potentials in response to Cues after repetitive, predictable learning, suggesting greater cued recall in this condition. Pseudo-randomized learning, on the other hand, lead to an enhanced late positivity during the test phase in response to Conflicting targets but not Associated or Non-associated targets, suggesting greater utilization of contextual information in memory to resolve such conflicts. Together, these results suggest that different modes of learning distinguished only by the order and predictability of trials can engage different brain systems during subsequent expression of memory. Results are interpreted with regards to distinctions between human and animal memory research, which often differ in consistency and predictability of training, potentially promoting involvement of different brain systems.

Disclosures: **D.R. O'Young:** None. **J.L. Voss:** None.

Poster

646. Human Long-Term Memory: Encoding Retrieval Interactions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 646.16/SS15

Topic: F.02. Animal Cognition and Behavior

Support: Ontario MRI ERA

Canada Foundation for Innovation

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Krembil Foundation

CIHR

Title: Gamma-band oscillations in the human hippocampus during memory retrieval

Authors: *O. A. ARBIV[1], M. C. DRAGAN[1], T. K. LEONARD[1], R. MONTEFUSCO-SIEGMUND[1], T. A. VALIANTE[2], K. L. HOFFMAN[1];
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Abstracts: Gamma-band (30-100 Hz) synchrony is thought to facilitate the communication, integration, and selection of information in the brain. In the hippocampus of rodents, gamma-band synchrony is a correlate of both encoding and retrieval of memories, with fast gamma band (60-100 Hz) hypothesized to facilitate encoding of memories through plasticity across adjacent phases of the gamma oscillations, whereas slower gamma oscillations (30-60 Hz) could support memory retrieval by allowing for communication amongst neurons that is too slow for LTP to take place, thereby maintaining the connection strengths across the assembly of cells supporting the memory trace (Bragin et al., 1995; Colgin & Moser, 2010). In humans, gamma oscillations are stronger during successful encoding (Sederberg et al., 2007), but little is known about whether or not hippocampal gamma oscillations are involved in memory retrieval. We addressed this question using a memory-guided visual search task that requires hippocampal integrity (Chau et al., 2011). In Experiment 1, 6 patients with intractable epilepsy, implanted with hippocampal depth electrodes for clinical purposes, performed a flicker target-detection task. The task involves visual exploration of a flickering scene that alternates presentation of one object in the scene (‘target’). Target detection (fixation) was faster on repeated trials that were remembered than on novel or forgotten trials, an effect that requires an intact hippocampus (Chau et al., 2011). In the time leading up to target detection, we observed an increase in oscillatory power in the gamma-band (60-100 Hz) in remembered trials, compared to the same time period in novel trials, $t(5) = 3.40$, $p = .02$. In Experiment 2, 3 of the aforementioned patients performed an uncued (non-flicker) target detection task. In this task, participants viewed naturalistic scenes for 5 seconds, after which a target object was revealed in the image. In repeated viewings of the scenes, participants were asked to fixate upon the remembered (but otherwise uncued) location of the target object. We observed a similar memory-related increase in the gamma-band spectral power (limited to 50-80 Hz) in the 1-second period preceding

successful recall compared to fixations balanced for time or location during novel trials. These results suggest that gamma-band synchrony may indeed be related to memory retrieval. In contrast with the putative role of fast gamma in encoding, we observed fast-gamma oscillations during successful recall, perhaps indicating a different role than previously suggested (Colgin & Moser, 2010).

Disclosures: O.A. Arbiv: None. **M.C. Dragan:** None. **T.K. Leonard:** None. **R. Montefusco-Siegmund:** None. **T.A. Valiante:** None. **K.L. Hoffman:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 647.01/SS16

Topic: F.01. Human Cognition and Behavior

Title: Neurofeedback training of large-scale brain networks

Authors: *R. LORENZ[1,2], A. A. FAISAL[2], M. DINOV[1], I. R. VIOLANTE[1], R. LEECH[1]; [1]Med., [2]Bioengineering, Imperial Col. London, London, United Kingdom

Abstracts: Recently, the focus in cognitive neuroscience has shifted in support of the view that cognition results from an interaction between large-scale brain networks rather than is attributable to isolated brain areas (Bressler & Menon, 2010). The active modulation of such intrinsic brain networks by means of real-time (rt-) fMRI neurofeedback has great therapeutic potential in improving impairments in high-level cognitive functions such as attention and memory. In our pilot study, we investigate the feasibility of such an rt-fMRI neurofeedback approach for voluntarily controlling activity of the default mode network (DMN). A subject-specific map of the DMN was derived from a standard template independent component (Smith et al., 2009) using a dual regression approach on a four minute resting state scan prior to the experiment. The subject-specific map was used to calculate an estimate of DMN function for each repetition time (TR). Real-time fMRI motion correction, preprocessing, registration as well as feedback calculation and display were performed within the open-source FSL-integrated toolbox FRIEND (Sato et al., 2013). Offline fMRI analyses were performed in FSL. Subjects reported a perceived neurofeedback control and how this varied across fMRI runs. Offline fMRI analyses indeed showed a significant relationship between subjective assessment of control and activation within the DMN, indicating network activity modulation. Although the vast majority of rt-fMRI research focused on the volitional control of activity in well-circumscribed brain

regions, the field is now mature enough to incorporate rt-feedback of large-scale brain networks and their connectivity. In the future we are interested in modulating functional connectivity between the DMN and executive control networks in healthy subjects and traumatic brain injured patients.

Disclosures: R. Lorenz: None. **A.A. Faisal:** None. **M. Dinov:** None. **I.R. Violante:** None. **R. Leech:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 647.02/SS17

Topic: F.01. Human Cognition and Behavior

Support: NSF SMA-0835976, 2

National Security Science and Engineering Faculty Fellowship to BGSC

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CompNet Travel Award

Title: Selective attention in a dynamic auditory scene

Authors: *I. CHOI, H. GOLDBERG, H. BHARADWAJ, B. SHINN-CUNNINGHAM; Boston Univ., Boston, MA

Abstracts: To communicate in social settings, we focus selective attention on one voice while simultaneously monitoring novel voices that unexpectedly arise from other locations. This study investigates the behavioral and neural consequences of "social monitoring" during selective auditory attention. We hypothesized that when a specific location is the focus of attention, sensory inputs from other locations are strongly inhibited, but that when listeners anticipate having to reorient to unexpected events, inhibition is weaker, degrading the ability to focus on the original target. Listeners heard either two or three spatially separated sequences of syllables spoken by the same talker. Both a center stream and a concurrent distractor stream on the left (-700 μs interaural time difference or ITD) were always played. In 2/3 of trials, a stream on the right (+700 μs ITD) started at a random time after the second syllable of the center stream. On

each trial, a visual cue 2s before the auditory stimulus denoted whether the trial was "Fix" or "Switch." On “Fix” trials, listeners had to report the syllables from the center stream. On “Switch” trials, listeners had to report the syllables from the delayed, right steam if it occurred (2/3 of trials), but report the center stream on other trials (1/3). Throughout, scalp potentials were measured using 64-channel electroencephalography. Inter-channel pairwise phase consistency (PPC) of alpha (8-14Hz) oscillations was analyzed from the preparatory period (after the visual cue but before auditory stimulus). We compared behavioral performance for Fix and Switch conditions in the trials where no right stream was present (i.e., physically identical trials with only center and left streams, report center). Performance was significantly better in the Fix condition, revealing the behavioral cost of social monitoring. Moreover, alpha-oscillation PPC over parieto-occipital electrodes was larger for the Fix condition than the Switch condition. Our results are consistent with previous studies associating alpha oscillations with inhibition of sensory inputs. In addition, we find that listeners pay a cost to enable dynamic switching of attention to new events, a result of reducing the inhibition that focuses selective attention.

Disclosures: I. Choi: None. **H. Goldberg:** None. **H. Bharadwaj:** None. **B. Shinn-Cunningham:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 647.03/SS18

Topic: F.01. Human Cognition and Behavior

Title: Failure of the default mode network to deactivate precedes attentional lapses: An intracranial EEG study

Authors: *A. D. MEHTA[1], P. MEGEVAND[2], Z. YANG[4], D. M. GROPPE[3], C.-G. YAN[5], F. CASTELLANOS[6], M. P. MILHAM[4];
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Abstracts: “Everyone knows what attention is”, wrote W. James over a century ago, and yet its neuroanatomical and neurophysiological underpinnings remain uncertain. Recent evidence has begun to link attention with the default mode network (DMN), a set of brain regions whose

activity is generally greater at rest than during task performance. Specifically, functional MRI studies suggest that failure of the DMN to deactivate during task execution might underlie attentional lapses. Intracranial EEG (iEEG), with its millisecond level temporal resolution and centimeter scale spatial resolution, offers a unique opportunity to test whether activity in the DMN causally determines attentional capabilities. In this preliminary iEEG study, we report on the correlation between trial-to-trial DMN activity and behavioral performance. iEEG was recorded with subdural electrodes over multiple brain regions within and outside the DMN in patients with drug-resistant epilepsy who performed a warned four-choice reaction time (RT) task. The iEEG electrodes' anatomical location was determined by co-registering pre- and post-implant CT and MRI scans. High-gamma amplitude (HGA, an index of local neuronal firing) was extracted using the Hilbert transform on the bandpass-filtered iEEG (4-order Butterworth filters with 10-Hz passband incrementally from 65 to 175 Hz), demeaned over the complete recording session (96 trials, ~20 min). We looked for changes in HGA power in the second following the appearance of the target stimulus. We also correlated single-trial RT with slow HGA fluctuations (<= 1 Hz) in the 1-s period immediately preceding stimulus appearance. The motor strip, supplementary motor area (SMA), and middle frontal gyrus (MFG) showed stimulus- and response-related HGA increases, whereas decreases were seen in the orbitalis portion of the inferior frontal gyrus (IFG) and the superior frontal gyrus (SFG), especially its medial and anterior part. Importantly, these areas with task-related HGA decreases are known nodes of the DMN. Crucially, we found positive correlations between longer RT and pre-stimulus high HGA in the same DMN areas. These preliminary findings support the notion that higher DMN activity precedes attentional lapses.

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Poster

647. Attentional Networks: Brain-Behavior Relations

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Program#/Poster: 647.04/SS19

Topic: F.01. Human Cognition and Behavior

Support: FNS grant 320030_149781

Région Ile-de-France (Dim Cerveau & Pensée)

Title: The functional neuroanatomy of sustained non-selective attention

Authors: C. P. COSTE[1], *A. KLEINSCHMIDT[2];
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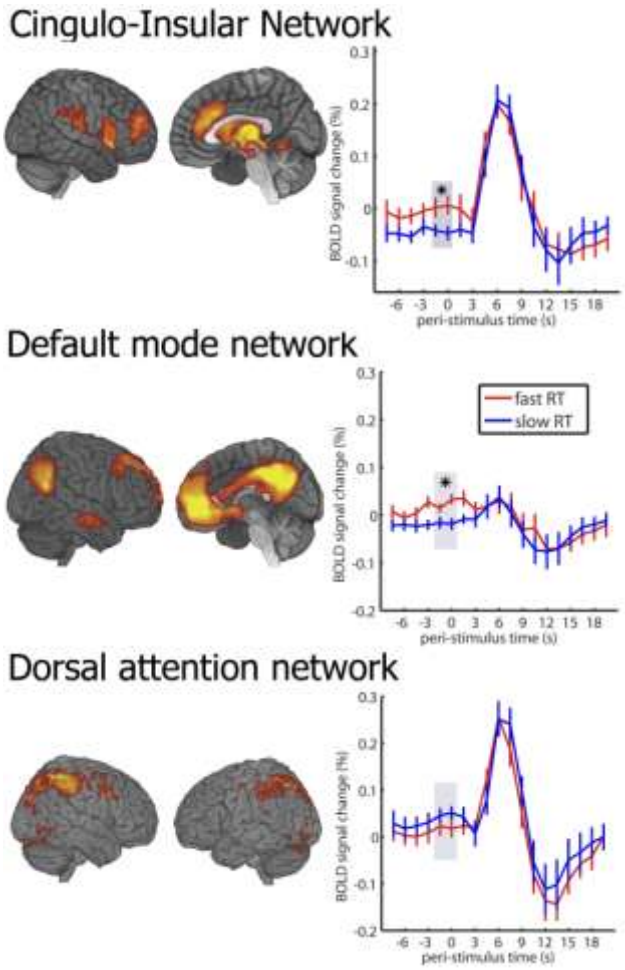
Abstracts: Paradigms investigating the neural basis of tonic alertness or vigilance have usually employed single targets and often been conducted in sleep-deprived subjects, thereby collapsing over effects from arousal, alertness, and selective attention. Here, we sought to selectively target tonic alertness by a task requiring merely sustained non-selective attention. We used functional magnetic resonance imaging and a sparse event-related design with many randomly appearing and interleaved auditory and visual trials, all of them targets. Moreover, to fully avoid signal contributions from selective attention once a given stimulus was presented, we focused on the influence of variations of ongoing brain activity prior to task appearance on reaction times. We analyzed effects in several intrinsic functional connectivity networks and early sensory cortices and compared pre-stimulus activity for quickly and slowly reported targets. We found higher pre-stimulus activity in a cingulo-insular network but also the default mode network to result in faster response speed, although only the former activated in response to the paradigm. Conversely, dorsal attention network activity was overall irrelevant and on auditory trials even detrimental to performance. Effects in sensory cortices suggested non-significant contributions from randomly switching selective attention: they were confined to the respective modality or, for visual trials, most pronounced in the relevant retinotopic representation. In a paradigm where no information predicted timing, modality or properties of a subsequent stimulus, our results hence establish the central role of the cingulo-insular network for sustained alertness and demonstrate a neural dissociation, both anatomically and functionally, between non-selective and selective attention.



Disclosures: **C.P. Coste:** None. **A. Kleinschmidt:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

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Program#/Poster: 647.05/SS20

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R01MH086530

Title: Humans expressing a subcapacity choline transporter variant: Attenuated right prefrontal activation during challenges to attention

Authors: *M. SARTER[1], A. S. BERRY[1], R. D. BLAKELY[2], C. LUSTIG[1];
[1]Psychol, Univ. Michigan, ANN ARBOR, MI; [2]Vanderbilt Univ., Nashville, TN

Abstracts: Studies in rodent models indicate elevated cholinergic neurotransmission in right prefrontal cortex (PFC) is essential for maintaining attentional performance, especially in challenging conditions. Conceptually paralleling the right PFC cholinergic increases in rodents, fMRI studies in humans reveal right PFC activation increases in response to elevated attentional demand. Enhanced activation in right Brodmann Area (BA) 9, associated with cognitive control, is thought to scale with greater attentional effort during distractor challenge. In the present study we examined the possible role of human cholinergic function in modulating BA 9's response to distraction consisting of a rapidly-changing background during signal detection. Specifically, we tested participants with a polymorphism thought to limit choline transport capacity (Ile89Val variant of the choline transporter gene SLC5A7, rs1013940) and matched controls. We previously showed that Ile89Val heterozygotes were more vulnerable to external distraction; here we assessed whether this polymorphism is associated with attenuation of the typically robust right BA 9 response to attentional demands. Relative to controls, Ile89Val participants showed blunted enhancement of right BA 9 activation in response to increased attentional demand. Further, pattern classification analyses of activation within this region significantly predicted participant genotype. These analyses also suggested Ile89Val may have differentially recruited orbitofrontal cortex and parahippocampal gyrus to maintain attentional performance: Activation in these regions weighed more heavily in discriminating between distraction and no-distraction conditions for Ile89Val relative to controls. The present study contributes to a growing body of translational research clarifying the role of cholinergic signaling in human attention and functional neural measures, and begins to outline the risk and resiliency factors associated with potentially suboptimal cholinergic function.

Disclosures: **M. Sarter:** None. **A.S. Berry:** None. **R.D. Blakely:** None. **C. Lustig:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

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Program#/Poster: 647.06/SS21

Topic: F.01. Human Cognition and Behavior

Support: Lockheed Martin Corporation (project # 13051318), USA

Title: Objective assessment of cognitive workload during varying degrees of task difficulty using a dry EEG system: Relevance for ecological validity

Authors: *K. JAQUESS[1], J. C. RIETSCHEL[2], L.-C. LO[1], M. W. MILLER[3], H. OH[1,4], Y. TAN[1,4], B. D. HATFIELD[1,4], R. J. GENTILI[1,4,5];
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Abstracts: The assessment of cognitive workload in individuals can inform the allocation processes of brain resources during demanding tasks which is critical to understand the underpinning principles of cognitive-motor performance in human performance. A possible solution to assess cognitive workload is to combine behavioral performance along with brain signals such as EEG. Although many studies have examined cognitive workload during performance, they employed EEG-gel based (i.e., wet EEG using conductive gel) systems that are well suited for laboratory, but not field, settings. Such wet EEG systems require a high preparation time; can be uncomfortable for participants and the gel can dry-up over time requiring adjusting the quantity of conductive gel during performance. Therefore, the use of wet EEG systems is relatively limited when examining cognitive-motor processes such as cognitive workload during ecologically valid performance. To address this shortcoming, several EEG systems that do not require conductive gel were developed (i.e., dry EEG). The present investigation aims to compare signal quality of between wet and dry EEG systems at four electrode sites (Fz, FCz, Cz, & Pz) and their respective capabilities to provide brain signatures to assess cognitive workload during a previously employed paradigm where participants played a videogame (Tetris®) at three levels of challenge (easy, medium, and hard). Cognitive load was assessed by employing self-report (VAS and NASA TLX) as well as EEG analyses in the spectral and time domain (P3 component). P3 amplitudes were subjected to a series of four 2 x 3 (System [wet vs dry] x Challenge [easy, medium, hard]) repeated-measures ANOVAs which revealed i) no differences between wet and dry EEG signals, ii) no significant interactions, and iii) significant differences between levels of challenge. These results are promising in support of measurement of cognitive load using dry EEG during ecologically valid human cognitive-motor performance. This research was supported by the Lockheed-Martin Corporation (project # 13051318), USA.

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Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

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Program#/Poster: 647.07/SS22

Topic: F.01. Human Cognition and Behavior

Support: NWO TopTalent to P. de Weerd

Title: Differences in alpha band lateralization between young adults and elderly when performing a lateralized delayed match-to-sample task

Authors: *M. LEENDERS[1,2], D. LOZANO SOLDEVILLA[1], O. JENSEN[1], P. DE WEERD[2,1]; [1]Donders Inst. For Brain, Cognition & Behaviour, Nijmegen, Netherlands; [2]Dept. of Cognitive Neurosci., Fac. of Psychology and Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstracts: Aging has been associated with degraded connectivity in the brain, as well as with declined memory performance and processing speed. Performance in delayed match-to-sample (DMS) tasks relies crucially on these abilities, which makes such tasks a powerful tool to study aging. Here, we compared young adults and elderly by assessing structural connectivity and functional activation of brain regions, using diffusion MRI and MEG, respectively. DMS tasks generally consist of to-be-remembered target stimuli, followed by a retention interval in which the stimuli are absent, and a memory probe which the participant has to compare with the items in memory (cf. Vogel et al., Nature, 2004). We used a lateralized version of this task, which shows target stimuli in a cued (attended) hemifield and similar (ignored) distracters in the other hemifield, while participants fixate centrally. In young adults (n=25, 18-24y), a relative comparison between hemispheres showed a relative increase in alpha band (8-12 Hz) power in the hemisphere ipsilateral to the target stimuli, and a decrease contralateral to the target stimuli. This fits with the proposed inhibitory role for alpha (see Jensen & Mazaheri, Front. Hum. Neurosci., 2010). Importantly, this alpha lateralization pattern was stronger in the stimulus-to-probe retention interval than in the cue-to-stimulus interval. This might reflect the protection of items in memory from distracting signals during retention. Strikingly, older adults (n=35, 60-75y) showed the opposite pattern: they showed weaker alpha lateralization in the retention interval than in the cue interval; a finding paired with declined memory performance compared to the young population. Further analysis will reveal whether the reduced lateralization during retention in elderly reflects an alpha power decrease within the ipsilateral hemisphere (suggesting a declined ability to suppress distracters) or an increase within the contralateral hemisphere (suggesting declined processing of targets). Alternatively, target processing might recruit both hemispheres, reducing alpha power. Bilateral recruitment in elderly where young people show lateralized activity has been a common finding in aging studies. Analysis of diffusion data is currently ongoing. In summary, compared to young adults, elderly have less capacity to maintain lateralized attention and inhibition during maintenance of items in working

memory, as indexed by changes in alpha band lateralization. Hence, deficiencies in alpha lateralization might underlie at least in part working memory problems in the elderly.

Disclosures: M. Leenders: None. **D. Lozano Soldevilla:** None. **O. Jensen:** None. **P. de Weerd:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

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Program#/Poster: 647.08/SS23

Topic: F.01. Human Cognition and Behavior

Support: MH045573

Title: The striatum is anatomically linked to large-scale distributed networks

Authors: *E. CHOI[1], Y. TANIMURA[2], S. N. HABER[1];
[1]Dept of Pharmacol. & Physiol., Univ. of Rochester Med. Ctr., Rochester, NY; [2]Kyoto Univ., Kyoto, Japan

Abstracts: Monkey anatomical tract-tracing studies have revealed that frontal cortex projections to the striatum segregate into a broad topography of motor, cognitive, and limbic functional zones in the striatum. Other studies have further identified cortico-striatal projections from nearly the entire cerebral cortex. Consistent with these more global projections, resting-state fMRI of the human striatum shows that striatal regions are functionally coupled to specific large-scale functional networks consisting of regions distributed throughout the cerebral cortex (Choi, Yeo, and Buckner 2012). However, only a few studies have investigated the anatomical organization with conflicting conclusions as to whether connected frontal and non-frontal cortical regions have convergent projections in the striatum (Yeterian and Van Hoesen 1978; Selemon and Goldman-Rakic 1985). A limitation of these studies is their use of anterograde tracer injections in a restricted number of cortical regions. Here, we investigate the potential anatomical network organization of the macaque striatum using retrograde tracer injections in the striatum to comprehensively identify inputs from throughout the cerebral cortex. We found that a retrograde tracer injection in the rostral dorsomedial caudate labels cortical cells in 46, 9, 45, and 7a. These cortical areas are anatomically interconnected and part of a potential cognitive network. Similarly, a retrograde tracer injection in the rostral ventromedial caudate labels cells in 25, OFC areas, and the temporal pole. Likewise, these cortical regions are interconnected and

part of a limbic network. Taken together, our results provide evidence that there are striatal regions receiving convergent projections from frontal and non-frontal members of anatomically and functionally linked cortical networks. This architecture indicates that the striatum may play a key role in integrating large-scale distributed networks.

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Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 647.09/SS24

Topic: F.01. Human Cognition and Behavior

Title: Frontoparietal cortices show superior to inferior gradients of preferential structural and functional connectivity with visual and auditory regions

Authors: *R. M. BRAGA, P. J. HELLYER, R. J. S. WISE, R. LEECH;
Imperial Col. London, London, United Kingdom

Abstracts: Introduction: Frontoparietal cognitive control networks are thought to control the allocation of attention to auditory and visual sensory inputs. However, it is not clear whether the same frontoparietal regions are active for auditory and visual sensory processing. Moreover, dorsal regions of the superior parietal lobe and frontal eye fields contain retinotopic maps and elicit saccades when microstimulated. We investigated whether frontoparietal cortices show differential structural and functional connectivity with auditory and visual primary cortices. **Methods:** *Structural connectivity*: We used diffusion tensor imaging (DTI) data from the Human Connectome Project and unconstrained probabilistic tractography to assess the structural connectivity of each voxel in the prefrontal and parietal cortices. We compared the number of streamlines projecting to visual and auditory primary cortices to compute a measure of preferential connectivity to each sensory modality. *Functional connectivity*: We used independent component analysis in a resting-state fMRI dataset to decompose the activity of heteromodal cortices into components. This revealed non-dominant signals within a 215 voxel searchlight that was passed across the parietal and frontal cortices. We assessed these subsignals for functional connectivity with primary auditory and visual sensory cortices to compare relative functional connectivity preference of each searchlight with each sensory modality. **Results:** In

both prefrontal and parietal cortices we observed a superior-inferior gradient of preferential connectivity to visual and auditory regions, respectively. Dorsal regions had stronger functional and structural connectivity with visual regions, while ventral regions communicated more strongly with auditory centers. **Discussion:** Our results suggest that audio-visual sensory processing and attentional control may be mediated by different frontoparietal networks, rather than a single cognitive control network. Importantly, our results suggest that sensory modality can also be encoded in the parietal and prefrontal cortices by the relative position of activation along the superior-inferior axis. This has implications for our understanding of how cognitive control of sensory information is organised in the cortex.

Disclosures: R.M. Braga: None. **P.J. Hellyer:** None. **R.J.S. Wise:** None. **R. Leech:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

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Program#/Poster: 647.10/SS25

Topic: F.01. Human Cognition and Behavior

Support: NIH grant 5R37AG-006265-25

Title: Decreased segregation of brain systems across the healthy adult lifespan

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Abstracts: Healthy aging has been associated with changing patterns of specialization in brain function. Though much research has focused on descriptions of functional specialization at the level of neurons and areas, less work has explored systems-level descriptions of organization and function. We analyzed patterns of resting-state functional correlations (RSFC) using a graph-theoretic framework to describe systems-level changes in the brain networks of individuals sampled across the adult lifespan. Individuals were a subset of participants from the Dallas Lifespan Brain Study (DLBS) who had completed a resting-state fMRI scan and a neuropsychological battery (N=210; age range: 20-89 years). Brain network nodes were constructed from a recently published area parcellation map (Wig et al. 2014). In this parcellation map, area borders are defined in relation to probabilistic transitions in patterns of RSFC. Each node was labeled according to a RSFC-defined functional system (Power et al.

2011). For each individual in the DLBS sample, brain network edges were defined as the Fisher's z-transformed Pearson correlation of the resting-state time series between each pair of nodes. Young adults' brain systems exhibited a balance of within- and between-system correlations that promoted a modular and segregated organization. Increasing age was accompanied by decreasing segregation of brain systems across a variety of network metrics that focused on patterns of within- and between-system correlations. Interestingly, not all systems exhibited the same pattern of age-related reductions in system segregation. While systems mediating processing operations (e.g., visual, sensory and motor) demonstrated a linear pattern of age-related reduction in system segregation, systems mediating control operations (e.g., frontal-parietal control, cingulo-opercular control, dorsal attention) exhibited a quadratic pattern, with accelerated reductions in segregation following age 50. Of particular importance, a partial correlation revealed that the magnitude of control system segregation was predictive of long-term memory performance, independent of an individual's age. Together, these results provide strong evidence for systems-level changes that accompany healthy aging, and suggest that the organization of brain systems has a direct relationship to cognitive function.

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Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 647.11/SS26

Topic: F.01. Human Cognition and Behavior

Support: Rackham graduate research grant

Title: Breaking away from perceptual attention: Electrophysiological signatures of shifts from monitoring to signal-associated response in typically-developing children and those with obsessive-compulsive disorder

Authors: *A. S. BERRY, M. SARTER, G. L. HANNA, W. J. GEHRING, C. LUSTIG; Univ. of Michigan, Ann Arbor, MI

Abstracts: Many situations require monitoring the environment for signals - the stoplight turning green, the beep of the microwave - and activating their associated behaviors - hit the gas, retrieve the popcorn. Distinctions between externally-directed (perceptual) processes involved in

monitoring and internally-directed (reflective) processes involved in activating signal-associated representations are fundamental to the organization of attention. However, only recently has the field engaged with questions of how these attentional modes interact or how processing switches between them (see discussion by Chun & Johnson, 2011). Our previous fMRI research tested adults in a visual signal detection task to investigate the neural activity associated with shifts from perceptual to reflective processing. Specifically, we found increased rostrolateral PFC (RLPFC) activation for hits (signal detections) occurring after extended periods of perceptually-oriented, non-signal processing relative to hits that followed a previous hit and thus did not require as large a shift from perceptual attention (Howe et al., 2013). A complementary event-related potential (ERP) investigation likewise found a right PFC signature for this contrast (larger P3a amplitude). For both studies, shift sequences were associated with increased frontoparietal functional connectivity. Here we investigated this frontoparietal neural activity in typically developing children and those with obsessive-compulsive disorder (OCD) (n = 20 per group; ages 8 - 16). These populations were of particular interest due to known changes in frontoparietal structure and function throughout childhood, and possible dysregulation of rostral PFC activity in OCD. Results suggest that compared to young adults in our previous studies, healthy children show an attenuated right prefrontal response, which is further disrupted in OCD for trials involving shifts from perceptual to reflective attention. Altered RLPFC response in OCD may reflect an impairment in the ability to flexibly shift attention or "break away" from a current task representation.

Disclosures: **A.S. Berry:** None. **M. Sarter:** None. **G.L. Hanna:** None. **W.J. Gehring:** None. **C. Lustig:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

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Topic: F.01. Human Cognition and Behavior

Support: ICM P10-001-F

P09-015-F

CONICYT 21110696

Title: Visual attention redirection interferes with saccadic performance

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Abstracts: Based on behavioral evidence, it has been proposed that orientation of visual attention is coupled to oculomotor planning giving that saccade planning leads to a facilitation of the visual processing of targets at the same visual locus. However, there are evidence of a complete mechanistic independence between an oculomotor planning and attentional orientation. We aimed to examine this dependence during a task that requires visual redirection during a saccade execution. Ten subjects (2 male) performed a dual task (adapted from Deubel and Schneider, 1996) in order to track the location of an attentional focus by discriminating the identity of a peripheral target which is indicated with a central cue, while they have to perform a saccade to a target that is in the same location of the primary task, or to a neighboring site. 25% of the trials were “invalid”, because we presented the discrimination target in the opposite side of the one indicated by the central cue. Each trial begins with the gaze on a central fixation while all possible locations for targets presents a premask. The central fixation is replaced by an arrow which direction and color indicate the side of the discrimination target and the position of the saccade, respectively. The saccade has to be withheld during a variable time (900-1400ms) until a new arrow appears indicating the saccade was to be executed (Go signal). The identification target appears 60ms post Go signal and held for 120 milliseconds, and then replaced by a mask. There were significant differences in saccadic performance between valid and invalid trials. In the valid trials subjects obtained a significant higher percentage of hits to the cued location (40.13%, SD 13.31) versus invalid trials (2.6% , SD 1.53), ($p=0.000$; $F=78.4$). Bonferroni test showed a significant difference between congruent valid trials (53.1%, SD 21.0) and invalid congruent trials (21.0%, SD 3.8), $p=0.001$, $F=50.48$. These results suggest that, during invalid trials, the absence of the discrimination target in the cued side triggers an attentional reorienting, interfering the execution of the current saccade. A possible explanation for this phenomenon is the existence of shared resources between attentional orienting and oculomotor planning, causing structural interference between these two processes when attention is reoriented.

Disclosures: J.R. Torres: None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 647.13/SS28

Topic: F.01. Human Cognition and Behavior

Title: Distributed attention is implemented through theta-rhythmic gamma modulation

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Abstracts: When subjects monitor a single spatial location, target detection depends on the pre-target phase of an ~8 Hz brain rhythm. When multiple locations are monitored, performance decrements suggest a division of the 8 Hz rhythm over the number of locations. This suggests that different locations are sequentially sampled. Indeed, when subjects monitor two locations, performance benefits alternate at a 4 Hz rhythm. These performance alternations followed a reset of attention to one location. Although resets are common and important events for attention, it is unknown, whether in the absence of resets, ongoing attention operates rhythmically. Here, we examined whether spatially specific attentional sampling can be revealed by ongoing pre-target brain rhythms. Specifically, visually induced gamma-band activity plays a role in spatial attention and therefore, we hypothesized that performance can be predicted by a theta-rhythmic gamma modulation. Brain rhythms were assessed with MEG, while subjects monitored bilateral grating stimuli for a unilateral target. The corresponding contralateral gamma-band responses were subtracted from each other to isolate spatially-selective, target-related fluctuations. The resulting lateralized-gamma activity (LGA) showed opposite 4 Hz phases prior to detected versus missed targets. The 4 Hz phase of pre-target LGA accounted for a 14% modulation in performance. These findings suggest that spatial attention is an ongoing theta-rhythmic sampling process, with each sampling cycle implemented through gamma-band synchrony. This extends previous findings by demonstrating that in the case of distributed attention, gamma-band synchrony is shaped by the slower sampling rhythm that governs performance benefits.

Disclosures: **A.N. Landau:** None. **S. Van Pelt:** None. **H.M. Schreyer:** None. **P. Fries:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

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Program#/Poster: 647.14/SS29

Topic: F.01. Human Cognition and Behavior

Title: The slope relating neural frequency to power and attentional control: Computational mechanisms of visual anticipation

Authors: E. WHITE[1], *J. J. BENGSON[2,1];
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Abstracts: As a complex system, the cortex has the intriguing ability to shift between states of high and low entropy. In the present work, we examined attention-induced broadband shifts of the slope of the power spectrum of the electroencephalogram (EEG) recorded from human subjects. Our results indicate that the spectral slope becomes significantly more negative over occipital regions contralateral to an attended region of space. This shift is indicative of an increased amount of order (lower entropy) within the visual cortex as a consequence of anticipatory attention. Importanly, this shift occurs in the absence of significant frequency-specific changes in power, indicating a novel computational mechanism by which anticipatory attention prepares sensory regions for impending stimuli.

Disclosures: E. White: None. **J.J. Bengson:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

Location: Halls A-C

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Program#/Poster: 647.15/SS30

Topic: F.01. Human Cognition and Behavior

Title: Cortical and sub-cortical brain regions modulated during spatial attention: A BOLD fMRI study in monkeys

Authors: *A. R. BOGADHI, R. J. KRAUZLIS;
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Abstracts: Studies of spatial attention have focused on neocortical circuits linking sensory cortex with areas in parietal and frontal cortex (e.g., MT/MST, LIP, FEF). However, recent findings demonstrate that subcortical regions (e.g., superior colliculus, SC) play a crucial role in spatial attention (Lovejoy & Krauzlis, 2010) and act down stream of visual cortex (Zenon & Krauzlis, 2012), but the circuit through which the SC acts is not known. Because fMRI data could be useful in identifying potential brain regions that partner with the SC to control spatial attention, we trained a monkey to perform a covert spatial attention task inside a vertical fMRI

scanner. The stimulus sequence on a single imaging run (~550s) consisted of three different blocks: Rest (R), Foveal Attention (FA) and Peripheral Attention (PA) blocks, each lasting 27.5s. R blocks were interleaved between FA and PA blocks. In R block trials, the relevant stimulus was a central fixation point that dimmed at random times. FA block trials were similar to R block trials but added a peripheral motion-change stimulus as an irrelevant distracter. In PA block trials, the fixation point did not dim and the peripheral motion-change was the relevant stimulus. The task of the monkey was to report the relevant stimulus change (fixation dimming in R & FA blocks, peripheral motion change in PA blocks) by releasing a lever to get a juice reward. Eye movements were recorded during each imaging run. Blocks in which eye position fell out of the 20 radius fixation window during any trial were excluded from analysis. A total of 656 R blocks, 296 FA blocks and 141 PA blocks across eight sessions were included. For our preliminary analysis, we defined two anatomical ROIs (MT/MST, SC) on the high-resolution anatomical images of the monkey as the overlap between the stereotaxic atlas coordinates (Saleem & Logothetis, 2007) and the functional contrast between R and FA blocks. A majority (79%) of the voxels in MT/MST (PA: 0.27±0.001%; FA: 0.17±0.001%) and all voxels in SC (PA: 0.24±0.001%; FA: -0.05±0.001%) showed BOLD activation for attention to motion (PA); the activity was significantly modulated when peripheral motion was task-relevant (Wilcoxon rank sum test; $P<0.001$). A minority (21%) of the voxels in MT/MST (PA: -0.58±0.005%; FA: 0.27±0.002%) showed BOLD suppression in blocks with attention to visual motion (PA); this suppression was significantly different from the activation in FA blocks (Wilcoxon rank sum test; $P<0.001$). These results corroborate the findings from physiological studies in monkeys and validate the use of fMRI in monkeys to identify brain regions involved in the control of spatial attention.

Disclosures: **A.R. Bogadhi:** None. **R.J. Krauzlis:** None.

Poster

647. Attentional Networks: Brain-Behavior Relations

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Topic: F.01. Human Cognition and Behavior

Support: NSF SMA-0835976

Title: Topography and temporal dynamics of resting state network signatures in high-density EEG

Authors: *S. E. SAPERSTEIN[1], R. SEKULER[3], J. W. BOHLAND[2];
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Abstracts: The analysis of resting state functional connectivity using fMRI has gained widespread acceptance and has identified consistent networks engaged at rest, but such approaches remain underdeveloped in EEG. Here we propose methods to identify sensor networks representing resting brain states that can be used to characterize the topography and temporal dynamics of those states. Nine subjects participated in either a sustained attention or working-memory task preceding and following periods of rest with eyes open and fixated while high density (128-channel), high impedance EEG signals were recorded. The resulting sensor time series were manually quality controlled, band-pass filtered, and split into epochs one-second in duration. The maximum values (across a range of lags) of pairwise cross-correlations between electrodes, for each epoch, were compared to an empirical null distribution to generate a percentile rank for each edge, representing functional connectivity between electrodes. A Mann-Whitney U-test identified functional connections that are stronger in either the pre- or post-task resting periods compared to the task period. Comparing the sensor pairs more strongly coupled during each rest period than the task period, a hypergeometric test confirmed that the network overlap, within a session, was highly significant across a range of p-value thresholds from the U-test. The resulting common network can be considered "rest-enhanced," and we compare the topography of these connections across subjects and sessions using a method that combines sensors from nearby locations to account for variability in sensor placement. Using these subject-specific networks as probes, we then calculate an activation time course across the full experiment to determine if this network is transiently activated during the task period, potentially interfering with task performance. In a second analysis, vectors of functional connections and node degrees for each epoch formed the inputs to k-means cluster analyses, for a range of values of k. Patterns of occurrence of the resulting clusters were compared between experimental periods, across k, to find network states that occur preferentially in each experimental period. We found that network clusters tended to recur preferentially during the pre- and post-task resting periods in each subject. Our approach identifies common sensor correlation patterns that occur preferentially during rest, offering the potential to study fast temporal interactions between rest- and task-specific networks during more natural experiments which entail interleaved and unpredictable periods of task and rest.

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Poster

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Program#/Poster: 647.17/SS32

Topic: F.01. Human Cognition and Behavior

Title: Strength of task-relevant networks at rest predicts sustained attention performance

Authors: *M. D. ROSENBERG[1], E. S. FINN[2], X. SHEN[4], D. SCHEINOST[4], X. PAPADEMETRIS[4,3], R. T. CONSTABLE[2,4,3,5], M. M. CHUN[1,2,6];
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Abstracts: We tested whether the strength of complex brain networks at rest can predict performance on a working memory task requiring sustained attention. Participants performed 3-minute blocks of a go/no-go task (90% go) interleaved with 30-second fixation blocks ("breaks") during high-resolution fMRI (voxel size = 2.5mm^3; TR = 1s). Two 6-min resting state runs were also collected. For functional connectivity analyses, network nodes were defined via a novel functional parcellation scheme (Shen, Tokoglu, Papademetris & Constable, 2013). Connectivity matrices were then calculated, for each participant, from data collected during (1) task performance, (2) resting state runs, and (3) each mid-task break. Using across-subjects rank correlation, we identified connections whose strength during the go/no-go task related to performance (sensitivity, or *d'*). To characterize these task-relevant networks at rest, we summed the z-scores of each component connection in the resting-state matrices. We then correlated summed strength at rest with *d'* to determine whether resting-state connectivity predicted overall task performance. In an attempt to predict performance on individual task blocks, we performed analogous analyses of each mid-task break. Specifically, we summed the strength of task-relevant networks in the break matrices, and correlated these sums with the *d'* of the task block immediately preceding and following the break. We identified networks (consisting of ~3% of 36,000 possible connections) whose strength during task performance correlated with overall *d'*. The summed strength of these networks at rest predicted *d'* across subjects ($|r|$ values > 0.7; p values < 0.01). In addition, we observed a trend such that connectivity during 30-sec task breaks also predicts performance: Network strength during rest breaks correlated with *d'* of the preceding and following (mean $r = 0.46$) task blocks. Thus, resting-state functional connectivity predicts performance on a sustained attention task. In addition, connectivity during brief periods of mid-task fixation can predict proximate attentional performance, suggesting a way to measure the effectiveness of these short breaks. Together, these results highlight the complexity of attention networks, and suggest that task-relevant networks are present to a meaningful degree during both resting state epochs and short task breaks.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH55714

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Title: Dorsal anterior cingulate cortex mediates decisions about where to attend: Evidence from graph-theoretic analysis of network connectivity

Authors: *Y. LIU[1], X. HONG[1], J. J. BENGSON[1], M. DING[3], G. R. MANGUN[2]; [2]Departments of Neurol. and Psychology, [1]Univ. of California, Davis, Davis, CA; [3]The J Crayton Pruitt Family Dept. of Biomed. Engin., Univ. of Florida, Gainesville, FL

Abstracts: A hallmark of human experience is the sense of volitional control over our actions. Converging evidence from studies of human neuroimaging, electrophysiological recordings, cortical stimulation, and focal brain lesions has pointed to an extensive network consisting of areas in the medial and lateral frontal cortices as well as those in the parietal cortex in the generation of volitional acts. Despite the growing body of evidence, how regions within this network dynamically interact, and how it interacts with other functional networks, in tasks involving internally generated intentions remain unknown. We addressed this issue by recording functional magnetic resonance imaging (fMRI) data from subjects performing a willed attention task where they freely chose which of two spatial locations to selectively attend. Applying graph-theoretic analysis to inter-regional functional connectivity, it was found that the dorsal anterior cingulate cortex (dACC), along with bilateral insula and the right frontopolar cortex, mediated the communication between the dorsal attention network and areas in frontal and parietal cortices involved in volition. Such network community structure was absent during a control condition where the subjects were cued to attend to one of the spatial locations; in this case of instructed attention, the dACC interacted most strongly with the dorsal attention network. Further analyses revealed that in willed attention, dACC served as an "inter-community hub" between the dorsal attention network and the volitional network, exhibiting high values of both betweenness centrality (the fraction of shortest paths traversing a network node) and participation index (connectedness within versus between sub-networks). Moreover, this network configuration is behaviorally relevant, with faster reaction times being associated with higher levels of dACC's participation in mediating inter-community communication under willed

attention. In contrast, during the control condition of instructed attention, faster reaction times were associated with lower dACC participation indices, suggesting stronger dACC connections with the dorsal attention network when subjects performed better. Taken together, our results suggest that dACC dynamically configures its functional interaction with various network constituents to support the generation and execution of instructed and volitional acts.

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Poster

647. Attentional Networks: Brain-Behavior Relations

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Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 25870802

Title: Detecting the feeling of somatic discomfort using near-infrared spectroscopy on prefrontal cortex

Authors: *K. SAKURAI[1], Y. ONO[1], G. KOBAYASHI[2], R. HAYAMA[3], R. IKUTA[2], H. WAKE[3], K. TAMAKI[2,3];
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Abstracts: The numbers of patients that have perceived physical symptoms (discomfort, pain etc.) in orofacial area despite the absence of inflammation or trauma are increasing greatly. A support for quantitatively evaluate the perceived amount of discomfort is required both for patients and doctors for treatment. We aim to develop a machine-learning based classifier that can quantitatively evaluate the severity of occlusal discomfort. Sixteen participants without any perceived symptoms in orofacial area (10 male, average 22.6 years old) participated in this experiment. We used functional near-infrared spectroscopy (ETG-7100; Hitachi Medical Corporation, Tokyo, Japan) to measure the hemodynamic responses from the prefrontal area. Occlusal discomfort was simulated by grinding the first molar with a stacked metal strips (aluminum foil for dental examination, 12mm thickness). The two thickness of metal strips tested were (1) thickness equal to the recognition threshold and (2) Thickness causing maximum

discomfort. Subjects repeated 30s of grinding for 5 times with each thickness of the metal strips with 40s of interval between trials. Total 10 Oxy-Hb data from each participant were used to generate a 2-class classifier that judges the presence of discomfort based on Linear Discriminant Analysis. We extracted 3 features for the classifier; the maximum amplitude, maximum slope, and integral of raw Oxy-Hb response during grinding. We generated classifiers using each of the three features as well as combinations of them to determine an appropriate combination of features. We used cross validation method to evaluate the accuracy of the classifier.Sixteen channels out of total 22 prefrontal channels are significantly higher in discrimination rates than chance level (50%; t-test) in most single or combinations of features. Highest discrimination rate of 80±3.9% was achieved using single feature of integral in dorsolateral prefrontal cortex (DLPFC, BA 9 and 46). Channels corresponding to the bilateral frontal pole (Brodmann area (BA) 10) and the left DLPFC (BA 9 and 46) showed high discrimination rate.Since DLPFC has been known to control the perception of not preferable somatosensory stimuli and play a role in an affective evaluation, the response in the DLPFC might represent the evaluation and regulation of discomfort feeling regarding to the somatic stimuli. Considering the role of the frontal pole in the working memory related to decision making, the responses in the frontal pole with different perceived discomfort might reflect the comparative activity between the currently-perceived, altered somatic stimuli and their original body image.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant P20GM103629

Title: Cortical representations of absolute and relative sound locations during an auditory spatial attention task

Authors: ***M. J. SEAY**[1], J. R. MOCK[2], E. J. GOLOB[2];
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Abstracts: Successful human behavior requires allocating attention to the location of goal-related sounds. Yet we must also flexibly attend to unexpected sounds, as they may contain information important for survival. Behavioral studies show that the benefits of attending to a location diminish with distance, indicating an attention gradient. We recently used cortical event-related potentials (ERPs) to measure attention gradients in orienting responses by presenting distractor sounds at varying distances from an attended location. We found a three-phase process in which the slope of the gradient peaked positively over frontal areas, reversed over parietal areas, and again peaked frontally before returning to baseline. The current study sought to better define ERP gradients in terms of their cortical sources and timing. We recorded EEG during a spatial target detection task in which subjects (n=35) heard white noise bursts at five azimuthal locations (from left to right: -90°, -45°, 0°, +45°, +90°) and responded to a target whose location varied between conditions (-90° or +90°). Independent component analysis was used to identify distinct clusters of cortical responses in auditory, frontal, and parietal regions. To quantify the attention gradient we measured the slope of a linear fit across each cluster's constituent response to distractors with increasing distance from the target. Consistent with the first phase, a frontocentral cluster had a positive slope from 200-360 ms ($p<.001$), and two frontal clusters showed a positive slope in the same time range when contralateral to the target (left: $p<.001$, right: $p=.01$). Consistent with the second phase, a midline posterior parietal cluster had a negative slope from 360-500 ms ($p<.001$). Midline and lateralized posterior parietal clusters also showed an event-related decrease in α and β oscillatory power between 250-550 ms that had a negative slope and contralateral pattern. Finally, a cluster originating in left posterior parietal cortex showed an earlier (120-200 ms) ERP response whose amplitude was dependent on the absolute angular location of the distractor ($p<.001$). Our results show that an attention gradient is represented in the brain dynamics that support orienting, predominantly in regions contralateral to the attended location. Distinct frontal and parietal areas function at different times in concert to support our ability to flexibly orient attention among competing environmental sources.

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Poster

647. Attentional Networks: Brain-Behavior Relations

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The John Templeton Foundation

Title: Decoding the locus of attention from the full correlation matrix of the human brain

Authors: *J. HUTCHINSON, Y. WANG, N. B. TURK-BROWNE;
Dept Psychology, Princeton Univ., Princeton, NJ

Abstracts: The ability to selectively process a subset of all available perceptual input is critical for efficient behavior. Such selective attention has been linked to a distributed set of regions in the brain, suggesting that selection arises from dynamic interactions within this network. However, these regions are typically identified because they show univariate activity in attention tasks, not because they have meaningful interactions with other regions in these tasks. Indeed, studies that have linked attention behavior to correlated or synchronous activity between areas have done so based on regions-of-interest defined from activity levels. Here we avoid this bias, applying full correlation matrix analysis (FCMA) to decode where attention is allocated in space from the pairwise correlations of every voxel with every other voxel. In a block design fMRI task, participants were presented with a series of spatial pairs of scene images, with one scene on the left of fixation and the other on the right. Participants were instructed to make a perceptual judgment about the scene on one side for an entire block, while ignoring the other side. We first examined univariate activity for left vs. right attention over the whole brain and then performed a searchlight analysis in which multivariate pattern analysis (MVPA) was applied over small volumes centered around each voxel. For FCMA, we calculated the full pairwise correlation matrix for each block and submitted these massive matrices to MVPA with nested feature selection and across-subject cross-validation. Preliminary results showed that both MVPA and FCMA were able to robustly decode which hemifield was attended. Importantly, the voxels which were most important for classification in these two analyses only partially overlapped with each other and with voxels that showed univariate differences. Taken together, these findings suggest that selective attention may be implemented by regions whose average activity, in both univariate and multivariate analyses, is uninformative. Instead, attentional goals may be represented in these regions in terms of the set of other regions in the brain with which they interact.

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UAB Comprehensive Center for Healthy Aging

Title: Task-related modulations of functional connectivity in primary visual cortex are eccentricity-dependent

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Abstracts: Primary visual cortex (V1) is organized so that the most posterior regions correspond to central vision, whereas anterior regions correspond to peripheral vision. While there is growing evidence that centrally and peripherally responsive regions of V1 exhibit distinct anatomical and functional characteristics, the effects of eccentricity on the functional connectivity of V1 to other brain regions are not understood. In this study, we investigate the effects of eccentricity on functional connectivity between V1 and a set of brain regions associated with top-down attentional control. We compare functional connectivity at rest and background connectivity during a task requiring sustained visual attention. We used fMRI to collect 10 minutes of resting state data and 28 minutes of task data from 16 healthy young adults. The task data were collected while participants performed either a lateralized peripheral visual discrimination task with auditory and visual distracters or an auditory discrimination task with visual distracters. For each participant, we divided V1 into nine 10mm regions of interest that represented centrally and peripherally responsive parts of cortex. We also defined 41 regions of interest associated with task control networks based on the literature. Resting state and

background functional connectivity measures were calculated from each V1 region of interest to each task control network region of interest. Resting state functional connectivity analyses revealed eccentricity-dependent patterns of connections between V1 and task control network regions. Background functional connectivity analyses revealed strong, dynamic eccentricity and task-dependent patterns of functional connectivity between V1 and various task network regions including frontal, temporal, parietal and higher visual regions. Our results indicate that centrally and peripherally responsive regions of V1 exhibit distinct patterns of functional connectivity to other brain regions previously associated with control of task performance. Importantly, we found that these connections are strongly modulated by a participant's task set, suggesting that functional connections from different regions of V1 to task control areas are dynamic and depend on an individual's cognitive and behavioral state.

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Poster

647. Attentional Networks: Brain-Behavior Relations

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Title: Target and oddball detection produce opposing effects on global functional connectivity in human cerebral cortex

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Abstracts: Both goal-driven and stimulus-driven factors govern everyday behaviors, controlling how we work to complete current goals while balancing the need to react to novel or salient stimuli. While strong evidence points to the control of goal-driven attention by a dorsal network and control of stimulus-driven attention by a more ventral network, these networks likely do not

operate in isolation to control attention. Further, the impact of target detection and of attentional capture by task-irrelevant 'oddball' stimuli on the brain's functional connectivity has yet to be examined beyond restricted network-level comparisons. Graph theory analysis, combined with fMRI, provides an ideal means for characterizing the large-scale community structure of functional connectivity data for detection of both goal-relevant targets as well as task-irrelevant oddballs. Performed at ultra-high field strength on 14 participants, the present study sought to characterize and contrast the neuro-architecture of functional connections associated with conscious detection of a target in a masking paradigm with the presentation of rare, task-irrelevant oddball images. Functional connectivity between each region of interest (ROI) was assessed via pair-wise psychophysiological interactions (PPI) analysis (Mclaren et al., *NeuroImage*, 2012) in order to examine the interaction of connectivity seed and target/oddball processing. Cortical and subcortical ROIs were defined using a published set of 264 coordinates parsed into 14 distinct networks (Power et al., *Neuron*, 2011). The PPI measures of connectivity for each ROI pair were then submitted to graph theory analyses in order to assess the whole-brain measures of functional integration and segregation. The results suggest that conscious target detection is associated with a decrease in global network modularity (measuring separability of networks), concomitant with an increase in functional participation (measuring the strength of connections between networks). This finding provides evidence in favor of target-induced widespread increases in functional network connectivity brought about by increases in inter-network connectivity. By contrast, detection of an oddball stimulus produced opposing effects on topology: increased modularity, decreased participation, and decreased local efficiency (a measure of functional integration). We conclude that whereas detection of a target produced a more globally connected topology, oddballs shifted graph properties in the opposite direction, favoring a more segregated and less efficient network structure.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Paradoxical increase of the P3 event related potential in oddball tasks requiring motor responses vs. mental counting suggests the need to expand its cognitive domain

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Abstracts: One of the best studied physiological windows on attention is the P3 potential (also called P300 or late positive component). The P3 can arguably be named the most intensely studied event related potential. However in spite of more than 40 years of research, fundamental questions regarding its true meaning and the nature of its neural generators still remain unresolved. The 3-stimuli oddball task, most often used to evoke the P3, presents the subject with rare, frequent and distractor stimuli. In active oddball tasks subjects are required to respond to targets while ignoring frequents and distractors. In terms of the required response, most active oddball tasks fall into one of two categories: motor response (button presses, finger raises, vocalizations etc.) and mental counting of targets (with no overt motor responses). We conducted a review of the last 121 published papers on the P3 which revealed that 70% of studies used motor responses, 21% mental counting and 9% were passive oddballs (not response required). Since any kind of motor response generates negative (5-15 μV) motor potentials that superimpose themselves on the positive P3 (5-15 μV), choosing this type of oddball should result in a reduced and more variable P3, compared to mental counting. If the former hypothesis were to be proven accurate, the majority of published studies might be using a sub-optimal variant of the oddball task and future studies might consider using the mental count variant. To test the hypothesis of reduced P3 potentials in motor oddballs we conducted 64-channel EEG recordings on 60 subjects with a visual and auditory oddball in both the motor and mental count variant. The measured maximum P3 amplitudes belonging to each task variant revealed a rather paradoxical result: motor oddballs of both sensory modalities generated statistically significantly ($p<0.001$) larger P3 potentials (5-15% increase in amplitude) even though they were clearly “contaminated” by negative motor potentials. When the superimposed motor potentials were subtracted from the P3 using independently recorded subject-specific motor potentials, the motor response P3 was 10-35% larger in terms of maximum amplitude ($p<0.001$) compared to the mental count P3. These results suggest that the list of cognitive processes the P3 is supposed to signify (categorization, memory, attention) might need to be extended to also include processing, preparation or cognitive control of goal-oriented motor behavior since it seems that oddball tasks requiring overt motor responses produce large increases in P3 amplitude simply due to final task demands and irrespective of stimuli categorization, memory and attention effects.

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Poster

647. Attentional Networks: Brain-Behavior Relations

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James S. McDonnell Foundation Scholar Award

Title: Leftward prism adaptation modulates PPC-M1 interactions within both hemispheres: A twin-coil paired-pulse TMS approach

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Abstracts: Prism adaptation (PA) to a rightward optical displacement (RPA) is one of the most promising techniques for treating neglect - a complex syndrome that frequently occurs after right parietal damage. While RPA can ameliorate neglect symptoms in patients, leftward PA (LPA) can induce neglect-like behaviour in healthy subjects. The underlying mechanisms of these effects are poorly understood. In patients with neglect, applying inhibitory repetitive transcranial magnetic stimulation (rTMS) to the left (contralesional) posterior parietal cortex (PPC) reduces neglect symptoms. In contrast, applying rTMS to the right PPC in healthy subjects induces neglect-like behaviour. It has been suggested that in neglect patients RPA might decrease the (hyper-) excitability of the left hemisphere and in healthy subjects LPA might decrease the excitability of the right hemisphere. A previous study in neglect patients revealed hyper-excitability within left parietal-motor interactions (PPC-M1) using a twin-coil paired-pulse TMS approach (ppTMS). We used the same technique to examine the effects of LPA in healthy subjects by measuring PPC-M1 interactions in the left (n=13) and right (n=13) hemispheres before and after a single session of LPA. Specifically, we delivered a sub-threshold conditioning pulse (90% resting motor threshold) to either the left or right PPC (P3 or P4 EEG coordinate) at four different inter-stimulus intervals (2 4 6 or 8 ms) followed by a test pulse to the ipsilateral M1 that produced a motor evoked potential of 1mV in the contralateral first dorsal interosseus. Our hypothesis was that LPA would differentially modulate PPC-M1 interactions in the left and right hemispheres. We found that a single session of LPA differentially modulated the PPC-M1 connectivity in the two hemispheres - decreasing the excitability of PPC-M1 interactions in the left hemisphere and increasing them in the right hemisphere. These results suggest that LPA modulates PPC-M1 interactions at rest in opposite directions in each hemisphere. This

modulation could reflect a push-pull pattern induced by LPA, which is consistent with the 'hemispheric rivalry' model that predicts reciprocal interhemispheric competition.

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Poster

648. Executive Function I

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Title: Proactive control and motor urgency: An fMRI study of the stop signal task

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Abstracts: Background: We showed previously the neural processes of proactive control in the stop signal task (Hu et al., 2013 SfN; under review). Specifically, the anterior pre-supplementary motor area (pre-SMA) responds to increased likelihood of the stop signal or p(Stop), as computed by a Bayesian model (Ide et al., 2013 J Neurosci) and posterior pre-SMA and bilateral anterior insulae respond to reaction time (RT) slowing as a result of stop signal anticipation. These findings describe a neural circuit for the sequential effect - a linear correlation between p(Stop) and RT. On the other hand, approximately a quarter of all participants (n=161) did not exhibit a significant sequential effect (pCorr(p(Stop), RT)>0.05). Here, we examined the role of motor urgency - or the fore-period (FP) effect - on the manifestation of sequential effect and its neural correlates. Methods: Participants were studied with 40 minutes of fMRI of the stop signal task. We modeled fMRI signals midway through the FP (1-5 s, random) in a GLM, with individual events parametrically modulated by p(Stop) and fore-period. In a second GLM, fMRI signals are modeled at go signal onsets with go trials parametrically modulated by p(Stop) and RT. Results: Regional activations are identified for longer FP in posterior pre-SMA and SMA,

left M1, bilateral anterior insulae, and the thalamus. These regional activations overlap those in response to RT slowing, while longer FP is associated with faster RT, suggesting that the FP effect counteracts the influence of proactive control of response time. In contrast, the anterior pre-SMA decreases activations to longer FP, consistent with a weakening influence of its role in proactive control during prolonged FP. Additional analyses are to dissect these interacting effects in individuals who show and do not show a sequential effect. Conclusions: The current results highlight interacting influences of proactive control and motor urgency in determining response time in the stop signal task.

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Poster

648. Executive Function I

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Topic: F.01. Human Cognition and Behavior

Title: Rethinking the bilingual advantage: Evidence from ERP data for a differential impact on control processes

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Abstracts: Control of cognitive operations involves executive functions (EF). Due to their lifetime experience in using more than one language, bilinguals usually show better capacity in executive control processes such as conflict monitoring, interference suppression or switching (Diamond & Lee, 2011; Heidlmayr et al., 2013; Kroll & Bialystok, 2013). However, to date the question of whether the different executive control processes are shared by different domains (linguistic, non-linguistic, motor) remains open. Under the hypothesis that domain-general control processes are involved in managing multiple language use, a bilingual advantage should be found in linguistic and in motor control tasks. The goal of the present study was to test this hypothesis by examining whether bilinguals show a benefit for controlling irrelevant distracting information using a linguistic task, i.e. the Stroop task and a motor task, i.e. the saccade task. For this purpose, two EEG experiments were designed: (1) Forty-four French native speakers, amongst which 22 with frequent use of German (bilinguals) and 22 with little foreign language

use (monolinguals), were tested in the Stroop task; (2) 20 new participants in each group were tested in the saccade task. Event-related potential (ERP) analyses on the Stroop task showed that both the Stroop-N400 effect and the late sustained potential were reduced in bilinguals compared to monolinguals, suggesting better interference suppression abilities in bilinguals. In the saccade task, the cue-locked positivity effect reflecting a larger positivity for the pro-saccade than for the anti-saccade and usually peaking around 250 ms was smaller in bilinguals than in monolinguals. We interpreted this finding as the indication of better efficiency for bilinguals in conflict monitoring in the task preparation phase. Moreover, the N2/P3 ERP complex thought to reflect inhibitory processes in the task implementation phase was reduced in bilinguals compared to monolinguals, suggesting a benefit for the former in response inhibition. In contrast, for motor task switching no such bilingual advantage was observed. Therefore, caution is needed before drawing firm conclusions regarding a possible spreading of cognitive advantages on executive control processes observed in bilingualism towards the motor domain. Taken together, the present findings suggest that multiple language use can increase the efficiency of some executive control processes but not of some others. Our data are consistent with the view proposing that in a system of limited resources the strengthening of specific processes and network connections can cause others to weaken.

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Poster

648. Executive Function I

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Topic: F.01. Human Cognition and Behavior

Title: Neural mechanisms for neurofeedback based on EEG using functional near-infrared spectroscopy (FNIRS)

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Abstracts: Control of one's brain state through meditation is thought to enhance mental wellbeing, but learning to meditate effectively is difficult due to the inadequacy of verbal descriptions. A possible tool for learning to regulate brain state is neurofeedback, which visualizes real-time EEG frequency components in the theta (4-9Hz), alpha (10-12Hz), beta (13-

17Hz), and gamma (41-50hz) wave domains. We hypothesize that executive control systems of the frontal lobe are related to volitional changes in brain state. We aim to develop an experimental paradigm as well as recording and analysis tools for investigating the neural mechanisms of brain state control. The neurofeedback device (Jedi Force trainer, Uncle Milton Industries, Inc., CA) consists of a single dry electrode placed at the forehead (prefrontal cortex) and a Neurosky chip that provides frequency bands of EEG and visualizations of low frequency EEG spectrum in the form of a "levitating" ball. Through volitional shifts in their EEG spectra, subjects can move the ball up and down 0-3 levels, with 3 requiring the most effort and concentration. In a pilot experiment, subjects were instructed to maintain a cued level of meditation, which was indicated by the height of the ball at 0, 1, 2 or 3 levels. Each subject received 10 hours of training prior to the experiment, and repeated it twice. The experiment consists of 14 blocks of meditation. The durations of the blocks were random (mean=20 and std=3.5 sec). Neural activity was recorded with either a 10-20 channel EEG cap (G.Tech, Austria) or a 22 channel fNIRS system (Shimadzu OMM-3000) covering right frontal and temporal lobes, in addition to the single dry electrode of the feedback device. ICA analyses were done on EEG time frequency data and fNIRS data. We observed sustained elevations in EEG theta and alpha spectra during meditation states, indicating that subjects were capable of controlling the brain state. In addition, we observed transient spikes of beta and gamma spectra associated with the sustained theta and alpha waves. Functional NIRS data showed activity related to brain state changes that was distributed and anti-correlated between medial and lateral frontal lobe. Both EEG and fNIRS data suggest that major volitional efforts in brain state control are engaged transiently when subjects alter the intensity of their meditation. Functional NIRS yields spatial localization of BOLD signals and is a promising tool for investigating the neural mechanisms of neurofeedback.

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Poster

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Title: The neural cascade of processing underlying response time variability in visual search

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Abstracts: Visual search, the process of finding target items among distracters, is a critical cognitive function that is at the core of numerous daily and professional activities. While it is important to be able to rapidly detect targets, there is substantial trial-to-trial variability in this ability. Here we explored the neural mechanisms that may underlie this variability by comparing reaction times (RTs) and various electrophysiological markers as 17 participants performed a popout visual search task. In this task, participants were presented (50 ms duration) with search arrays that each contained a lateralized green popout target and, in the opposite hemi field, a red popout nontarget, among a field of 46 blue distracters. The participants' task was to orient their attention to the green target ellipse and indicate whether it was "vertical" or "horizontal" with either a left- or right-hand button response. We subsequently extracted from the electrical recordings the event-related potential (ERP) components that index attentional orienting to a target item (the N2pc) and that index motor response initiation (the lateralized readiness potential [LRP]). In addition, neural oscillatory activity was extracted for time intervals both before and after the stimulus occurrence, which was then related to the stimulus-locked ERP markers and behavioral RTs to describe the neural cascade that precedes faster or slower neural processing and RTs. Results demonstrated that the attentional-orienting-related N2pc was significantly earlier for trials with fast RTs compared to slow ones, and this was followed by earlier response preparation, as indexed by earlier motor-related LRPs. Faster RTs were also associated with post-stimulus decreases in fronto-central oscillatory theta activity (4-7 Hz) and increases in beta oscillations (15-25 Hz), indicating both markers reflect processes that ramify to cognitive task performance. Although bilateral prestimulus alpha levels (8-12 Hz) did not covary with RT, lower alpha contralateral to the target side (which is typically associated with more focused spatial attention) predicted larger N2pc amplitudes to those targets, suggesting that greater prestimulus neural engagement to that side resulted in more robust attentional orienting. Contralateral prestimulus alpha did not, however, manifest in faster RTs, suggesting that such variations do not necessarily ramify into faster RTs, at least for these highly salient popout stimuli. Collectively, the results delineate the cascade of neural processes before and after a stimulus array that do and do not lead to response-time variability in pop-out visual search.

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Poster

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Support: MITACS

Rosetta Stone

Title: Relationship between performance on mobile brain fitness exercises and cognitive assessment

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Abstracts: Mobile cognitive training applications can serve as an effective way to deliver and collect data for cognitive training research. To gather insight into how different mobile cognitive training tasks relate to each other, we are analyzing data from existing mobile training applications by Fit Brains/Rosetta Stone. From over 147 million scores from the Fit Brains Trainer and Cognitive Assessment database, we correlated maximum scores per user from 12 Trainer games and 7 Cognitive Assessments, and studied for performance and relationship between games assigned to 5 different categories (logic, memory, concentration, visual and speed). From analysis of the Trainer data, we found performance in most games within the same category correlated strongly with each other, and performance in some games correlated closely with games in different categories. Memory and visual games had the strongest within category correlation, focus and executive function games had moderately strong within category correlation, and speed games had moderate correlation within category except for “quick blocks”, which correlated more strongly with games in the executive function category. On the other hand, performance in another speed game, “speed sort”, correlated well with performance in the memory, focus and visual category, which might indicate some involvements of similar cognitive processes for these games. The speed game “find it fast” did not correlate easily with any other games, which might suggest a distinct cognitive process might be required in this particular game compared to other games. For the Brain Assessment app, we found performance within the 3 memory assessments correlate strongly with each other. Performance in the speed assessment didn’t correlate with any other domains. Interestingly, this dissociation between performances in the speed category from the rest of the categories was also found in the analysis

in the Trainer app. Overall, our results demonstrated that performance in Trainer games and Cognitive Assessments assigned to the executive function, memory, focus and visual correlated well with each other, while performance in the speed category correlated better with performance in other categories. Subsequent analysis will focus on correlating performances with users between the Trainer and Cognitive Assessment in order to further describe the relationship of user performance between the Trainer and Cognitive Assessment. Further analysis can investigate additional correlations between user training habits, age, gender, medical conditions, geographic region, education, and lifestyle on the performance in Cognitive Assessment.

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Poster

648. Executive Function I

Location: Halls A-C

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Program#/Poster: 648.06/SS46

Topic: F.01. Human Cognition and Behavior

Title: Inhibitory control over unwanted memories is mediated by hippocampal GABA concentration

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Abstracts: Sometimes, we are reminded of memories we would prefer not to think about. When this happens, people often seek to suppress retrieval of the unwanted memory, a process shown to impair retention of the excluded trace. A fundamental question concerns how cognitive control is deployed to suppress memories, and the modulatory processes that may underlie memory disruption. In this multimodal Functional Magnetic Resonance Imaging (fMRI) and ^{1}H Magnetic Resonance Spectroscopy (MRS) study, we assessed whether concentrations of GABA in the dorsolateral prefrontal cortex (DPLFC) and the hippocampus, a putative 'top-down' pathway known to be involved in memory suppression, differentially predict inhibitory control over unwanted memories. We investigated this question using a well-established manipulation of

retrieval stopping, the Think/No-Think (TNT) paradigm. In the initial phase of the paradigm, participants learned word pairs. In the TNT phase, participants viewed one of the two words in the pair (cue) and either intentionally retrieved (T) or suppressed (NT) retrieval of the associated target word. FMRI was acquired during the TNT task, and forgetting was measured in a post-task memory probe unanticipated by participants. In a separate session, we obtained ^{1}H MRS from voxels in the right DLPFC, right hippocampus, and visual cortical control site. Our behavioural and fMRI data replicate prior findings obtained from the TNT paradigm. Specifically, we observed that significantly fewer intentionally suppressed (NT) words were recalled compared to both retrieved (T) words, and baseline words presented during the learning phase but not the TNT phase. Moreover, compared to retrieval, suppression evoked higher activation in the right DLPFC, lower activation in the right hippocampus, and increased DLPFC_hippocampal connectivity. We next assessed whether our neural indices of retrieval stopping in the DLPFC_hippocampal pathway related to molecular indices of GABA concentrations in this same pathway, and if so, how these two sources of variation give rise to individual differences in memory suppression capacity. We found that hippocampal GABA concentration predicts both disrupted retention of suppressed memories, and stronger suppression of hippocampal activity during retrieval stopping. These relationships were not observed in the DLPFC. Finally, hippocampal GABA concentration mediated the relationship between hippocampal suppression and forgetting of suppressed items. Our results suggest that the ability to suppress unwanted memories is influenced by GABA concentration in the hippocampal target, rather than the DPFC source, of executive control.

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Poster

648. Executive Function I

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Topic: F.01. Human Cognition and Behavior

Support: The Santos Family Foundation

US DOT Region I New England University Transportation Center

Title: Utilizing functional near-infrared spectroscopy to identify cognitive processes contributing to workload in a dual-task environment

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Abstracts: Mental 'workload' is the amount of cognitive effort required to complete a task. However many factors such as expertise, fatigue, distraction and concurrent tasks can affect the workload on any task. Since exceeding one's cognitive capacity can negatively affect performance, the ability to monitor and potentially reduce workload in naturalistic environments, could have significant real-world impact (e.g. for drivers, pilots or other machine operators). Peripheral physiological measures such as galvanic skin response, heart rate and pupil size have previously been used as a proxy for mental workload during naturalistic tasks; however, these measures can be confounded by physical arousal or exertion. Conversely, the measurement of cognitive activity during complex tasks has been thoroughly examined using brain-imaging techniques such as fMRI and EEG. However, electromagnetic interference and space constraints often inhibit naturalistic validity of traditional brain-imaging techniques. Functional near-infrared spectroscopy (fNIRS), like MRI, detects changes in oxygenated hemoglobin in the cortex, but utilizes lightweight, scalp-mounted optical sensors. The combination of fNIRS portability with high spatial and temporal resolution, may thus allow a disentanglement of potentially distinct cognitive activities that contribute to what we call workload such as working memory, task-switching and attention during safety-critical activities such as driving. Here, we recorded levels of oxygenated and deoxygenated hemoglobin using fNIRS along with electrocardiograph (EKG) and electrodermal activity (EDA) from 21 participants while driving in fixed-base driving simulator. In addition to baseline driving, participants also completed a secondary cognitive task (the digit n-back), often used to increase mental workload. Peripheral physiology resembled previous work from our lab revealing an approximately linear increase in both heart rate and skin conductance levels (SCL) as secondary-task difficulty increased. However, fNIRS sensors over the dorsal lateral pre-frontal cortices (DLPFC)(~Fp1-Fp2 in the international 10-20 system) detected first increases, then decreases in oxygen levels as task difficulty increased. Since the n-back task changes from a pure working memory task at the low levels, to include task switching or 'branching' activity as numbers are recalled, replaced and remembered up to 3 places back, we propose the workload captured in peripheral physiology is coupled with the migration of cortical activity from the DLPFC to pre-frontal areas associated with 'branching' activity such as the frontal pole.

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Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.08/SS48

Topic: F.01. Human Cognition and Behavior

Support: NWO VIDI

Title: Cortical oscillatory networks of synesthetic conflict processing as revealed by MEG source reconstruction

Authors: *J. VAN DRIEL[1], R. ROUW[1], A. HILLEBRAND[2], T. H. DONNER[1], M. X. COHEN[1];
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Abstracts: Grapheme-color synesthetes are often confronted with conflicting perceptual information (e.g. a brown "A" might feel 'wrong'). In general, mechanisms of goal-directed action monitoring after conflict have been linked to oscillatory activity in the theta (4-8 Hz) band, within and between medial and lateral frontal brain regions. Moreover, these frontal theta dynamics have been shown to exert top-down control, via phase synchronization, over lower sensory and motor areas to optimize future behavior. Here, we used synesthesia as a novel framework to elucidate these adaptive control processes when triggered by anomalous color sensations. Ten "projector" synesthetes (who report perceiving synesthetic color as appearing in the outside world) performed an adapted Stroop task in which they responded to printed color of letters congruent or incongruent with synesthetic color experience, while we recorded their brain activity with MEG. Behaviorally, we found typical Stroop-effects which were modulated by congruency of the preceding trial (the "Gratton" effect). Sensor-level time-frequency analyses showed, as expected, conflict-related theta power increase over midfrontal sensors. Using minimum-variance beamformer analysis with subject-individual MRI-scans, we furthermore localized the cortical sources underlying these electrophysiological scalp dynamics to anterior cingulate as well as lateral frontal and parietal regions. In addition, we performed connectivity analyses in which we applied source weights of an independent color localizer to the Stroop task data, revealing large-scale fronto-occipital network interactions when synesthetes experienced grapheme-induced conflict. These results thus provide a unique insight into the neurophysiology of frontally mediated top-down control of visual information, even when such visual information is not physically present in the outside world.

Disclosures: J. Van Driel: None. **R. Rouw:** None. **A. Hillebrand:** None. **T.H. Donner:** None. **M.X. Cohen:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.09/SS49

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS071221

Title: Functional parcellation of right inferior frontal cortex and anterior insula: The widely-attended and often-neglected regions in inhibitory control

Authors: *W. CAI[1], S. RYALI[1], T. CHEN[1], C.-S. R. LI[2], V. MENON[1];
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Abstracts: Inhibitory control, an ability to withhold or override a prepotent response, is crucial in everyday life. A decade of neuroimaging research has repeatedly reported co-activation in the right inferior frontal cortex (rIFC) and its neighboring region, right anterior insula (rAI), in inhibitory control. In contrast to the rIFC whose role has been extensively discussed, the role of the rAI in inhibitory control is underappreciated, and functional dissociation between these regions remains unclear. Here we adopted a data-driven approach to examine whether the rIFC and rAI have different physiological and functional features and to study functional dissociation between the two regions in inhibitory control. We conducted meta-analysis using GinglerALE on 73 inhibitory control studies, including Go/NoGo and Stop-signal tasks (SST), to generate an activation mask in the right frontal operculum. We computed two different features from three different datasets: intrinsic functional connectivity of the voxels within the mask from a resting-state fMRI dataset and task evoked hemodynamic responses in two SST datasets. Using these features, we parcellated voxels within the mask into an optimal number of stable clusters using a novel consensus based clustering algorithm. We then compared the physiological and functional characteristics of the clusters obtained from the two datasets. The parcellation produced two most stable clusters, one at rAI and the other at rIFC, consistently across resting-state and task fMRI datasets. Strikingly, corresponding clusters were highly similar between the two datasets with above 70% of voxels overlapped, despite the vastly different features used in the parcellation algorithm. More importantly, the two clusters (rAI and rIFC) showed distinct physiological and functional characteristics. First, rAI had strong intrinsic connection with anterior cingulate cortex and striatum whereas rIFC had strong intrinsic connection with lateral and dorsomedial prefrontal cortices. Second, although the rAI and rIFC both had stronger activation in successful stop than go trials, the rAI had even greater activation in unsuccessful

stop trials than the IFC. Third, activation in the right IFC had significant correlation with individual's inhibitory control ability while the AI did not. Taken together, our study provides robust and convergent evidence to demonstrate that the rIFC and rAI play different mechanistic roles in inhibitory control.

Disclosures: W. Cai: None. **S. Ryali:** None. **T. Chen:** None. **C.R. Li:** None. **V. Menon:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.10/SS50

Topic: F.01. Human Cognition and Behavior

Title: Neuroanatomical modeling of executive functioning: The algorithmic and reflective minds

Authors: *J. PAN[1,2], A. MIELE[3,2], D. GANSLER[1], S. DEONI[2];
[1]Psychology, Suffolk Univ., Boston, MA; [2]Advanced Baby Imaging Lab., Brown Univ., Providence, RI; [3]Neurodevelopmental Ctr., Providence, RI

Abstracts: Background: In clinical and research practices, performance-based and report-based measures are both used to tap into executive functioning. However, there is growing empirical support that the two types of measures actually tap into distinct constructs that represent different levels of higher cognitive functioning, termed the "algorithmic" and "reflective" minds (Stanovich, 2009). To date, the neural correlates of the algorithmic and reflective minds have not been investigated in a pediatric population. Toward that purpose, we compared the neural correlates of a performance-based and ratings-based measure of inhibition, considered to be a key aspect of executive function, predicting that the ratings-based reflective mind measure would be more strongly associated with default mode network areas. **Methods**: Twenty-four children participated in longitudinal study of brain development (mean age = 8.2, SD = 1.8). Myelin content was estimated throughout the brain using an MRI-based multicomponent relaxometry approach (mcDESPOT). All children completed the Inhibition subtest from the Developmental Neuropsychological Assessment (NEPSY), and parents/guardians also completed one report-based measure, the Behavior Rating Inventory of Executive Functioning- Inhibition Scale (BRIEF). Non-parametric correlations, corrected for multiple comparisons, were examined between myelin content and neurocognitive measures throughout the brain. **Results**: Both performance- and report-based scores yielded significant correlations to the myelin content of the dorsolateral prefrontal cortex (DLPFC) (p values ranged from 0.001 to 0.05), indicating that both

types of measures are related to working memory. While both the BRIEF Inhibition subscores and NEPSY Inhibition subscores were related to the dorsal anterior cingulate cortex (ACC) (*p* values ranged from 0.001 to 0.05), only the former was distinctly related to the ventral ACC (*p* values ranged from 0.001 to 0.05). This is a region theorized to be involved in emotion-based decision making. **Conclusions**: Consistent with a priori hypotheses, only a ratings-based reflective mind measure of inhibition was associated with ventromedial frontal regions associated with the default mode, while both performance and ratings-based measures were related to other frontal regions. These findings give credence to the distinction between the algorithmic and reflective minds, which are present in children as young as 6 years of age.

Disclosures: J. Pan: None. **A. Miele:** None. **D. Gansler:** None. **S. Deoni:** None.

Poster

648. Executive Function I

Location: Halls A-C

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Program#/Poster: 648.11/SS51

Topic: F.01. Human Cognition and Behavior

Support: NSF HSD0527698 awarded to JPS

Title: Parametric Manipulations in Simon and Go/NoGo reveal specificity of neural mechanisms of Response Selection and Inhibition

Authors: *S. WIJEAKUMAR, M. W. VOSS, V. A. MAGNOTTA, A. T. BUSS, R. E. HAZELTINE, J. P. SPENCER;
Univ. of Iowa, Univ. of Iowa, Iowa City, IA

Abstracts: Increased activation in cortical and sub-cortical regions has been reported in response to infrequent inhibition. However, it is unclear whether this increase in activation is due to infrequent inhibition or infrequent trials in general. Further, it is unknown how neural activation changes in response to increased stimulus-response mappings within the context of response selection. We used functional magnetic resonance imaging to investigate the effects on behavior and neural activation for two parametric manipulations in a Simon and a Go/NoGo (GnG): a working memory load manipulation implemented by varying the number of stimulus-response mappings and a proportion manipulation implemented by varying the proportion of excitatory trials. It has also been shown that functional networks involved in task performance have enhanced synchrony during the resting state directly following task engagement. Therefore, we

compared change in resting state networks before to after each task to determine whether the tasks would elicit distinct network interactions. Seed-based analyses were used to extract the default-mode network (DMN), salience network (SALN), executive control network (ECN) and a sensorimotor network (SMN). Behavioral results revealed increased reaction times on infrequent trials and also an increase in reaction times with an increasing number of stimulus-response mappings (load) for both tasks. A broad network of cortical, sub-cortical and cerebellar areas were activated by both tasks. Activation was greater on Go/compatible than Nogo/Incompatible trials at areas involved in motor-planning and control of movement. Bilateral lingual gyrus was selectively activated and de-activated in the Simon and GnG tasks respectively, reflecting a role specific to visuo-spatial attention. Insular-thalamic regions were selectively activated on infrequent events across both tasks. With regard to the load manipulation, there was a decrease in activation in the right inferior parietal lobule at higher loads. Thus, in contrast to the typical increase in neural activation with greater working memory demands, we found a decrease in activation suggesting that an associative memory mechanism underlies the stimulus-response mappings. Reliable resting state networks were identified across all resting-state runs. Both the SALN and ECN showed changes in resting state synchrony following both Response selection tasks. On the other hand, change in synchrony of the posterior cingulate cortex with the DMN was observed following the GnG task. Future work will integrate current theories and models of response selection with changes in neural activation.

Disclosures: S. Wijeakumar: None. **M.W. Voss:** None. **V.A. Magnotta:** None. **A.T. Buss:** None. **R.E. Hazeltine:** None. **J.P. Spencer:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.12/SS52

Topic: F.01. Human Cognition and Behavior

Support: Mind and Life Institute Varela Research Award

Title: Mind-wandering on the good, the bad, and the useful: Distinctive neural correlates of spontaneous thoughts differentiated by emotional valence, utility, and spontaneity

Authors: *K. C. FOX[1], M. L. DIXON[1], M. ELLAMIL[2], M. L. PUERTOLAS[3], A. RAUSCHER[1], K. CHRISTOFF[1];

[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Rotman Res. Institute, Baycrest Ctr., Toronto, ON, Canada; [3]NIH, Bethesda, MD

Abstracts: 'Mind-wandering' (MW) has been most famously tied to activity in brain regions of the default mode network (DMN). However, studies to date have aimed at delineating a general, undifferentiated picture of the neural correlates of MW. We sought to refine this understanding by examining the neural correlates of different kinds of thoughts, specifically investigating the spontaneity, utility, and emotionality of spontaneous thoughts. During an fMRI scan, we allowed subjects to rest and think freely, interrupting their thinking at random intervals with occasional thought probes. Probes asked subjects about (i) whether their thoughts arose spontaneously, or whether they were intentionally directing them; (ii) whether thoughts were related to their current concerns and goals in life, or not; and (iii) whether they were emotionally pleasant, unpleasant or neutral. We used an event-related design to examine neural activity during MW (just prior to the thought probe) according to subjects' responses. We found distinctive neural activity, as well as functional connectivity, underlying spontaneously arising vs. intentionally directed thoughts; thoughts related vs. unrelated to current concerns and goals; and emotionally pleasant vs. unpleasant thoughts. Counter to the prevailing view, thoughts were mostly emotionally positive and related to concerns and goals, suggesting an adaptive function. Our results refine the current understanding of 'the' neural correlates of MW, suggesting that distinctive forms of spontaneous thinking recruit distinctive sets of brain regions. They also speak to clinical disorders involving dysfunctional forms of spontaneous thought, e.g. depressive rumination, by showing that negative, non-useful thoughts are neurally distinct from positive, useful thinking.

Disclosures: K.C. Fox: None. **M.L. Dixon:** None. **M. Ellamil:** None. **M.L. Puertolas:** None. **A. Rauscher:** None. **K. Christoff:** None.

Poster

648. Executive Function I

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Program#/Poster: 648.13/SS53

Topic: F.01. Human Cognition and Behavior

Support: DA13165

Title: Humans and a non-human primate show similar behavior in a novel context-dependent stop signal task

Authors: *K. Z. XU[1], V. STUPHORN[1], S. YANTIS[2], S. COURTNEY[2];
[1]Psychological and Brain Sciences; The Zanvyl Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Psychological and Brain Sci., The Johns Hopkins Univ., Baltimore, MD

Abstracts: Human lesion and imaging (fMRI) studies have demonstrated that the inferior frontal cortex (IFC) is critical for stopping action, but the specific function of IFC activity in stopping is debated. While some argue for a direct role of IFC in response inhibition, others have argued for a role of IFC in either guiding attention to external events, or in encoding which task rules are behaviorally relevant. To test these hypotheses, we previously trained a monkey to perform a novel context-dependent variant of the selective stop signal task. We found that the activity of movement and fixation neurons in frontal eye field was sufficient to control gaze. In contrast, the activity of neurons in ventrolateral prefrontal cortex (VLPFC, which includes IFC) represented context-dependent rules and might be involved in switching between sets of task rules. This supports the hypothesis that VLPFC represents the abstract rules that guide inhibition, but does not instantiate inhibition itself. In order to compare behavior across species to this novel context-dependent stop-signal task, we administered an analogous behavioral task in humans. Each participant received 3 trial types in a pseudo-randomized order: no-stop-signal trials (NSS), stop-signal (SS) trials, and continue-signal (CS) trials, presented in a pseudo-randomized order. All trials started when participants acquired central fixation at a context cue. The shape of the context cue (square or triangle) indicated the rule mapping on that trial, and was visible briefly before it was replaced by a central fixation point. The fixation point was then extinguished and, simultaneously, a peripheral target appeared. On NSS trials, participants were required to generate a speeded saccade to the target. On NS and CS trials, a yellow or blue central point reappeared after a variable delay. In one context, a yellow point cued the participants to cancel the saccade while a blue point cued the participants to ignore it and make a saccade to the peripheral target. In the other context, the rule was reversed. The context alternated every 8,10 or 16 trials, randomly and without prior warning. The first trial following the context alternation was termed a “switch” trial. Humans demonstrated behavior that was similar to the monkey’s: reaction times in CS trials were longer than in the NSS trials, and the non-canceled SS trials yielded the shortest reaction times. On switch trials, reaction times increased and accuracy decreased. Importantly, the behavioral similarity across species will allow us to meaningfully compare primate electrophysiology and future human fMRI experiments.

Disclosures: K.Z. Xu: None. **S. Courtney:** None. **S. Yantis:** None. **V. Stuphorn:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.14/SS54

Topic: F.01. Human Cognition and Behavior

Title: EEG correlates of individual differences in motor sequence planning and accuracy

Authors: M. A. NIERMEYER[1], *M. J. EULER[2], Y. SUCHY[1], J. MATYI[1], H. SCHRYVER[1], D. CHON[1], V. REDDY[1], P. GIBBS[1], E. VACCARIELLO[1];
[1]Psychology, [2]Univ. of Utah, Salt Lake City, UT

Abstracts: Complex motor sequencing has long been recognized as a sensitive measure of executive dysfunction in neurological disorders. In particular, the Push Turn Taptap (PTT) task from the Behavioral Dyscontrol Scale-Electronic Version has been shown to detect subclinical neurocognitive dysfunction in both mild TBI and older adults (Suchy, Euler, & Eastvold, 2014; Kraybill, Thorgusen, & Suchy, 2012). Prior fMRI research has established a relationship between PTT planning time and accuracy and SMA-basal ganglia connectivity (Marchand et al., 2013). In order to further explore the neural correlates of PTT planning time and accuracy, the current study developed an EEG analogue of the PTT task. It was hypothesized that fronto-central EEG activity would predict both accuracy and intermediate planning times, reflecting performance monitoring processes during complex motor sequencing. *Methods*: Forty-five healthy college students completed the standard PTT task while a subgroup of 27 also completed the EEG analogue. Planning time and accuracy were assessed in both tasks. Each trial of the analogue task involved the presentation of a fixation cross, followed by a slide presenting the correct four-movement sequence. To elicit activity related to response planning as well as performance monitoring, the EEG analogue manipulated response certainty by varying the correct sequence on 10% of trials. *Results:* Planning time and accuracy were correlated across the two tasks (r = .50, p=.009; r = .475, p = .014). Averaging of correct trials time-locked to the onset of participants' first movement revealed a negative potential over fronto-central electrodes beginning at approximately -750 ms and peaking -35 ms prior to the response. When measured at Cz, the amplitude of this negative potential correlated with accuracy in both the EEG analogue (r = .496, p = .01) and the original PTT task (r = .610, p = .001); greater negativity was associated with fewer errors. In addition, the potential showed a curvilinear relationship with analogue planning time such that greater negativity was associated with intermediate planning times (b = .463, β = .520, p = .019). Fewer errors were also associated with intermediate planning times (b = 13.367, β = .398, p = .011). *Conclusions:* These results suggest that medial-frontal performance monitoring processes facilitate accurate responding during complex motor sequencing in the PTT task. The relations between performance and EEG activity in the analogue task suggest that the observed neural correlates support executive functions that balance competing task demands of efficient response planning and accuracy.

Disclosures: **M.A. Niermeyer:** None. **M.J. Euler:** None. **Y. Suchy:** None. **J. Matyi:** None. **H. Schryver:** None. **D. Chon:** None. **V. Reddy:** None. **P. Gibbs:** None. **E. Vaccariello:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.15/SS55

Topic: F.01. Human Cognition and Behavior

Title: Neural correlates of attention in two relaxation techniques

Authors: *S. DEEPESHWAR[1], S. A. VINCHURKAR[2], K. NAVEEN[1], S. TELLES[3]; [1]Yoga and Life Sci., [2]Swami Vivekananda Yoga Res. Fndn., Bangalore, India; [3]Patanajali Res. Fndn., Haridwar, India

Abstracts: Meditation and relaxation have both been known to influence the cognitive abilities of human beings. The aim of the present study was to assess the effect of meditation on stroop task using event related potentials. Fifty healthy right-handed male participants with age ranging between 19-32 years (group age mean, SD; 23.8±6.2 years) recruited from a Yoga University, Bengaluru. Participants had no history of current or past neurological or psychiatric illness. The participants had a minimum of one year experience with 'Cyclic meditation'. Each participant was assessed in two sessions, i.e., Cyclic meditation and supine rest lasted in 23 min and was preceded and followed by an Stroop task. Both sessions were recorded on two consecutive days and consisted of two states i.e., Before and After. EEG was acquired using a 128-channel HydroCel Geodesic Sensor Net (EGI, Eugene, OR). All electrode impedances were kept below 50KΩ. Recordings were referenced to Cz. ERP data was processed using the NetStation software. Scalp potentials sources estimates were modeled using the GeoSource imaging software with the sLORETA constraint to derive source locations based on the MNI coordinates. Activations were seen in the inferior temporal gyrus, middle temporal gyrus for the incongruent and congruent condition whereas middle temporal gyrus for neutral condition. Paired Sample t-test was used to examine scores of the three conditions of Stroop task i.e., neutral, congruent and incongruent before and after meditation and control sessions. Results suggest that there was significant reduction in N1 latency ($p<0.01$) at parietal and temporal regions in Stroop task after meditation, whereas increased latency at left tomporo-occipital and right parietal regions after supine rest indicated that meditation practice facilitated the focusing of attention resources. We also found reduction in amplitude in late negative component N450 at frontal and central regions

following meditation which reflect meditation practice reduced the recruitment of resources during object recognition processes and conflict processing. The mean reaction times were longer in stroop task for neutral ($p<0.05$) and incongruent (0.01) conditions, after supine rest, whereas the mean reaction times were shorter for neutral ($p<0.01$), congruent ($p< 0.05$) and incongruent ($p<0.05$) conditions after meditation. This suggest that meditation improves efficiency, possibly via improved sustained attention with the reduction of interference in Stroop task. To conclude, the results suggest that meditation may alter the efficiency of allocating cognitive resources, leading to improved self-regulation of attention.

Disclosures: S. Deepeshwar: None. **S.A. Vinchurkar:** None. **K. Naveen:** None. **S. Telles:** None.

Poster

648. Executive Function I

Location: Halls A-C

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Program#/Poster: 648.16/SS56

Topic: F.01. Human Cognition and Behavior

Support: DARPA

Title: The Plaza: Executive function improvement through innovative gameplay

Authors: *T. F. NUGENT III[1], A. KRUSE[1], C. CRAWFORD[2], S. WOLOSIN[2], J. YUILL[2], F. PIERCE[2], J. GARCIA[2], J. WILLIAMS[2], A. MILHOLLUN[2];
[1]Intific, Inc., Alexandria, VA; [2]Intific, Inc., Austin, TX

Abstracts: As the demand for cognitive enhancement tools continues to grow at a rapid pace, it has become apparent that there are definitive and identifiable shortcomings in the standard method and expected impact through their use. This effort looks to improve upon two major facets within this space: tackling the concern of maintaining interest with a sense of replay value and targeting development of an application that has the potential for long standing improvement of cognitive capability. Focusing on cognitive skills tied to executive function, the authors have developed a cognitive enhancement tool entitled The Plaza. With distinct emphasis on creating an engaging narrative, The Plaza leverages the known benefits of established neuropsychological cognitive tests into a story driven single player experience while attempting to address concerns over practice effects and diminishing interest through repeated play. Additionally, The Plaza incorporates cutting edge neuroscience software tools to allow for integration of real-time

biophysiological sensor systems to create an optional, closed-loop experience for the user. Utilization of cognitive state metrics through devices like electroencephalography (EEG) allows for unique, tailored gameplay experiences to each individual, every time they engage the software training tool. Applications of The Plaza could leverage neurofeedback capabilities in addition to the presentation of suggested learning strategies associated with working memory, problem solving, planning, and other executive function based cognitive functions. The tool will measure and train cognitive skills via gameplay in a way that is both motivating and meaningful. This tool is aimed at an older target audience (teen to adult) but the mini games for executive function training could be applied to any age group with the appropriate narrative setting. Effort is funded through the Information Innovation office of the Defense Advanced Research Projects Agency.

Disclosures: **T.F. Nugent III:** None. **A. Kruse:** None. **C. Crawford:** None. **S. Wolosin:** None. **J. Yuill:** None. **F. Pierce:** None. **J. Garcia:** None. **J. Williams:** None. **A. Milhollun:** None.

Poster

648. Executive Function I

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National Institutes of Health Grant 5T32EY007135 to the Vanderbilt Vision Research Center

Title: No truly domain-general resource in the human cerebral cortex

Authors: *B. J. TAMBER-ROSENAU[1,2], A. T. NEWTON[3,4], R. MAROIS[1,2,5];
[1]Dept. of Psychology, [2]Vanderbilt Vision Res. Ctr., [3]Vanderbilt Univ. Inst. of Imaging Sci., [4]Dept. of Radiology and Radiological Sci., [5]Ctr. for Integrative & Cognitive Neurosci., Vanderbilt Univ., Nashville, TN

Abstracts: Classic psychological theories posit domain-general limited resources whose exhaustion by one task – regardless of demands imposed on any specific sensory or motor modality, cognitive process, or representational format of task-relevant information – interferes with the performance of other tasks. Most primate electrophysiological research instead suggests broadly domain-specific information processing wherein task-relevant variables are coded even in frontal or parietal cortex, generally considered to be home to the most abstract and domain-general representations in the brain. Human functional MRI has addressed these disparate accounts, reporting a frontoparietal multiple-demand (MD) network recruited across a wide variety of tasks that vary on modality, process, and format (e.g., Duncan, Trends Cog Sci 2010). We recently used fMRI and multivariate pattern analysis (MVPA) to show that most MD regions code modality during response selection (RS) tasks (Tamber-Rosenau et al, J Neurosci 2013). Here, we extend these results by examining coding of two other task dimensions, cognitive process and representational format. Sixteen participants were scanned while performing experiments that manipulated either cognitive processes or representational formats in separate 7-Tesla fMRI sessions. We then used MVPA to determine whether information about the specific process or representational format was differentially encoded in the brain. In the Process experiment, we compared a 6-alternative arbitrary stimulus-response mapping task – which loads heavily on RS processes – to No-Go trials of a Go/No-Go task – which load heavily on inhibition processes. In the Representational Format experiment, we compared object identity and object location visual working memory tasks. We defined regions of interest (ROIs) based on overlapping univariate activation for the two tasks in each experiment, respectively. In each ROI, we calculated event-related timecourses of MVPA decoding between tasks within-experiment. We found broad decoding of both process and format: in both sets of ROIs, only subcortical regions and subsets of visual cortex failed to support decoding in each experiment. Furthermore, in additional ROIs drawn from our previous modality-coding work (Tamber-Rosenau et al 2013) and classic MD work (Duncan 2010), we observed similar results. Taking these results together with our previous (2013) work, all tested cortical regions proved to be sensitive to one or more of

modality, process, or format. Thus, we find no evidence for truly domain-general information processing in the human cortex.

Disclosures: **B.J. Tamber-Rosenau:** None. **A.T. Newton:** None. **R. Marois:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.18/SS58

Topic: F.01. Human Cognition and Behavior

Title: The influence of a single session of aerobic exercise on cortical activity during a flanker task

Authors: ***K. B. BEYER**[1], M. D. SAGE[2,3], W. E. MCILROY[1,2,3,4];
[1]Univ. of Waterloo, Waterloo, ON, Canada; [2]Rehabil. Sci., Univ. of Toronto, Toronto, ON, Canada; [3]Mobility Res. Team, Toronto Rehabil. Inst., Toronto, ON, Canada; [4]Canadian Partnership for Stroke Recovery, Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstracts: Aerobic exercise benefits the brain in many ways but appears to influence specific networks differently. Both higher fitness levels and long-term aerobic training are associated with more activity in a network underlying selective attention and less activity in a network underlying action monitoring. Even after a single session of aerobic exercise, the selective attention network is more active but it is not known how the action monitoring network is influenced. We used electroencephalography to probe electrophysiological markers of the activity of these two networks in response to a single session of aerobic exercise. Participants performed a modified flanker task before and after a 40-minute bout of moderate cycle ergometry (i.e., 50-60 % VO_2peak). From continuous EEG, we examined two ERP components: the stimulus-locked P3, which is associated with attention, and the response-locked error-related negativity (ERN), which is associated with action monitoring. Initial results reveal evidence that a single session of aerobic exercise influences electrophysiological markers of attention and action monitoring differently. Following exercise, P3 amplitude was higher indicating more activity in the attention network. Conversely, ERN peak latency was longer signalling a decrease in activity in the action monitoring network. These findings support previous research showing that fitness and long-term aerobic training modulate specific brain networks differently by revealing this phenomenon after just a single session of aerobic exercise. Ongoing analysis will explore event-related activity in the frequency domain and communication between these two

networks to further examine the relationship between aerobic exercise and cortical activity linked to attention and action monitoring.

Disclosures: K.B. Beyer: None. **W.E. McIlroy:** None. **M.D. Sage:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.19/SS59

Topic: F.01. Human Cognition and Behavior

Title: The relationship between manual dexterity and executive function in healthy older adults

Authors: *O. VASYLENKO, C. RODRIGUEZ-ARANDA;
UiT The Arctic Univ. of Norway, Tromsø, Norway

Abstracts: Aging is associated with cognitive and motor decline. Among the affected cognitive processes is executive function (EF). Manual dexterity also declines with aging. Studies have shown a relationship between decline in dexterity and executive dysfunction in cognitively impaired older adults. In normal aging, however, evidence of this relationship is lacking. A better understanding of the link between manual dexterity and cognitive function can provide information about how and why hand motor control changes with age. Objective: To investigate the relationship between EF and the kinematics of manual dexterity in healthy older adults. Methods: 15 healthy elderly and 15 young adults were tested with two EF tests: the Stroop Color and Word Test and the Trail Making Test. Dexterity of the dominant hand was assessed with two Purdue Pegboard subtests, and recorded with Vicon Motus. Movement times were obtained for each transport (reach to grasp, reach to insert) and manipulation (grasping, inserting) movement. Kinematic variables calculated for each movement were peak and mean angular displacements (PD, MND), peak and mean angular velocities (PV, MNV), times to peak angular displacement and velocity (TPD, TPV), number of changes in angular displacement and velocity (NCD, NCV). To compare EF scores and movement times between groups, we used t-tests. For the movements that showed time differences between groups, kinematics were analyzed by MANOVA. Pearson correlations were used to measure the association between the kinematics and EF scores. Results: Compared to the young group, the elderly group had lower EF scores and longer movement times during grasping and inserting. The elderly group showed lower MNV during grasping on both tasks. On the pins task, the elderly group had lower MND and longer TPD during inserting. On the assembly task the elderly showed larger NCV and NCD during

grasping and larger NCD during inserting. On the pins task, MNV during grasping and MND during inserting were positively related to EF scores. On the assembly task, NCD during grasping and inserting was negatively related to EF scores. Movement variability during grasping and inserting the first 3 parts was inversely related to overall performance. Conclusions: Age differences in manual dexterity were evident during manipulation only. Older adults were slower to manipulate objects on both tasks and showed increased movement variability on the more difficult task. Some of the kinematics were correlated with EF, which suggests that manual dexterity might be dependent on EF in healthy older adults.

Disclosures: O. Vasylenko: None. **C. Rodriguez-Aranda:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.20/SS60

Topic: F.01. Human Cognition and Behavior

Support: 2KL2RR024157-06

K12 NS080223

Dana Foundation

Title: Distinct representation of conflict, response, and feedback selectivity by individual neurons in human dorsal anterior cingulate cortex

Authors: *G. HORGA[1], M. K. MIAN[2], S. R. PATEL[2], E. N. ESKANDAR[2], M. M. BOTVINICK[3], S. A. SHETH[4];
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Abstracts: The dorsal anterior cingulate cortex (dACC) is a main focus of neuroscience research given its central role in regulating adaptive behavior and its involvement in the pathophysiology of neuropsychiatric illness. Although a unique role for the human dACC remains elusive, current models suggest a complex role with simultaneous contributions to multiple cognitive functions, from computation of effort to reinforcement learning to cognitive control. Research in non-human primates suggests that dACC distinctively encodes multiple decision variables, even at the level of individual neurons, but evidence in this respect is lacking in humans. Here, we

recorded from individual human dACC neurons during a neurosurgical procedure while subjects performed the multi-source interference task (MSIT). On each trial, the cue consisted of a set of three numbers, and subjects were instructed to respond to the identity of the unique number ('target') that differed from the other two numbers ('distractors') via button press. Decision conflict emerged on trials in which the identity of distractor numbers corresponded to possible responses (Eriksen-conflict trials), the position of the target number was incongruent with its identity (Simon-conflict trials), or both. Neutral feedback alternated with non-neutral feedback in a block-wise manner. We used a sliding GLM (ANOVA) to classify individual neurons based on changes in their firing rates as a function of conflict, response selectivity, and feedback sensitivity during three target 500-ms periods: post-cue, choice, and outcome periods. We also evaluated population-level effects using a hierarchical GLM approach. Of the 59 neurons we studied, 27.1% exhibited a significant conflict effect in at least one of the three target periods, 25.4% exhibited response selectivity, and 18.6% exhibited feedback sensitivity; 40.7% showed only one effect and 15.3% showed more than one effect (all $p<0.05$, corrected). None of the neurons exhibited an interaction between conflict and response selectivity or time-on-task effects. Population activity showed a positive bias for conflict, response, and feedback selectivity. Contrary to prior non-human findings, population activity in response neurons did not account for conflict effects. Our findings support the functional heterogeneity of neuronal populations in human dACC, consistent with single-unit recordings in non-human primates. Our data further support the existence of pure conflict signals in individual neurons of human dACC, a finding with relevant implications for integrative models of dACC function and human imaging studies.

Disclosures: G. Horga: None. **M.K. Mian:** None. **S.R. Patel:** None. **E.N. Eskandar:** None. **M.M. Botvinick:** None. **S.A. Sheth:** None.

Poster

648. Executive Function I

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.21/SS61

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DA026452

Title: Suppressing a motivationally-triggered action tendency reduces future provocation

Authors: *S. FREEMAN, D. ALVERNAZ, A. TONNESEN, D. LINDERMAN, A. R. ARON; UCSD, San Diego, CA

Abstracts: In Freeman, Razhas and Aron (2014), we showed that withholding an instrumental response while provoked by a motivating stimulus recruits top-down response suppression. Here we tested if repeatedly performing such response suppression leads to a change in stimulus-generated motivational provocation. To examine this, we used a hybrid Pavlovian-instrumental transfer and Go-NoGo paradigm in thirsty human subjects. In the first stage, Instrumental, participants continuously pressed a button to obtain juice (Go trials), while, on NoGo trials, they were required to not respond; in the second stage, Pavlovian, participants learned to associate one color with juice (CS+) and the other color with no juice (CS-); the third stage, Transfer, resembled the instrumental phase, with the addition of the CS+ or CS- appearing in the background of the Go/NoGo cue and the CS+ serving as the motivating stimulus. We tested three independent groups of participants who had varying proportions of NoGo CS+ trials in the transfer phase (10%, 25%, and 40%). We examined how provoked subjects were by the CS+ (compared to the CS-) on Go trials, with the dependent measure being RT to press for juice. ANOVA revealed an interaction between Stimulus (CS+/CS-) and Group (10%, 25%, 40%), $p = 0.01$, with reduced CS+ provocation in the groups with a greater proportion of NoGo CS+ trials. The change across groups was highly linear ($r = 0.99$, $p = 0.004$, for the difference between Go RT on CS+ and CS- trials). Subsequent experiments using single-pulse transcranial magnetic stimulation as a probe of corticospinal excitability showed that the group-level changes in CS+ provocation were best explained by the engagement of a response suppression mechanism that selectively monitors for and suppresses motivational provocation on the following trial. These results suggest that when a motivating stimulus is detected in a context where it is inappropriate and response suppression is triggered, the response suppression mechanism stays active for a period of time sufficient, at least, to mitigate motivationally-driven responses on the next trial

Disclosures: S. Freeman: None. **D. Alvernaz:** None. **A. Tonnesen:** None. **D. Linderman:** None. **A.R. Aron:** None.

Poster

648. Executive Function I

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Program#/Poster: 648.22/SS62

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH082957-01A2

Title: Conflict-Specific cognitive control mechanisms for task rules: Switching between task rules and resolving cue incongruence

Authors: *Y.-S. SHEU[1], S. M. COURTNEY[2];
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Abstracts: To achieve behavioral goals, cognitive control permits flexible use of limited cognitive resources by configuring brain processes according to the currently relevant task rule. This control is critical when the task rules are constantly shifting or when resolution of conflicting task rules is required. The mechanism of cognitive control for task rules has been widely studied using either task-switching paradigms or bivalent/incongruent cue paradigms. In both cases, there is a competition between a relevant and an irrelevant task rule, and participants are required to select the one that is relevant to the current behavioral goal. However, the source of conflict originates differently: in a task-switching paradigm, the conflict arises from the previously activated task rule, whereas in a congruence paradigm, the conflict arises when different dimensions of the cue are simultaneously mapped onto conflicting rule. Hence, an unanswered question is whether different sources of task conflict are associated with dissociable, conflict-specific, cognitive control mechanisms, or whether a single, domain-general, cognitive control mechanism is recruited to resolve all types of task conflict. To test this, we independently manipulated task switching (switch/repeat) and cue congruency (incongruent/congruent) using a single paradigm. This allowed for direct comparison of neural activation patterns associated with these two sources of task conflict. Specifically, participants switched between two different task rules instructed by either the shape or the color dimension of the cues, after extensively learning of cue-rule mappings. Cue congruency was manipulated by having the color and shape dimensions of the cue stimulus associated either with the same task rule (congruent) or different ones (incongruent). Behaviorally, we found a significant main effect for both switching and cue congruency, but no significant interaction between these two. Furthermore, there was no significant correlation between switch cost (switch - repeat) and congruency cost (incongruent - congruent) across participants. The neuroimaging data further revealed distinct neural circuits associated with task switching and cue congruency. A conjunction analysis later confirmed that no common area was shared between these two control processes. Overall, these results indicate that the cognitive control mechanism involved in task switching is different from that involved in resolving cue congruency, supporting the hypothesis that conflict resolution for task rules is achieved via multiple cognitive control mechanisms specific to the source of the conflict.

Disclosures: Y. Sheu: None. **S.M. Courtney:** None.

Poster

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.23/SS63

Topic: G.06. Computation, Modeling, and Simulation

Title: Single time-point classification of brain activity during emotional feelings using multivariate pattern analysis

Authors: *A. GOTSOPOULOS, H. HEIKKILÄ, I. P. JÄÄSKELÄINEN, M. SAMS, L. NUMMENMAA, J. LAMPINEN;
Deparment of Biomed. Engin. & Computat. Sci., Aalto Univ., Espoo, Finland

Abstracts: Multivariate pattern analysis (MVPA) refers to a set of methods that are able to detect patterns of neural responses. This is an advantage over univariate analysis methods, which measure magnitude of an effect at a single voxel level. All higher-level brain functions, such as emotions, are subserved by connected activity in multiple brain regions. Therefore, MVPA is a promising method for studying their neural basis. Here we introduce a non-linear classification scheme based on artificial neural networks with leave-one-subject-out cross-validation to analyze emotional responses using fMRI data. We exploit the ability of the classifier to classify brain states in single time points, hence unveiling a temporal classification pattern that provides further information about task-related neural signatures. Analysis of the trained neural network's structure provides information on the importance of each voxel in each classification category, as we can recalculate the output of the classifier after removing each voxel separately from the input. This output difference reveals the importance of each voxel for each output class, yielding spatiotemporal brain importance maps for each category and each time point. We applied our method to fMRI data acquired in two experiments involving induction of emotional feelings. In the Movies experiment, 21 subjects were instructed to watch 10-second movie clips that invoked four basic emotions (disgust, fear, happiness, sadness) and a neutral state. In the Words experiment, emotions were induced in 14 subjects by mental imagery of emotional states belonging to six categories after seeing a cue word (anger, fear, disgust, happiness, sadness, surprise). Fixation period and inter-trial interval time points were also included in the analysis to verify our hypothesis of chance-level accuracy during these periods. Our classifier exhibited above-chance accuracy for time points of interest (47.5% against 20% chance level in the Movies experiment and 38.8% against 16.6% chance level in the Words experiment). Classification accuracy over time indicates the duration that a classifier can still detect distinctive activation patterns while the classification importance maps pinpoint the responsible regions for

the classification. The voxels contributing to the classification covered a wide and temporally varying range of brain regions such as frontal pole, posterior cingulate, precuneus, occipital lobe and limbic regions.

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Poster

648. Executive Function I

Location: Halls A-C

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Program#/Poster: 648.24/SS64

Topic: F.01. Human Cognition and Behavior

Title: Optimizing cognitive task designs to improve learning rates in a large online population

Authors: *N. NG, A. KALUSZKA, J. L. HARDY, M. D. SCANLON;
Lumos Labs, Inc, San Francisco, CA

Abstracts: Though many cognitive training studies have shown improvements in task performance over repetitions, few have sought to optimize these tasks to extend and accelerate learning rates. Recent wide-scale, online studies have shown the feasibility of quickly detecting different learning curves after manipulating multiple psychophysical parameters of specific games in an online cognitive training system (Kaluszka et al 2013). To continue this research, we set out to test the effect of varying other cognitive task parameters (eg up/down rule) on performance on the task over time. We hypothesized that optimizing the level of challenge of cognitive training tasks would lead to accelerated learning of the tasks. Participants were drawn from a pool of millions of users undergoing cognitive training through an online system (Lumosity). These individuals were randomly assigned to different versions of a training task as part of a battery of cognitive tasks that they performed daily. For example, we varied the up/down rule of a visuospatial short-term memory task inspired by the Visual Patterns Test (Della Sala et al 1997). Each session of the task consisted of 15 trials which required subjects to recall the location of a number of highlighted tiles within a grid. The up/down rule is a psychophysical parameter that dictates when the level of task difficulty between trials, as defined by the number of highlighted tiles, moves up or down. This random assignment method was applied to several other cognitive training tasks for which different relevant psychophysical parameters were varied. For all manipulations, participants were blinded to the differences in versions, and performance metrics such as percent correct and maximum span were recorded

across sessions for individuals and compared across groups. This study suggests that optimizing cognitive task difficulty to better match subjects' skill levels leads to faster learning. Additional investigation is needed to determine whether this accelerated learning curve extends to other non-trained cognitive tasks.

Disclosures: **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. **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.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

648. Executive Function I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 648.25/SS65

Topic: F.01. Human Cognition and Behavior

Title: Neural correlates of conflict during interpersonal communication observed in dorsal lateral prefrontal cortex using NIRS

Authors: *S. V. YAHIL[1], X. ZHANG[1], A. NOAH[1], P. LAPBORISUTH[1], M. BIRIOTTI[2], J. HIRSCH[1,3];
[1]Dept. of Psychiatry, Brain Function Laboratory, Yale Sch. of Med., New Haven, CT; [2]Med. Humanities, Univ. Col. of London, London, United Kingdom; [3]Dept. of Neurobio., Yale Sch. of Med., New Haven, CT

Abstracts: Although cognitive conflict has long been studied within individual brains, little is known about conflict processing in interpersonal interactions. Prefrontal cortex, known as an executive control center, is targeted as the ROI for this study. We present results of a novel, interpersonal Stroop-like task using natural communicative gestures. Audio or written forms of "yes" and "no" were superimposed on video recordings of actors performing simple

communicative gestures, such as thumbs up/thumbs down and head nodding/head shaking, thereby generating congruent and incongruent stimuli. Subject task was to identify the communicative intent of the gesture as either a “yes” or a “no.” Stimuli were presented for 3s with jittered inter-stimulus intervals of 2.5-3.5s. A single run consisted of 64 trials, for a total run time of 6-7 minutes. Trials were grouped 4 at a time into 8 congruent-dominant or 8 incongruent-dominant blocks, ensuring pseudo-random presentation. Congruent-dominant blocks contained 3 congruent trials and 1 incongruent trial, while incongruent-dominant blocks contained 3 incongruent trials and 1 congruent trial. All trials and blocks were presented continuously. BOLD activation from prefrontal cortex was measured with a portable, Bluetooth-enabled functional near-infrared spectroscopy (fNIRS) system (Astem Hb-13). Standardized Polhemus digitizations were acquired for each subject and used to assure homologous fNIRS optode placement across subjects’ prefrontal cortex. We predicted that our task would elicit the canonical Stroop effect as an increased reaction time for incongruent trials and conflict-related processing in prefrontal cortex. Behavioral results consisted of increased reaction time for incongruent trials over congruent ones, indicating Stroop-like conflict in a multisensory, interpersonal domain. Task-related BOLD activation in prefrontal cortex differed for congruent and incongruent trials, suggesting that frontal structures play a specific role in processing interpersonal conflict. In particular, signal amplitudes were suppressed for conflict-related trials relative to congruent cases. The findings suggest that prefrontal cortical regions are sensitive to types of social information.

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Poster

649. Human Social Cognition II

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Topic: F.01. Human Cognition and Behavior

Support: Wyncote Foundation

AG043503

AG017586

AG032953

AG038490

NS044266

NS053488

Title: Learning from our mistakes: Apoe4 influences misdiagnosis of alzheimer's disease and frontotemporal degeneration

Authors: *J. L. HALEY, M. GROSSMAN, C. MCMILLAN, K. RASCOVSKY, D. IRWIN, D. WOLK, J. TROJANOWSKI, V. VAN DEERLIN, B. MCCARTY WOOD, L. SHAW; Neurol., Univ. of Penn, Philadelphia, PA

Abstracts: FTD and AD are pathologically distinct neurodegenerative diseases but have considerable clinical overlap that make accurate diagnosis challenging. The Ɛ4 allele of the Apolipoprotein E gene (APOE4) is a risk factor associated with late-onset AD that can influence clinical presentation, but has received little attention in FTD. We assessed whether APOE4 status contributes to misdiagnosis of AD and FTD. Furthermore, we utilized neuropsychological test results to determine if there was a difference in cognitive profile related to APOE4 status. Clinically diagnosed AD (n=545) and FTD (n=263) patients were assessed using a detailed neuropathological exam at autopsy or using an autopsy-validated analysis of cerebrospinal fluid (CSF). A previously autopsy-validated CSF total-tau to beta-amyloid ratio was used to classify patients as AD (>0.34) or FTLD (<0.34) pathology. DNA samples were collected via blood draw using standard genotyping methods to determine APOE4 genotype. We categorized patients as carriers of an E4 allele (APOE4+) or non-carriers (APOE4-). We assessed cognition with a standard neuropsychological battery examining the following domains: global (MMSE), working memory (Digit Span Backwards, Logical Memory), executive functioning (Trails B, Digit Symbol), word fluency (Animal and Vegetable Naming) and language (30-item Boston Naming Task). Overall, 84% of clinically-diagnosed AD and FTLD patients revealed consistent pathology with their diagnosis. An assessment of APOE4 status in clinical AD revealed that 66% (n=330/498) of patients consistent with having AD pathology were APOE4+ while only 43% (n=20/47) of inconsistent cases were APOE4+. In contrast, 25% (n=47/184) of clinical FTD patients consistent with having FTLD pathology were APOE4+ while 35% (n=28/79) of inconsistent cases were APOE4+. Together, these findings suggest inconsistent AD cases are more likely to be APOE4- (X^2=13.28; p=.001) while inconsistent FTD cases are more likely to be APOE4+ (X^2=5.66; p=.05). Furthermore, an assessment of Logical Memory revealed significant differences across diagnoses and APOE4+/- status (F=4.36, p<0.001). Our findings suggest that APOE4 status may influence the clinical presentation of AD or FTD and thus may contribute to pathological misdiagnosis. Logical Memory scores may help differentiate between AD and FTD. Future investigations should identify if there are regions of atrophy associated with APOE4 status in AD and FTD that may facilitate differential diagnosis.

Disclosures: **J.L. Haley:** None. **M. Grossman:** A. Employment/Salary (full or part-time):; University of Penn. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH. **C. McMillan:** None. **K. Rascovsky:** None. **D. Irwin:** None. **D. Wolk:** None. **J. Trojanowski:** None. **V. Van Deerlin:** None. **B. McCarty Wood:** None. **L. Shaw:** None.

Poster

649. Human Social Cognition II

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: MEXT

Title: Social value orientation and choice type dependent activity in the dorsal prefrontal cortex and amygdala

Authors: ***A. S. R. FERMIN**[1], T. KIYONARI[2], Y. MATSUMOTO[3], Y. LI[1], M. SAKAGAMI[1], T. YAMAGISHI[4];
[1]Brain Sci. Inst., Tamagawa Univ., Machida, Tokyo, Japan; [2]Aoyama Univ., Sagamihara, Japan; [3]Hokkaido Univ., Sapporo, Japan; [4]Tokyo Univ., Tokyo, Japan

Abstracts: Although the neural basis of social cooperative behavior has been recently investigated, little is known whether neuro-anatomical and neuro-functional differences exist in humans with distinct social choice preferences. Here, we investigated these issues in subjects with prosocial or proself value orientations while they played a sequential non-matrix prisoner's dilemma game inside the fMRI scanner, and acquired their anatomical and functional brain data. Subjects played the game, as the first player to make a choice, against trial-by-trial randomly matched anonymous human partners. Subjects received an endowment and had a delay period of 4s-6s to decide to either cooperate or defect. If the subject chose to cooperate the partner received two times the endowment value, but if the subject chose to defect the partner received nothing and the subject received only the actual endowment value. The partner's choices were preprogrammed to cooperate with probability 0.6 if the subject cooperated or with probability 0.1 if the subject defected. Analysis of choice behavior found that subjects with prosocial orientation cooperated significantly more than subjects with proself orientation. The VBM analysis using multiple regression and ROIs revealed that proselves have larger DLPFC GM

volume, whereas prosocials have larger amygdala GM volume. A positive correlation was found between the left amygdala size and cooperation rate. The voxels identified in the VBM analysis were used as mask images to estimate the BOLD signal as subjects made their choices during the delay period. Overall, proselves showed increased BOLD signal in the DLPFC regardless of choice type. Prosocials, on the other hand, had increased BOLD signal in the left DLPFC only when choosing to defect. Amygdala BOLD signal increase was observed only in prosocials regardless of choice type. These results suggest that the DLPFC is required for the implementation of the rational economic decision to defect by both proselves and prosocials, and that prosocials' decisions are guided by an emotional sensitivity of what their choices incur to their partners.

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Poster

649. Human Social Cognition II

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Program#/Poster: 649.03/SS68

Topic: F.01. Human Cognition and Behavior

Support: University of Georgia, Office of the Vice President of Research

Title: Neural correlates of consciously controlling interpersonal trust

Authors: *M. M. FILKOWSKI, I. W. ANDERSON, B. W. HAAS;
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Abstracts: Interpersonal trust is an important component of human social interaction. Previous research has investigated the tendency to trust to others. However it is currently unknown how people go about consciously controlling the tendency to either trust or distrust another person. This study was designed to examine how people use conscious control to change how much they trust or distrust another person. We recruited 60 healthy adults to participate in behavioral testing and neuroimaging. Each participant evaluated the trustworthiness of a series of faces presented on a computer screen. On a separate day, each participant underwent fMRI while they were instructed to control how much they either trusted or distrusted each face. The faces within the fMRI experiment were the same as the faces used during the initial trustworthiness behavioral evaluation. Following fMRI, each participant then performed a second trustworthiness evaluation

of each face. Control of distrust of trust was operationalized as the change of trustworthiness evaluation during pre fMRI to post fMRI. Results showed that participants became more distrusting of the faces paired with a distrust instruction, but did not consistently become more trusting of faces paired with a trust instruction. Within the brain, both control of distrust and trust conditions were associated with increased dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) activity. Lastly, we found that individual differences in the control of trust were associated with precuneus activity. Specifically, greater change of trust scores (pre to post) was associated with greater engagement of the precuneus during the control of trust condition. Together, these findings identify a brain network involved in the top down control of attitudes (DLPFC and ACC) engaged during the conscious control of interpersonal distrust and trust and show that the precunues serves to successfully consolidate the attempt to trust another person.

Disclosures: M.M. Filkowski: None. **I.W. Anderson:** None. **B.W. Haas:** None.

Poster

649. Human Social Cognition II

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Topic: F.01. Human Cognition and Behavior

Support: Strategic Research Program for Brain Sciences (D)

JSPS research fund (24653058)

Title: Neural mechanism of social preferences toward reference persons of the same and different gender

Authors: *K. YAMADA[1], S. C. TANAKA[2], R. KITADA[3], S. K. SUGAWARA[3], H. TAKAHASHI[3], F. OHTAKE[4], N. SADATO[3];
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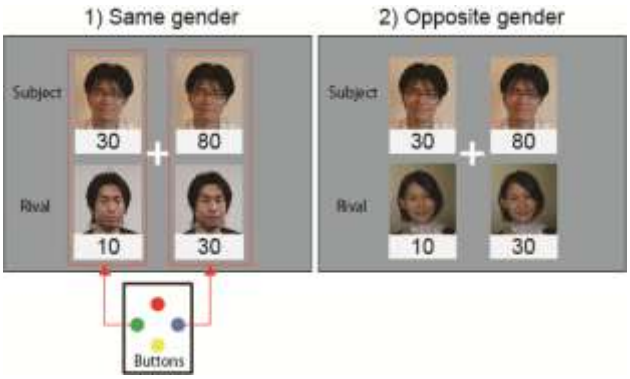
Abstracts: We examined neural mechanism of social preferences by addressing two novel features of the experiment. One is methodological: while much has been uncovered, one shared caveat in previous studies is that neural basis of social preferences was analyzed using self-reported evaluation scores of experimental situations. The reliance on self-reported evaluation

scores would be problematic unless the same numerical sense on the scale was shared across subjects. We employed a new experimental paradigm on social preferences that has been developed in economics and is free from the use of self-evaluation data. The other feature is our focus on the effect of gender of reference persons after a recent finding in economics that the effects of social preferences were asymmetric between genders. We measured brain activity by fMRI at the timing when subjects (N = 26, male 14; female 12) chose, rather than gave scores on, a preferred option out of two, each of which consisted of combination of reward for the subjects and for their fictive male or female rivals (see Figure). Key parameters of the intensities of social preferences toward reference persons of each gender were estimated from choice patterns in the tasks via the Conditional Logit model. By looking at the correlation of estimated parameters of social preferences and the BOLD signal in the DLPFC and the insula, we obtained the following results. Regarding the DLPFC, the correlations of BOLD signal associated with reward information of reference person and estimated parameter of social preference were significant in all combinations of gender of subjects and that of reference persons. For subjects who were estimated to be more jealous via the behavioral data, neural responses representing the information of rivals' reward were stronger in DLPFC. Hence, in terms of gender, DLPFC seems to play an universal role of social preferences. On the other hand, regarding the insula, that correlation was significant only in the case when female subjects compared to male rivals. Hence, the insula plays a very specific role of social preference in term of gender.



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Poster

649. Human Social Cognition II

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Program#/Poster: 649.05/TT2

Topic: F.01. Human Cognition and Behavior

Support: PRIN

Title: The selective involvement of frontal lobes in naming social groups

Authors: *L. PIRETTI[1], A. CARNAGHI[2], F. CAMPANELLA[3], E. AMBRON[1], E. SOMACAL[2], M. SKRAP[3], R. RUMIATI[1];
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Abstracts: Introduction: Social groups are defined as categories of individuals that share category-relevant characteristics and/or features (Mason & Macrae, 2004). Preliminary evidence from both neuropsychological and neuroimaging studies indicates that the knowledge about this category may be represented independently of the knowledge about other categories such as animals, plants and tools. Aim: We want to study whether social groups have a representation of their own relative to other categories of knowledge and, if so, where these concepts are stored in the brain. Methods: We tested twenty-nine patients with frontal and temporal brain tumors in the left or right hemisphere and nineteen healthy controls (HC) on three tasks (picture naming, word-to-picture matching and picture sorting) using three categories of stimuli: living things (N=15), non-living things (N=15) and social groups (N=15). The stimuli of the three categories were matched for letter length and word frequency ($p>.05$). Results: Left brain tumor patients (lBTP) performed significantly worse than right brain tumor patients (rBTP) and HC on naming non-living things ($p<.05$) and social groups ($p<.01$). In particular, lBTP named significantly worse non-living things than living things and social groups, while rBTP performed at HC level. Error analysis revealed a higher amount of anomias within the social groups category for lBTP respect to rBTP. All the patients performed at ceiling level on the remaining tasks (word-to-picture matching and picture sorting). Voxel-based lesion-symptom mapping (VLSM) showed that damage to the left inferior frontal gyrus led to the impairment on naming living things, damage to the left inferior temporal cortex led to a deficit in naming non-living things, and damage to left superior and middle frontal gyri gave rise to a deficit in naming social groups. Conclusion: Our results, in particular the lesion analyses, confirmed that the concepts about social groups are represented in specific brain structures, different from those in which other categories of knowledge are represented. Furthermore, the brain areas that when lesioned give rise to naming errors with social groups are similar to those reported in neuroimaging studies concerning social stereotypes.

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Poster

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Program#/Poster: 649.06/TT3

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R01 MH096914-01A1

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Title: Mentalizing regions explicitly code quality and source information about other's beliefs

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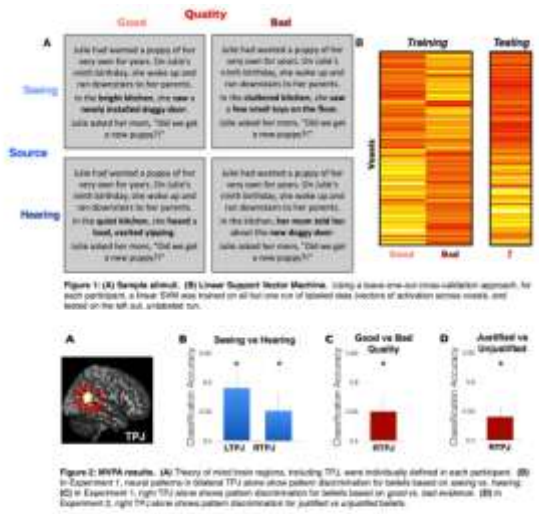
Abstracts: Predicting and explaining other's behavior depends on understanding mental states: theory of mind (ToM) reasoning. A key open question asks what neural computations underly this ability. ToM reliably recruits specific brain regions, including bilateral temporoparietal junction, precuneus, and medial prefrontal cortex. We used multivoxel pattern analysis in two fMRI experiments to examine how these regions code information about others' beliefs. We probed features that are informative when evaluating someone else's belief: the source and quality of their evidence and the justification of their belief. In Exp. 1, 20 participants listened to 48 stories in which a protagonist is presented with evidence and comes to a conclusion; in each story, we varied the quality (seeing something clearly vs ambiguous evidence) and perceptual source of the evidence (seeing vs hearing). Using a linear support vector machine to identify patterns of neural activity across voxels within independently localized ROIs, we find that bilateral TPJ codes perceptual source of beliefs, and that RTPJ alone distinguishes between good and poor visual evidence. In Exp. 2 we ask whether the discrimination between good and poor visual evidence in the RTPJ generalizes beyond representations about the protagonist's perceptual evidence (how well could they see?) to information about the justification of their belief (how well does their evidence support the inference?). 19 participants read 54 stories in which a protagonist comes to a conclusion (e.g. that their child will wait to get into the swimming pool when asked to do so), that is justified or unjustified given the past experience of the protagonist (e.g., the child always vs never does what they are told). Using the same analysis parameters from Experiment 1, we find that the pattern of response in RTPJ alone distinguishes between justified and unjustified beliefs. Together, the current evidence suggests that the RTPJ

explicitly codes features of beliefs that are crucial in belief evaluation, including the source, quality, and overall justification of the belief.



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Poster

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Topic: F.01. Human Cognition and Behavior

Support: MIUR grant (PRIN 2010XPMFW4_008; I meccanismi neurocognitivi alla base delle interazioni sociali) to SFC

Title: Empathy impairment in the behavioral variant of frontotemporal dementia: Evidence from resting-state brain activity

Authors: ***N. CANESSA**[1,2], S. CAMINITI[1,2], C. CERAMI[1,2], A. DODICH[1], C. CRESPI[1], A. MARCONE[2], S. IANNACCONE[2], A. FALINI[1,2], S. F. CAPPA[3,2];

[1]Univ. Vita-Salute San Raffaele, Milan, Italy; [2]San Raffaele Scientific Inst., Milan, Italy; [3]Inst. Universitario di Studi Superiori - IUSS, Pavia, Italy

Abstracts: Loss of empathy is a symptom of the behavioral variant of frontotemporal dementia (bvFTD), constituting a clue for early diagnosis. We have previously shown that the affective facets of empathy are more affected than cognitive ones, with this deficit reflecting gray matter atrophy in limbic and frontoinsular regions (Cerami et al., 2014). Here we investigated whether this impairment reflects in anomalous resting-state activity in 12 bvFTD patients compared with 30 healthy controls. We used Independent Components Analysis (ICA) to extract maximally independent and temporally coherent spatial networks from resting-state fMRI scans lasting 8 minutes (Allen et al., 2011). We used different outcome measures to characterize resting-state activity, as well as its relationship with scores related to affective empathy, cognitive empathy and the processing of causal inferences (baseline condition) in a non-verbal cartoon task. Namely, we assessed the power spectra of RSN timecourses (level of coherent activity within a network), the intensities of RSN spatial maps (connectivity and degree of coactivation within a network), as well as functional network connectivity (connectivity between networks). For all these measures, we assessed both group differences and an interaction between group and task performance. Patients displayed a shift from low to middle-high frequencies in spontaneous fluctuations of resting-state activity, as well as reduced inter-network connectivity, in the default-mode, fronto-temporal and attentional networks. They also performed worse than controls in both empathy tasks, and particularly in affective empathy. This pattern reflected in a significant "group by task" interaction, which was specific to the affective empathy condition. Only in the latter, in patients (compared with controls) lower performance was related to a stronger shift towards high-frequency activity in frontal and sensorimotor networks, as well as to reduced connectivity within the default-mode network. No such interaction emerged for cognitive empathy, nor for the processing of causal inferences. We thus confirmed an empathic impairment in bvFTD, involving particularly the ability to infer emotional states. This deficit is related to anomalous patterns of resting-state activity, likely reflecting altered functional connectivity within and between the brain networks supporting the empathic ability. Allen et al. (2011) A baseline for the multivariate comparison of resting-state networks. Front Syst Neurosci 5:2. Cerami et al. (2014) Neural correlates of empathic impairment in the behavioral variant of frontotemporal dementia. Alzheimers Dement.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: National Ataxia Foundation

Title: The cerebellar contribution to social cognition

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Abstracts: Background: Neuroimaging and brain lesion studies suggest that the cerebellum is incorporated into neural substrates for social cognition, including tests of emotion attribution (EA) and Theory of Mind Reasoning (TOMR) - the ability to interpret the motivations of others. Neuropsychiatric disorders resulting from cerebellar pathology include deficits in social cognition, and small studies in patients with cerebellar disorders have described single domain deficits of social cognition. It remains unclear which domains are affected in patients with cerebellar disease, and how these relate to other neuropsychiatric manifestations of impaired social interaction. Methods: We examined 55 patients with cerebellar pathology (n=25 isolated cerebellar pathology [IC], n= 30 complex cerebrocerebellar pathology [CC]) and 55 healthy controls. We evaluated the overall level of cognitive function as well as mood and behavioural changes. Using a battery of social cognition assessments, we performed tests of emotion attribution (EA) with the Eye Task (Baron-Cohen, 2001); TOM with TOM pictures (Janet C. Sherman, doctoral thesis); and social situation recognition with social situation stories. Results: Impairments were found on EA skills as well as TOM performance. Compared to controls, IC and CC patients committed more errors on the EA task ($p < .0000$; student's t- test) and provided fewer mental answers on TOM tasks ($p < .000$). Social situation recognition was not different between patients and controls. EA skills were similarly affected between IC patients and CC patients ($p = .45$). Higher scores on the eye task were positively correlated with higher numbers of mental interpretations of TOM pictures, whereas Eye Task scores were not correlated with language or visuospatial abilities. Discussion: IC patients were as impaired as CC subjects on tasks of emotion attribution; and EA deficits were positively related with deficits in TOM. These results provide support for the hypothesis that EA is necessary for TOM, and that cerebellum contributes to both emotion attribution and TOM.

Disclosures: F. Hoche: None. **J.D. Schmahmann:** None. **J.A. Harding:** None. **M. Vangel:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NICHD HD33113-17

Oak Tree Foundation

Title: Emotional Contagion as an index for positive emotional responses: Mapping the minds-eye with neural substrates in williams syndrome

Authors: *P. FILLET[1], R. NG[4,1], C. O'LOUGHLIN[5,1], M. DEWITT[1], Y. SEARCY[1], P. LAI[6,9,1], M. EARHART[7], T. BROWN[8], A. JÄRVINEN[2], J. KORENBERG[10,1], U. BELLUGI[3]; [2]Lab. for Cognitive Neurosci., [1]Salk Inst., La Jolla, CA; [3]Salk Inst., La Jollac, CA; [4]Developmental Psychopathology, Univ. of Minnesota, Twin Cities, MN; [5]Cognitive Sci., [6]Language and Communication Disorders, [8]Neurosciences, [7]Univ. of California San Diego, San Diego, CA; [9]San Diego State Univ., San Diego, CA; [10]Ctr. for Integrated Neurscience and Human Behavior, Univ. of Utah, Salt Lake City, UT

Abstracts: While empathy can be defined in many ways, there is consensus that at a simple level it refers to an ability to understand and respond to another person's emotional cues. Williams syndrome (WS) is a rare neurogenetic disorder caused by a hemizygous deletion on chromosome 7q11.23 (Korenberg et al., 2009). The unique social phenotype of individuals with WS includes an enigmatic and uneven profile of behaviors (Bellugi et al., 2000; Doyle et al., 2004, Järvinen et al., 2013). Toward this end, we employed the Emotional Contagion Questionnaire (ECQ) and the Salk Institute Sociability Questionnaire (SISQ) as part of a multi-layered ongoing study encompassing neural, behavioral and physiological assessments. The ECQ and SISQ best capture the positive emotional construct that is central to the reported prosocial and empathic sensitivities in WS. The SISQ is designed to tap into social functioning including empathic and approach behaviors while the ECQ is employed to assess the consistency of experiencing the five basic emotions (Doherty, 1997). The combination of behavioral measures with imaging tools, i.e., magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI), allows us to map the neural networks responsible for the positive and emotional biases. Our central aim in analyzing these questionnaires along with imaging methods to explore empathy in WS is to characterize a multifaceted behavioral model that links the underlying neural and genetic mechanisms responsible for some of the intriguing dissociations reported in WS individuals.

Current behavioral findings indicate that those with WS experience positive emotional states in higher intensity/frequency than typically developing individuals. Preliminary imaging results indicated that in WS adults, increased emotional contagion of sadness was associated with reduced cortical surface area of anterior cingulate cortex, whereas experience of love was associated with reduced fractional anisotropy of the cingulum cingulate. Currently, additional exploratory analyses are in progress for other regions of interest (i.e., anterior insular cortex and amygdala) implicated in social-affective functions. Recent studies have implicated the amygdala and insular cortex, and hormonal dysregulation, as specific regions of interest that define uniquely the neurobiological and genetic profile of WS individuals (Brown et al, 2012; Meyer-Lindenberg, et al., 2012; Korenberg et al., 2012). Therefore, our efforts to link these neural substrates to the prosocial tendencies will significantly contribute to the extant literature and further highlights WS are atypically hypervigilant to positive emotions.

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Poster

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Program#/Poster: 649.10/TT7

Topic: F.01. Human Cognition and Behavior

Support: Gift fund

Title: NIRS-based hyperscanning reveals sex differences in brain synchronization during cooperation and competition

Authors: ***N. LIU**, J. BAKER, X. CUI, P. VRTICKA, M. SAGGAR, A. REISS; Psychiatry, Stanford Univ., Stanford, CA

Abstracts: Very little is known about how the neural response patterns of multiple brains interact during real-time social interactions. Differences in the social roles played by males and females throughout evolution have led to assumptions that sexual dimorphisms during cooperation and competition are observable today[1]. However, the degree to which men and women differ during real-time neural processing of cooperative and competitive social interactions remains unknown. Here, functional near-infrared spectroscopy (fNIRS)

hyperscanning is used to measure the neural responses of mixed- and same-sex pairs as they engage in cooperative and competitive interactions. Unacquainted participants (n = 118, aged 18-35 y) were randomly assigned into male/male (MM), male/female (MF), or female/female (FF) pairs. Each pair underwent fNIRS hyperscanning while performing a task with their partner. The task[2] was divided into three parts, and required participants to either cooperate, compete, or respond independently, in a counterbalanced order, to earn points. Two primary regions of interest (ROI), the right prefrontal cortex (rPFC) and the right middle temporal cortex (rMTC), were identified based on their roles in social cognition related to cooperation and competition. A single fNIRS recording device, ETG-4000 optical topography system (Hitachi Medical, Japan), was used to measure the hemodynamic response of both participants simultaneously. Wavelet transform coherence (WTC) was used to assess the relationship between the fNIRS signals generated by participant pairs. Coherence increase (*CI*) for each part of the task was calculated by subtracting the average coherence of a rest period from that of two task blocks ($CI = C_{task} - C_{rest}$). The *CI* value for each ROI and pair was then submitted for statistical evaluation. Our results suggest that sex significantly mediates patterns of inter-brain coherence. Inter-brain coherence in the rPFC increased during cooperation for all MM pairs (p = 0.032). Furthermore, MM pairs demonstrated significantly greater inter-brain coherence in the rPFC during cooperation than FF pairs (p = 0.041). Inter-brain coherence in the rMTC was significantly higher for all pairs during competition than during the independent control condition (p = 0.014). Taken together, our results provide the first evidence that cooperative and competitive social interactions elicit different patterns of inter-brain coherence, and that the sex composition of an interacting pair significantly influences inter-brain coherence. References **1. M. Van Vugt, et al., *Psych. Sci.* 18, 19-23 (2007). 2. X. Cui, et. al., NeuroImage, 59(3), 2430-7 (2012).**

Disclosures: N. Liu: None. **J. Baker:** None. **X. Cui:** None. **P. Vrticka:** None. **M. Saggar:** None. **A. Reiss:** None.

Poster

649. Human Social Cognition II

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Topic: F.01. Human Cognition and Behavior

Support: R01-HD059852

Title: Stimulation of the periaqueductal gray alters social perception

Authors: *K. HAROUSH[1], A. SHARMA[2], Z. WILLIAMS[2];
[1]Neurosurg., [2]Harvard Med. Sch., Boston, MA

Abstracts: Perceiving another individual's emotional state is essential for social interaction. However, it remains unknown which brain areas directly mediate such judgment processes. The periaqueductal grey (PAG) is positioned in an intersection between ascending sensory pathways and inputs from higher areas modulating these signals. While functional Magnetic Resonance Imaging studies have suggested that certain brain areas may play a role in social perception, it is unclear which areas causally modulate this cognitive function. Here, pain patients who were implanted with Deep Brain Stimulation (DBS) electrodes in the ventrolateral PAG performed an emotional perception task in front of a computer screen. Morphed images composed of two distinct emotional expressions obtained from a single individual across three emotional axes crossing happy, sad and angry, were displayed at various ambiguity levels while a sub threshold electrical current was delivered via the participants' PAG implant on half of the trials. We find that PAG stimulation lead to a consistent bias in the social perception of another's emotional expression across all axes. Under conditions of maximal ambiguity, the other's expression was perceived as being sad rather than happy or angry and angry rather than happy. Control trials testing for the participants perceived pain levels were obtained every ten trials, ensuring that the participants comfort level was not compromised throughout the experiment and demonstrated no association with stimulation trials. These findings suggest that that the PAG is responsible for modulating sensitivity to certain emotions in another individual, and may thus play a central role in mediating social interchange.

Disclosures: K. Haroush: None. **A. Sharma:** None. **Z. Williams:** None.

Poster

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Program#/Poster: 649.12/TT9

Topic: F.01. Human Cognition and Behavior

Support: NIH RO1 MH091113

Title: The embedding of social status in person knowledge: An MVPA study

Authors: *J. KOSKI, J. A. COLLINS, I. R. OLSON;
Psychology, Temple Univ., Philadelphia, PA

Abstracts: Both humans and non-human primates rapidly assign status information to others based on a host of variables such as size, lineage, financial status, and reputation. We find the social status of others so salient that it shapes our attention and gaze patterns towards others, as well as our preferences and memories of others. Given the prominence of this variable in person processing, it seems possible that our neural representation of specific individuals contains status information. Here we asked whether the representations of specific individuals in the ventral face circuit (OFA, FFA, vATL, and amydgala) represent status information. Participants were trained to associated names and status information (high versus low ratings) with 8 different objects and 8 different faces over a two-day training regimen. These faces and objects were then presented to the subjects within a block-design fMRI experiment with a target detection task that was orthogonal to the variable of interest. In addition, we functionally localized regions in the extended face circuit, as well as object sensitive regions (LOC) using a localizer task. Data were analyzed using multivoxel searchlight analysis in FSL. The searchlight analysis revealed a network of status-sensitive clusters for faces that was more robustly represented in the right hemisphere than in the left. Regions sensitive to person status included the right vATL, right ventromedial prefrontal cortex, right fusiform gyrus, and bilateral superior temporal sulcus. In contrast, the status of objects was localized to a cluster in the left ventral anterior temporal lobe. Placing this work in the context of the relevant literature, prior work indicates that the anterior temporal lobes play an important role in storing and retrieving semantic information and that a region in the medial aspects of the ventral ATL - perirhinal cortex - contains a patch of neurons particularly sensitive to face identity as well as person-related semantic information such as biographical details. There is a predictable lateralization of this region with the left vATL more sensitive to verbal information such as names, while the right is more sensitive to nonverbal information such as faces. The present findings on the vATL are consistent with this literature. In addition, they suggest that status information becomes embedded into person identity representations stored in this region. We additionally found status-related activations across many regions in the social brain network, possibly because high status objects and people are attentionally salient.

Disclosures: J. Koski: None. **J.A. Collins:** None. **I.R. Olson:** None.

Poster

649. Human Social Cognition II

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Topic: F.01. Human Cognition and Behavior

Support: Gordon and Betty Moore Foundation

National Science Foundation

Title: A neuro-computational account of behavioral and neural patterns in altruistic choice

Authors: *C. HUTCHERSON[1], B. BUSHONG[2], A. RANGEL[1];
[1]Humanities and Social Sci., Caltech, PASADENA, CA; [2]United States Dept. of Def., Monterey, CA

Abstracts: An important open question in neuroeconomics and social neuroscience is characterizing the neuro-computational basis of altruistic behavior. We investigated this question in the context of a modified fMRI Dictator Game involving different tradeoffs between the participant's own payoffs and the payoffs of an anonymous partner. We fitted participants' choices to a computational model of social preferences, inspired by drift diffusion models of simple choice, that has five key parameters: the weight given to one's own payoffs, the weight given to the payoffs of others, the height of a choice-determining threshold, the rate at which this threshold decreases over time (to account for time pressure), and a non-decision time. Choices in the model result from the noisy accumulation of a relative value signal derived from the weighted sum of payoffs for self and others, and are made when the accumulated signal becomes strong enough to pass the criterion threshold for making a choice. Behaviorally, we observed a tight correspondence between predictions of the model and observed responses and reaction times: generous choices on average take longer to make, particularly for comparatively selfish individuals. Neurally, we observed that two key model variables (representations of one's own and other's payoffs) correlated with BOLD responses in distinct regions: with one's own payoffs coded in amygdala and ventral striatum, and the other's payoffs coded in TPJ. Rostral anterior cingulate cortex (rACC) was sensitive to both one's own and others' payoffs, and functional connectivity analyses are consistent with the hypothesis that rACC integrates inputs from the amygdala and TPJ to compute a 'net value' for the proposal being evaluated. The model also suggested that dorsal ACC and inferior frontal gyrus (IFG) may be recruited to resolve conflict during generous choices, particularly for less generous individuals. The model suggests a need for caution in interpreting recent work using reaction-time and neural data to support self-control models of generosity, and provides a flexible framework within which to understand not only non-instrumental altruism, but also social decision-making more generally.

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Poster

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Program#/Poster: 649.14/TT11

Topic: F.01. Human Cognition and Behavior

Title: Neural systems tracking popularity in real-world social networks

Authors: *N. ZERUBAVEL[1], P. BEARMAN[2], K. OCHSNER[1];
[1]Dept. of Psychology, [2]Columbia Univ., New York, NY

Abstracts: Successfully navigating our complex social world requires understanding the relative status of members of our groups. Sociologists and social psychologists have historically emphasized two kinds of status that have important implications for behavior: power-based status, where individuals vary in their control over resources and outcomes, and affiliation-based status, where individuals vary in the extent to which they are liked by other group members. To date, the majority of neuroscience research has focused on power-based hierarchies rather than affiliation-based popularity. Here we present the first imaging research to examine the neural systems tracking the popularity of members of real-world social networks. To do this we first used social network analysis (SNA) to determine the relative popularity of individuals in in the context of a friendship-based network to which they belonged. We then had members of each network view photographs of other group members and asked, on a trial-by-trial basis, how brain activity parametrically scaled with the popularity the target group member viewed on that trial. We found that activity in two kinds of brain regions tracked target popularity: systems involved in affective valuation (e.g. vmPFC, amygdala, ventral striatum) and social cognition (e.g. dmPFC, TPJ). Importantly, activity in the affective valuation systems mediated the relationship between target popularity and activity in social cognition regions, suggesting that a history of learning about the affective outcomes associated with popular individuals organizes our responses to them. These data have implications for models of affect, person perception and group behavior.

Disclosures: **N. Zerubavel:** None. **P. Bearman:** None. **K. Ochsner:** None.

Poster

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Support: NWO-VICI Grant 453-08- 002

Title: Altered communicative adjustments following ventromedial prefrontal lesions

Authors: *I. TONI[1], G. DI PELLEGRINO[2], D. D'IMPERIO[2], A. STOLK[1];
[1]Radboud Univ. Nijmegen, Donders Inst., Nijmegen, Netherlands; [2]Univ. of Bologna, Bologna, Italy

Abstracts: The ventromedial prefrontal cortex (vmPFC) has been consistently implicated in supporting behaviors guided by a mental model of other agents. For instance, moral judgements made by patients with vmPFC lesions are more strongly influenced by the outcome of a harmful action than by its underlying intention, i.e. whether the harm was attempted or accidental. Yet, during linguistic interactions, patients with vmPFC lesions are able to consider the knowledge of an interlocutor, for instance by using shorter utterances and definite references following repeated verbal exchanges. Here we test whether the inability of vmPFC patients to use a mental model of an interlocutor becomes evident once communicative interactions are not confounded with linguistic phenomena. Communicative abilities of patients with vmPFC lesions (N=8) were quantified in a controlled experimental setting involving the production of referential non-verbal behaviors with a communicative goal. Participants were asked to inform an addressee about the location of a token in a grid (visible only to the communicator) by means of their movements on the grid (visible to both communicator and addressee). Participants spontaneously marked the location of the token in the grid by waiting longer on that location as compared to other locations visited during their movements on the grid (TimeOnTarget effect). Crucially, participants were told this communicative game involved online interactions with a child and with another adult, in alternation. In fact, an adult confederate performed the role of both addressees, while remaining blind to which one of the two roles she was performing in any given trial. These tasks features allowed us to directly tap into patients' ability to spontaneously generate communicative adjustments to their mental model of an addressee, rather than retrieving knowledge from pre-established conventions (e.g. a common language). The specificity of the vmPFC-lesion effects was assessed by comparing communicative behaviors of vmPFC patients with those evoked in patients with brain lesions outside the vmPFC (N=8, lesion-controls) and in age-matched healthy participants (N=15, healthy-controls). Patients with vmPFC lesions communicated as effectively as lesion- and healthy-controls. Crucially, both lesion- and healthy-controls showed a larger TimeOnTarget effect when they thought they were communicating with a child, whereas vmPFC patients did not communicatively differentiate between the two addressees. These findings suggest that vmPFC is necessary for using social knowledge to bias current communicative decisions to the presumed characteristics of an addressee.

Disclosures: I. Toni: None. **G. di Pellegrino:** None. **D. D'Imperio:** None. **A. Stolk:** None.

Poster

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Program#/Poster: 649.16/TT13

Topic: F.01. Human Cognition and Behavior

Title: Self-other differences in EEG μ-suppression reflect familiarity and perspective-taking.

Authors: *C. C. WOODRUFF, A. GOODMAN, B. VELEZ, A. FORTIN, M. FECHTEL; Psychology, Northern Arizona Univ., Flagstaff, AZ

Abstracts: Previous research demonstrated a relationship between empathy and perspective-taking (PT) and suppression of electroencephalographic μ-rhythms, a putative measure of mirror neuron processing. This research suggested that PT is positively related to the size of the difference between self- and other-induced μ-suppression, but these findings were confounded because the task used involved motion by the participant during the self, but not the other condition. The current study addressed this confound by utilizing a self-condition that did not involve movement by participants. It further tested the size of self-other differences as a function of familiarity of the observed individual. Participants completed an empathic accuracy (EA) paradigm adapted from Zaki et al. (2009). We recorded μ-suppression while participants watched three different videos of facial emotional responses; 1) Self (S) - participant watches video of him/herself, 2) Familiar Other (FO) - watches video of best friend, 3) Stranger Other (SO) - watches video of a stranger. We predicted that self-other differences in μ-suppression would be smaller for S-FO than S-SO and that these differences would be related to EA and to self-report measures of empathy/PT. Results confirmed the prediction that S-FO differences were smaller than S-SO differences, and that the size of difference scores was positively related to PT. We failed however, to find a relationship between empathic accuracy and either simple μ-suppression or S-O μ-suppression differences scores and empathic accuracy. This failure may be due to a ceiling effect resulting from all participants having high accuracy. The results will be discussed in the context of theories of mirror neuron processing and self-other discrimination.

Disclosures: C.C. Woodruff: None. **A. Goodman:** None. **B. Velez:** None. **A. Fortin:** None. **M. Fechtel:** None.

Poster

649. Human Social Cognition II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 649.17/TT14

Topic: E.03. Behavioral Neuroendocrinology

Support: NHLBI-HR-90-13

NO1-HR-16044

NO1-HR-16045

NO1-HR-16046

NO1-HR-16047

NO1-HR-16048

NO1-HR-16049

Title: Consistent familial warmth may predict child cortisol levels

Authors: *S. M. DINCES[1], L. N. ROWELL[1], J. F. L. PINNER[1], S. N. HILE[1], M. EMERY THOMPSON[2], A. C. TANG[1,3], R. D. ANNETT[4];
[1]Dept. of Psychology, [2]Anthrop., [3]Neurosciences, Univ. of New Mexico, Albuquerque, NM; [4]Pediatric Neurol., Children's Hosp. of Colorado, Aurora, CO

Abstracts: Research from both animal models and humans has indicated that the quantity of nurturing (maternal care) offspring receive influences their Hypothalamic-Pituitary-Adrenal (HPA) development and function. Using the rodent model, reliability of maternal nurturing has also been shown to be influential in offspring HPA function and behavioral outcomes. Here, we attempt to translate these rodent findings to human children, and examine the relative contributions of both of these components of nurturing to child HPA functioning. The present study included 55 children with mild/moderate asthma and their caregivers from the NHLBI-funded randomized clinical trial (Childhood Asthma Management Program) who provided data for the analyses. Caregivers completed the Family Environment Scale (FES) at enrollment and annually. Familial warmth was calculated from two subscales of the FES ($Cohesion_{score}$ minus $Conflict_{score}$). Children's baseline and evoked cortisol levels were assessed at year three and utilized in the analyses. ANCOVAs revealed that variability in familial warmth across time, but not the amount of warmth across time, predicted child basal cortisol levels at year 3

(F(1,49)=5.758, p=.021; F(1,49)= .733, p=.396 respectively). As familial variability in warmth increases, child baseline cortisol also increases. Over the five years studied, consistency in family warmth, but not the amount of warmth, predicted child baseline cortisol levels, suggesting that predictability in family behaviors influence the development of child HPA function. These findings confirm previous empirical work investigating maternal care reliability in rodents and emphasize the need to look beyond a single time point or single measurement in order to obtain an understanding of contributions of the family environment to child biological regulation.

Disclosures: **S.M. Dinces:** None. **L.N. Rowell:** None. **J.F.L. Pinner:** None. **S.N. Hile:** None. **M. Emery Thompson:** None. **A.C. Tang:** None. **R.D. Annett:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

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Program#/Poster: 650.01/TT15

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP

CNPq

Title: Evidence of memory generalization in contextual locomotor sensitization induced by amphetamine: A key to addiction?

Authors: ***D. S. ENGELKE**[1], R. FILEV[2], J. G. SANTOS-JUNIOR[3], L. E. A. M. MELLO[2]; [1]NEUROPHYSIOLOGY, [2]UNIFESP, SAO PAULO, Brazil; [3]FCMSCSP, SAO PAULO, Brazil

Abstracts: Animal models of addiction are important to understand its neural basis, which comprises several neuroadaptations. Locomotor sensitization is used to investigate incentive salience and transition from use to abuse. There are studies reporting context influence in locomotor sensitization acquisition and expression. However, is not explored the maintenance and precision of contextual sensitization. Here, we propose a new insight about the transition from use and abuse to addiction. We ask whether contextual memory precision, maintenance and generalization are related with this transition? To test this, male C57bl/6 mice were conducted to contextual locomotor sensitization paradigm. Baseline activity of adult male C57bl/6 mice were accessed in contextual activity box. Animals were treated during five consecutive days: AMPH+CTX (N=40) - animals injected with amphetamine (1mg/kg, i.p) and submitted to

contextual activity box; AMPH+HC (N=40) - animals treated equally, but after acquisition they back to home cage; VEH (N=40) - animals treated daily with saline and submitted to contextual activity box every day. After three (3), Fourteen (14) or twenty eight (28) withdrawal days, all animals were challenged with amphetamine (1mg/Kg) in the same context (context A) or in an alternative context (context B). Locomotor sensitization was evaluated by 40 min. In context A, AMPH+CTX increased locomotion when compared to other experimental groups after 3rd, 14th and 28th days. In context B, after 3 days, all animals traveled the same distance. However, after 14 and 28 days, AMPH+CTX expressed an increased locomotion also in context B. We propose that it might be explained by memory generalization which animals are not able to discriminate between the original and novel contexts and drug effect expressing the locomotor sensitization in all contexts as long as the abstinence time. Thus we suggest that memory generalization could be a key to explain why there is a transition from use and abuse to the development of addiction.

Disclosures: D.S. Engelke: None. **R. Filev:** None. **J.G. Santos-Junior:** None. **L.E.A.M. Mello:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.02/TT16

Topic: F.02. Animal Cognition and Behavior

Support: DA014339 to RMC

T32 DA007244

Title: Voluntary wheel running reverses an established cocaine-induced negative affective state in a rodent model

Authors: *J. L. GREEN, L. A. DYKSTRA, R. M. CARELLI;
Psychology, Univ. of North Carolina, Chapel Hill, NC

Abstracts: As reported previously, when a palatable saccharin solution predicts impending, but delayed, cocaine availability the saccharin solution becomes devalued, as evidenced by the emergence of aversive taste reactivity during intraoral tastant infusion. Importantly, this negative affective state predicts the motivation to self-administer cocaine (Wheeler et al., Neuron, 57: 774, 2008). Exercise is a form of environmental enrichment that has been shown to reduce

cocaine-seeking in animal models. Given this well-established protective effect, we hypothesized that exercise may reverse or attenuate the negative affective state that develops following repeated taste-drug pairings. Male, Sprague-Dawley rats (n= 8) were singly housed and received 14 daily taste-drug pairings using this established behavioral design. Briefly, the task was conducted in two phases each day: 1) intra-oral saccharin infusions (0.15%; 0.2ml delivered over 3.5 sec/trial for 45 trials) and 2) cocaine self-administration (0.33 mg/inf; 2 hrs/session). Consistent with prior studies, rats initially showed appetitive taste reactivity and minimal aversive responses during the first phase on day 1 of training. However, following repeated taste-drug pairings (day 14), rats exhibited a decrease in appetitive taste reactivity and an increase in aversive taste reactivity in phase 1, indicative of a shift to a negative affective state. Next, rats were randomly assigned to either the exercise (EX, n=5) or sedentary (SED, n=3) groups. EX rats were given access to a running wheel in their home cages for 24 hrs/d for 7 wks while SED rats were placed in their home cages without wheel access. Preliminary findings show that access to the running wheel had a protective effect on the subsequent expression of negative affect during saccharin intraoral infusion. That is, EX rats exhibited an increase in appetitive taste reactivity and a corresponding decrease in aversive responses following the 7 week abstinence period. In contrast, rats in the SED group continued to show strong aversive taste reactivity during intraoral saccharin infusion, at similar levels to that observed prior to abstinence (day 14). Ongoing studies will increase the numbers of EX and SED rats. These preliminary studies suggest that voluntary wheel running reverses an established cocaine-induced negative affective state observed following repeated taste-drug pairings.

Disclosures: J.L. Green: None. **L.A. Dykstra:** None. **R.M. Carelli:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: DA029978

DA017318

Title: Prolonged abstinence from cocaine self-administration potentiates rapid dopamine signaling in the nucleus accumbens core

Authors: *C. M. CAMERON, R. M. CARELLI;
Univ. North Carolina, Chapel Hill, NC

Abstracts: Drug-seeking behavior progressively increases with longer periods of abstinence from cocaine self-administration, a phenomenon termed 'incubation of craving' (Tran-Nguyen et al., 1998; Grimm et al., 2001). We previously demonstrated a neurophysiological correlate of this incubated state, showing a dramatic (2-fold) increase in nucleus accumbens (NAc) core neural activity that encodes cocaine-associated cues and cocaine-seeking following a month of abstinence (Hollander & Carelli, 2005, 2007). Importantly, the NAc receives a dense dopaminergic input from the ventral tegmental area (VTA); however, less is known about changes in NAc DA release dynamics under cocaine abstinence conditions. Previous work using fast-scan cyclic voltammetry (FSCV) in anaesthetized rats has shown that even a short-term withdrawal paradigm (7 consecutive days of experimenter-delivered cocaine injections followed by 1 day of withdrawal) can cause a potentiation of DA signaling in the NAc in response to a subsequent cocaine challenge (Addy et al., 2010). However, it is possible that larger changes in DA signaling may be observed following a longer period of cocaine abstinence (one month) or as a result of self-administered versus experimenter-administered drug. To address this issue, we examined DA release and uptake dynamics in the NAc core following one month abstinence from cocaine self-administration. Rats underwent 14 days of cocaine self-administration training (0.33 mg/inf; FR1) followed by 1 day (D1; n=7) or 30 days (D30; n=7) of cocaine abstinence (placed in home cage with no drug access). After abstinence, all rats underwent a single extinction session (lever pressing had no programmed consequences). Rats were then deeply anesthetized and FSCV was used to measure DA release and uptake dynamics in the NAc core before and following a single cocaine injection. We found that a month of cocaine abstinence potentiated the peak concentration of electrically-evoked DA in the NAc following an acute injection of cocaine. Examination of release and uptake parameters revealed that the potentiated [DA]max observed in D30 animals was due mainly to a facilitation of release rather than a potentiation of uptake inhibition. Finally, in D30 (but not D1 animals) we observed a correlation between electrically-evoked DA release and behavioral responding during extinction such that a greater potentiation in DA was correlated with less extinction responding. Collectively, these findings reveal dynamic changes in NAc DA signaling following repeated cocaine self-administration and prolonged cocaine abstinence linked to alterations in drug seeking behavior.

Disclosures: C.M. Cameron: None. **R.M. Carelli:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA035322 to MPS

NIH Grant DA034021 to RMC

Title: Cocaine self-administration experience biases rats towards sign-tracking behavior in a subsequent Pavlovian task

Authors: *M. SADDORIS[1], X. WANG[1], D. R. TERRY[1], J. D. REID[1], R. M. CARELLI[1,2]; [1]Psychology, [2]Neurosci. Ctr., Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstracts: Cues associated with valued outcomes can come to elicit approach behaviors. For example, a Pavlovian cue predictive of a rewarding outcome (e.g., food) may elicit approaches to the location where the food will be delivered (Goal Tracking; GT), or may instead elicit autoshaped approaches to the cue itself (Sign Tracking; ST). Recent discoveries have demonstrated correlations between dopamine (DA) signaling and the form of the conditioned response. Specifically, enhanced DA release in the nucleus accumbens (NAc) appears to relate to greater ST compared to GT (Flagel et al., 2011; Saunders & Robinson, 2012), while drugs of abuse such as cocaine can elicit strong ST for drug-associated stimuli (Uslaner et al., 2006; Saunders & Robinson, 2010). However, it has recently been reported that rapid DA release in the NAc is strongly attenuated following repeated exposure to cocaine, and it is unknown whether this cocaine-induced dampening of the DA signal changes how rats subsequently respond to Pavlovian cues predictive of food reward. Here, thirsty rats self-administered either cocaine i.v. (2 wk, 2h/d, 0.33mg/inf) or water delivered to a foodcup (Controls) following lever presses. Following 30d abstinence, rats were food-restricted, then trained in a distinct context for 10d on a Pavlovian discrimination, where a CS+ cue light (10s) predicted food (3 sucrose pellets) and a CS- cue light (10s) was not reinforced (14 each cue type/d). ST (cue light approaches) and GT (foodcup approaches) for each session were recorded and scored by two experimentally-blind observers during the 10s prior to cue onset (baseline) and the 10s of each cue presentation. When the task was well-learned (day 10), rats in the control group showed a strong bias towards GT during cue presentations, while in contrast, cocaine rats showed the opposite bias towards ST during the cue ($\chi 2$: $p<0.05$). However, total associative behavior during the cue did not differ between groups. Further, the same cocaine-experienced rats displaying ST behavior during Pavlovian conditioning later displayed enhanced reinstated lever pressing (under extinction) compared to GT rats when returned to the original self-administration context. Voltammetric recordings of DA release in the NAc of cocaine-treated subjects taken during the task showed attenuated signaling during the cue compared to controls. However, within the control population, ST rats showed greater DA release than GT, consistent with previous results. These

findings demonstrate that shifts to ST can occur in the absence of enhanced DA release, and may be potentiated by chronic exposure to drugs of abuse like cocaine.

Disclosures: **M. Saddoris:** None. **X. Wang:** None. **D.R. Terry:** None. **J.D. Reid:** None. **R.M. Carelli:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIDA NIH Grant DA035196

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NIDA NIH Grant DA014339

Title: Neural encoding in the nucleus accumbens core during learning predicts subsequent test accuracy in a sensory preconditioning task

Authors: *D. H. CERRI[1], M. P. SADDORIS[1], R. M. CARELLI[1,2];
[1]Psychology, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]Neurosci. Ctr., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstracts: Environmental stimuli that predict natural reinforcers, such as food, can acquire salience through learned associations. Studies have shown that the nucleus accumbens (NAc) encodes important features of these associations, as evidenced by phasic changes in cell firing to the presentation of reinforcers and associated stimuli. However, it is not currently known whether this encoding is important for all associations, such as predictive relationships between neutral stimuli, or if instead the NAc only encodes motivationally salient associations between cues and valued outcomes. Here, electrophysiological recordings were taken from neurons in the NAc core during a sensory preconditioning task where rats were pre-exposed to neutral stimuli such as lights and tones. For rats in the Paired group (n=21), one of four stimuli (*A*; either tone, white-noise, flashing light, or solid light) was repeatedly followed by one of the other stimuli (*X*), while the remaining two stimuli (*B*; *Y*) were paired in the same manner. Alternatively, rats in the Unpaired control group (n=8) experienced random presentations of the same stimuli. Subsequently, in a first-order conditioning phase, all rats were trained that *X* predicted food, and

Y did not. Finally, during a test session, *A* and *B* were presented alone, and food-cup entries during stimulus presentation were recorded. Intriguingly, at test only a subset of Paired rats (n=9) showed evidence of successful preconditioning, as indicated by a selective increase in food-cup entry behavior to *A,* but not *B*. The remaining Paired rats (n=12) failed to behaviorally discriminate between *A* and *B* and did not differ in performance from Unpaired rats. Neural data from the NAc core was in accordance with these findings. “Good Learners” showed enhanced excitatory cell firing to each of the four stimuli presented during preconditioning relative to "Poor Learners" and Unpaired rats. Further, Good Learners, but not Poor Learners or Unpaired rats, exhibited increased excitatory cell firing to *A* relative to *B* during the test session. Unlike excitatory activity, differential inhibitory firing to stimuli was not observed at preconditioning or test. These data suggest that neural encoding in the NAc core may be important for the initial acquisition of associative relationships, even in the absence of explicit reward, and that these associative relationships can provide a critical foundation for the expression of later higher-order learning and behavior.

Disclosures: D.H. Cerri: None. **M.P. Saddoris:** None. **R.M. Carelli:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.06/TT20

Topic: F.02. Animal Cognition and Behavior

Support: DA014339 to RMC

Title: Dynamic shifts in nucleus accumbens neural encoding of reward-associated cues following reinforcer devaluation

Authors: *E. A. WEST[1], E. L. THOMAS[1], R. M. CARELLI[1,2];
[1]Psychology, [2]Neurosci. Ctr., Univ. of North Carolina, Chapel Hill, NC

Abstracts: Nucleus accumbens (NAc) neurons encode important features of stimulus learning and action selection associated with rewards. Additionally, the NAc is necessary for using information about expected outcome values to guide behavior as measured by reinforcer devaluation tasks (Singh et al., 2010). Based on its anatomical connections and role as a “limbic-motor” interface, it is likely that the NAc acts as a point of convergence between value encoding in limbic and prefrontal brain regions to modify behavior following changes in reward value.

Here, we investigated how NAc neurons encode reward-associated cues when the outcome value of the reward has been devalued. Male Long-Evans rats (n=11) were trained to press a lever following an illuminated cue light that predicted a specific reinforcer (e.g., raspberry flavored pellet). Rats received an alternative reinforcer in their home cages following training (e.g., peanut butter food pellets). Once rats achieved 90% accuracy during training (receiving a reward on 45 out of 50 trials), they were probed in a devaluation test under extinction conditions. Specifically, each rat was allowed ad libitum access to one of the two foods (selective satiation). Subsequently, we assessed NAc neural activity to the reward-associated cue (prior to lever extension) following either the devaluation of the same reinforcer received during training (devalued) or the different alternative reinforcer (nondevalued). On a separate day, the other food was devalued (counterbalanced). Behaviorally, rats responded significantly less when the same reinforcer received during training was devalued (52.5 +/- 11.5) compared to devaluation of the alternative reinforcer (nondevalued, 79.5 +/- 13.2; $p<.05$) showing successful outcome specific devaluation. Previous findings in other value based tasks suggest that NAc neurons encode the expected value of future rewards and promote action selection. Preliminary electrophysiological evidence from this study expands on these findings. Specifically, we observed a significant decrease in the percentage of NAc neurons that showed phasic responsiveness (i.e., cells that either increased or decreased firing) to the reward-associated cue when the same reinforcer received during training was devalued (7 out of 68, 10%) compared to the devaluation of the alternative different reinforcer (nondevalued, 21 out of 81, 26%). These data suggest that NAc neurons dynamically encode information regarding the value of the specific reward, and apply that new value to the reward-associated cue to guide behavior.

Disclosures: **E.A. West:** None. **R.M. Carelli:** None. **E.L. Thomas:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: DA014339 to RMC

T32 NS007431 to MAP

Title: Effects of prolonged abstinence on cocaine-induced negative affect and the encoding of this information by nucleus accumbens neurons

Authors: *M. A. PRESKER, JR, E. A. WEST, R. M. CARELLI;
Psychology, Univ. of North Carolina, Chapel Hill, NC

Abstracts: We previously reported that when a palatable saccharin solution predicts impending, but delayed, cocaine availability the saccharin solution becomes devalued as evidenced by the emergence of aversive taste reactivity and increased motivation to self-administer cocaine (Wheeler et al., Neuron, 57: 774, 2008). This shift in the affective state of the rat is dynamically encoded by a subset of nucleus accumbens (NAc) neurons: cells shift from exhibiting primarily inhibitory cell firing to mostly excitatory activity during infusion of the devalued tastant. It is not known, however, if the neural encoding of this negative affective state is altered by prolonged cocaine abstinence. Notably, 30 days of abstinence from cocaine leads to increased effort to obtain the drug, as well as increases in the percentage of NAc cells that encode cocaine-predictive cues and cocaine-seeking behavior (Hollander and Carelli 2007). Here, we extend previous findings to determine the effects of 30 days of cocaine abstinence on NAc cell firing and taste reactivity measures following 14 days of saccharin-cocaine pairings. Before abstinence, daily sessions were completed in two phases. In phase 1, saccharin (0.15%; 0.2 ml delivered over 3.5 sec/trial for 45 trials) was intra-orally infused. This was immediately followed by cocaine self-administration (0.33mg/kg/inf) for 2 hours (phase 2). Orofacial movements were measured in phase 1 while electrophysiological recordings were conducted in both phases. Behavioral data (n=5) indicate that aversive taste reactivity to the devalued saccharin tastant increased following 14 sessions prior to abstinence, as previously reported. Next, animals were placed in abstinence (homecage, no access to drug) for 30 days. Following abstinence, rats were tested in a single test session conduced in three phases, 1) intraoral saccharin delivery 2) extinction and 3) cocaine self-administration, and NAc cell firing and behavioral responses were measured. Preliminary findings indicate an increase in the number of aversive taste reactivity responses (gapes) to saccharin following abstinence, compared to that observed on day 14. Importantly, this was accompanied by an increase in NAc excitatory firing during saccharin delivery (n= 7/7 phasic cells, 100%) compared to immediately prior to abstinence (day 14; n=9/13 phasic cells, 66%). Although preliminary, these data suggest that cocaine abstinence has a profound ‘incubating’ effect on the negative affective state that develops following repeated taste-drug pairings and the encoding of this information by NAc neurons.

Disclosures: **M.A. Presker:** None. **E.A. West:** None. **R.M. Carelli:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: DA034021 to RMC

T32 DA007244 to DS

Title: Rapid dopamine signaling in the nucleus accumbens during a magnitude-based decision making task

Authors: *D. SACKETT, M. P. SADDORIS, X. WANG, R. M. CARELLI;
Psychology Dept., UNC Chapel Hill, Chapel Hill, NC

Abstracts: To maximize resources, organisms must choose actions that result in the most valuable outcome available and maintain that information to guide future behaviors. Integral to this decision making process is a neural circuit that includes the nucleus accumbens (NAc) and its dopaminergic input. In the rat, the NAc is divided into two discrete subregions, the core and the shell, believed to process information about reward learning, reward value, and decision making related to goal-directed actions. However, the precise role of those subregions in processing information about magnitude-based decisions remains unclear. Here, dopamine (DA) release in the NAc was monitored using fast-scan cyclic voltammetry during a magnitude-based decision making task. Male Sprague-Dawley rats (n=4) were trained to lever press following distinct visual cues that predicted the magnitude of future rewards. On Forced Choice Low Magnitude trials, a cue light predicted the opportunity to press a lever for a small reward (one 45mg sucrose pellet). On Forced Choice High Magnitude trials, another distinct cue light predicted the opportunity to press a different lever for a large reward (two 45 mg sucrose pellets). Finally, on Free Choice trials, both cue lights and levers were presented and rats were able to choose between both magnitude options. All rats accurately discriminated between cue types on Forced Choice trials and developed preferences for the high magnitude option on Free Choice trials. Preliminary electrochemical recordings from electrodes aimed at the NAc shell show increases in rapid DA release following presentation of Forced Choice cues, though peak DA concentrations did not differ for cues predictive of different magnitude rewards. However, preliminary results on the Free Choice trials indicate that peak DA during the choice cue was significantly greater when the animal subsequently chose the large magnitude option versus when it chose the small magnitude option. Despite this, there was no difference in peak DA during reward consumption of either magnitude. The current findings support previous reports that implicate the NAc shell in encoding comparative reward value during reward magnitude-based decision making. Ongoing studies are being completed to replicate these findings, and to examine DA release dynamics in the NAc core during the same task.

Disclosures: **D. Sackett:** None. **M.P. Saddoris:** None. **X. Wang:** None. **R.M. Carelli:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R00 DA024719

NIH Grant T32 DK007320-35

Title: Selective enhancement of performance in contingent reward assays, but not non-contingent assays, by DREADD activation of mPFC pyramidal neurons

Authors: *D. M. WARTHEN, P. S. LAMBETH, B. A. NEWMYER, R. P. GAYKEMA, M. M. SCOTT;
Pharmacol., Univ. of Virginia, Charlottesville, VA

Abstracts: The medial prefrontal cortex (mPFC) is implicated in a wide range of cognitive functions, including reward evaluation, decision making, memory extinction, mood, and task switching. The mPFC exerts its executive control function via projections composed exclusively of excitatory pyramidal neurons that innervate targets throughout the brain. We sought to determine the behavioral effects of varying excitatory outflow from the mPFC of mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADD) and a panel of behavioral assays. To drive mPFC excitatory output, we expressed Gq-coupled DREADD receptors in mPFC pyramidal neurons. We found that increasing excitatory output from the mPFC via DREADD enhances performance in high cognitive demand, instrumental food reward assays, while having no impact on performance in non-contingent, low cognitive demand assays. Specifically, activation of mPFC pyramidal neurons enhances operant responding for food reward, reinstatement of palatable food seeking, and suppression of impulsive responding for food reward. Activation of mPFC pyramidal neurons has no effect on non-contingent food intake, either when sated or when fasted, or when given a choice between two highly palatable diets (one high carb, one high fat). Activation of mPFC pyramidal neurons also has no effect on social interaction, behavior in an open field or elevated plus maze, or exploration of a novel environment. Furthermore, by testing our mice at varying concentrations of the DREADD receptor ligand clozapine-N-oxide (CNO), we found that the behavioral outcome is influenced by the degree of mPFC activation. Low CNO doses enhance operant responding, while higher doses are required to observe an effect on reinstatement and impulsivity. We hypothesize that this difference is due in part to alterations in neuronal activity in the mPFC, a hypothesis we are

testing by recording local field potentials in the mPFC before and during CNO challenge. Our data indicate that modestly increasing excitatory drive from the mPFC is sufficient to enhance performance of high cognitive demand, instrumental behaviors, while avoiding additional effects on low cognitive demand, non-contingent behaviors, such as have been reported by other groups using alternate stimulation methods.

Disclosures: D.M. Warthen: None. **P.S. Lambeth:** None. **B.A. Newmyer:** None. **R.P. Gaykema:** None. **M.M. Scott:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.10/TT24

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R00 DA024719

Title: Patterns of activation of excitatory and inhibitory neurons in the mouse medial prefrontal cortex associated with palatable food ingestion and food driven exploratory behavior

Authors: *R. P. GAYKEMA, X.-M. NGUYEN, J. M. BOEHRET, P. S. LAMBETH, J. JOY-GABA, D. M. WARTHEN, M. M. SCOTT;
Pharmacol., Univ. of Virginia Sch. of Med., Charlottesville, VA

Abstracts: The medial prefrontal cortex (mPFC) controls many aspects of executive function, which include attentional control and goal selection. Food-seeking behavior has been shown to involve activation of the mPFC, both during the execution of strategies designed to obtain food and during the consumption of food itself. As these behaviors likely require differential engagement of the prefrontal cortex, we hypothesized that the pattern of neuronal activation would also be behavior-dependent. In this study we describe patterns of Fos expression in different layers and cell types of the dorsal (prelimbic/anterior cingulate) and ventral (infralimbic/dorsal peduncular) subdivisions of the mouse mPFC following both the consumption of palatable food and exploratory activity directed at obtaining food reward. While both manipulations led to increases of Fos expression in principal excitatory neurons relative to the control group, food-directed exploratory activity produced a significantly greater increase in Fos expression than observed in the food-consuming condition. Consequently, we hypothesized that interneuron activation would be differentially engaged in a similar fashion. However, Fos

induction in interneurons depended heavily on interneuron subtype and did not exhibit the same differences in intensity between experimental groups as seen in the excitatory neuronal populations, illustrating how the differential engagement of subsets of mPFC interneurons depends on the behavioral state. In our experiments, both vasoactive intestinal peptide- and parvalbumin-expressing interneurons showed enhanced Fos expression only during the food-dependent exploratory task and not during food intake. Our data suggest that select activation of these cell types may be required to support high cognitive demand states such as observed during exploration while not being similarly engaged during the ingestion of freely available food.

Disclosures: **R.P. Gaykema:** None. **X. Nguyen:** None. **P.S. Lambeth:** None. **J. Joy-Gaba:** None. **D.M. Warthen:** None. **M.M. Scott:** None. **J.M. Boehret:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 650.11/TT25

Topic: F.02. Animal Cognition and Behavior

Support: NIDDK R01 DK085721

Title: Plasticity within the basolateral amygdala pathways to the prelimbic cortex during Pavlovian appetitive conditioning

Authors: *S. E. KEEFER, C. J. REPPUCCI, H. S. MAYER, G. D. PETROVICH; Psychology, Boston Col., Chestnut Hill, MA

Abstracts: The amygdala and medial prefrontal cortex (mPFC) are important for associative learning and memory, and these structures are heavily interconnected via topographically organized pathways. How these two areas communicate throughout different stages of learning is not well understood. Our laboratory recently found that the anterior and posterior basolateral nuclei of the amygdala (BLAa, BLAp) are differentially recruited during early and late stages of cue-food associative learning using a Pavlovian appetitive conditioning paradigm. Projections from the BLAa and BLAp to the mPFC are distinct, suggesting activation in these nuclei could differentially influence the mPFC throughout learning. Thus, we examined whether BLA neurons that send direct projections to the mPFC are selectively recruited during cue-food associative learning, and whether specific pathways from the BLAa and BLAp to the mPFC are differentially recruited across learning. To accomplish this, we used neuroanatomical tract

tracing combined with Fos induction analysis. Male Long-Evans rats received an injection of the retrograde tracer Fluoro-Gold (FG) into the prelimbic area (PL) of the mPFC via iontophoresis. Rats in the conditioned group (Paired) were given presentations of a tone (conditioned stimulus, CS) followed by immediate delivery of food pellets (unconditioned stimulus, US) in the conditioning chamber. Rats in the Control group received tones (CS) in the conditioning chamber and food pellets (US) in their home cage. Each training group received either one or ten days of training (one session per day) to examine early and late learning, respectively. Learning was measured as an increase in the percent of time rats spent at the food receptacle ('food cup behavior') during the tone presentations. The Paired group learned the tone-food association quickly and robustly, evident by a tone-specific increase in food cup behavior during training. Rats were sacrificed 90 minutes after either one or ten days of training, and brain tissue was processed with double-label fluorescent immunohistochemistry for FG and Fos detection. Neurons within the BLAa and BLAp were analyzed, and the number of double-labeled (FG + Fos) neurons was quantified for each nucleus. We found more FG + Fos neurons in the Paired compared to the Control group in the BLAa during late, but not during early training. Conversely, we found no differences between groups in the BLAp at either time point. Our findings indicate selective recruitment of the BLAa-PL pathway emerges by late training of the tone-food association, which suggests plasticity of this pathway across appetitive associative learning.

Disclosures: S.E. Keefer: None. **C.J. Reppucci:** None. **H.S. Mayer:** None. **G.D. Petrovich:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

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Program#/Poster: 650.12/TT26

Topic: F.02. Animal Cognition and Behavior

Support: NIDDKR01DK085721

Title: Male and female rats show differential Fos induction within distinct medial prefrontal areas during renewal of Pavlovian appetitive conditioned responses

Authors: *L. C. ANDERSON, G. D. PETROVICH;
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Abstracts: Recently, we began investigating contextual processing in associative learning and memory and found sex differences. In context-dependent renewal of extinguished appetitive Pavlovian conditioned responses we found that males showed renewal of responding while females did not. In that protocol, conditioning and extinction are conducted in different contexts and the renewal of responding is induced by return to the conditioning context ("ABA renewal"). In the current study, we examined recruitment of the medial prefrontal cortex (mPFC) during the test for renewal in male and female rats. We used Fos induction to evaluate distinct mPFC areas, the infralimbic (ILA), prelimbic (PL), and anterior cingulate (ACAd). First, male and female rats were conditioned to associate a tone (conditioned stimulus, CS) with food (unconditioned stimulus, US) in 5 acquisition sessions. Acquisition was followed by 2 extinction sessions with CS-only presentations. Rats were then tested for renewal with CS-only presentations in a single test session. Experimental groups experienced extinction in a context different than the acquisition context and then were tested for renewal in the original acquisition context (ABA, BAB). Control groups remained in the same context during acquisition, extinction, and test (AAA, BBB). A CS-specific increase in the expression of food cup behavior (conditioned response, CR) was the measure of learning. We found no sex differences in acquisition and similar responding in extinction. During the test males showed renewal of CRs, while females did not. There was a main effect of sex and group interaction for food cup responding ($F(1,28)=5.522$, $p < 0.05$), and a significant difference between male groups (Experimental Males: 40.12 +/- 6, Control Males: 17.9 +/- 7; $t(1,12)=1.045$, $p < 0.05$), but no differences between female groups (Experimental Females: 15.2 +/- 5, Control Females: 19.73 +/- 5; $p > 0.05$). Brains from these animals were processed with immunohistochemistry for detection of Fos and then total number of neurons with Fos analyzed. There was a significant sex by group interaction in the ILA and the PL (ILA: $F(23,1)=12.439$, $p < 0.01$; PL: $F(23,1)=12.439$, $p < 0.01$). In both the ILA and PL, Fos induction in males was significantly higher in experimental compared to control groups ($p < 0.05$). The pattern of Fos induction was the opposite in females: Fos induction was significantly lower in the experimental compared to control groups ($p < 0.05$). In contrast, within the ACAd there were no sex or group differences in Fos induction. Our results suggest distinct mPFC areas, specifically the ILA and the PL, are recruited during appetitive renewal in a sex-specific way.

Disclosures: L.C. Anderson: None. **G.D. Petrovich:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.13/TT27

Topic: F.02. Animal Cognition and Behavior

Support: NSERC 387224-2010

Title: A parametric study of appetitive Pavlovian conditioning and conditioned reinforcement in rats

Authors: R. I. TABBARA, P. BEHARRY, *N. CHAUDHRI;
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Abstracts: Objective: An appetitive Pavlovian conditioned stimulus (CS) can predict the delivery of an unconditioned stimulus (US), acquire incentive value, and reinforce new behavior. We examined the impact of water-deprivation and sucrose concentration on the acquisition of Pavlovian conditioning and expression of conditioned reinforcement (CR). We also tested the effect of nicotine (NIC) on CR. Methods: Male, Long-Evans rats (Harlan, 220-240 g on arrival, *n* = 96) were acclimated to 3% (3S) or 20% (20S) sucrose in the home-cage. Within each concentration, rats were then divided into two equal groups that received either unrestricted water (NWD: non water-deprived) or water for 1-hr per day (WD: water-deprived). All rats then underwent 7 Pavlovian conditioning sessions. For half of the rats in each deprivation and concentration condition (PAIRED), a 10-sec tone-light CS was paired with 0.2 ml of sucrose (16 trials/session; 3.2 ml/session). The remainder (UNPAIRED) received explicitly unpaired CS and US trials. Entries into the fluid port where sucrose was delivered were recorded. Next, tests for CR were conducted. Two novel levers were introduced. Presses on the 'active' lever produced the CS without the US, whereas presses on the 'inactive' lever had no consequences. Before one of these tests, rats received an injection of saline or nicotine (0.4 mg/kg, free base, s.c.). Results: For rats in the PAIRED but not UNPAIRED condition, the proportion of total port-entries that occurred during the CS (Percent CS) increased across session. NWD rats that received paired CS-US trials achieved higher Percent CS port-entries compared to WD rats. There was no impact of sucrose concentration on acquisition. During the test for CR, PAIRED rats in the WD, 20S condition responded more on the active lever than the inactive lever, and made more active lever responses than UNPAIRED rats. NIC selectively enhanced responding for CR in rats in all PAIRED conditions. Conclusions: A CS that is repeatedly paired with sucrose can come to predict the delivery of sucrose, and the acquisition of this association is influenced by water-deprivation. A CS can acquire predictive properties but not incentive value, as indicated by the finding that only a CS associated with 20S in WD rats served as a CR. NIC can non-associatively enhance the conditioned reinforcing properties of appetitive Pavlovian cues.

Disclosures: R.I. Tabbara: None. **N. Chaudhri:** None. **P. Beharry:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01AG043972

NIH Grant P30 NS47466

Title: The effects of a ghrelin agonist without increased energy intake on age-related cognitive, metabolic and circadian changes in SAMR1/SAMP8 mice

Authors: *I. KADISH[1], M. SHARPE[1], T. VAN GROEN[1], J. PAUL[2], K. L. GAMBLE[2], M. E. YOUNG[3], D. B. ALLISON[4];
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Abstracts: Caloric restriction (CR) is a long established paradigm which extends longevity and slows symptoms of aging through mechanisms which have yet to be clearly elucidated. Ghrelin is a hunger-inducing gut peptide, and the interoceptive cues caused by ghrelin are likely similar to those produced by CR. In this study we tested the novel hypothesis that a ghrelin agonist, when animals are not permitted to increase their energy intake, induces neuroendocrine effects consistent with hunger and thereby attenuates behavioral and cognitive decline, and also changes energy metabolism and circadian rhythm in aged SAMR1/SAMP8 mice, and that these changes involve interoceptive cues, rather than reduced energy intake per se. Two groups of 2 month old male SAMR1 and SAMP8 mice were used in this study. One group (control) from each strain had ad libitum access to food, while the second group (ghrelin) received the mean amount of diet consumed by the control group and a ghrelin agonist (LY444711; 30 mg/kg of LY in a 45 mg sucrose pellet) daily for 6 months. At the end of the experimental feeding protocol all mice were analyzed for body composition using quantitative magnetic resonance (QMR). A battery of behavioral and cognitive tests was carried out to analyze behavioral/cognitive differences. All animals were also assessed in CLAMS cages for energy expenditure and circadian rhythms. Biochemistry and immunohistochemistry analysis were carried out to assess the changes in brain tissue and plasma. NIH Image J was used to analyze protein expression changes, between-group ANOVA and Bonferroni post tests were used to determine the statistical significance. Six months of ghrelin agonist treatment did not alter the body composition of either group. Cognition

was significantly (p=0.03) improved in ghrelin group of SAMR1 mice compared to control group, but did not influence the cognitive outcome of SAMP8 mice. In a 12:12 light-dark cycle, both SAMR1 and SAMP8 mice exhibited typical 24-hour rhythmicity in general cage activity, respiratory exchange ratio (RER), and energy expenditure; however, SAMP8 mice exhibited pronounced ultradian (12-hour) rhythms in all measurements as well. Daily ghrelin administration to SAMP8 mice eliminated the ultradian phenotype and restored the amplitude of 24-hour rhythms in behavior and metabolism up to the level seen in SAMR1 controls. In conclusion, "hunger" without CR has similar cognitive and metabolic benefits compared to CR without the potential problem of weight loss in aging organisms.

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Poster

650. Appetitive and Incentive Learning and Memory II

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 650.15/TT29

Topic: F.02. Animal Cognition and Behavior

Title: Parallel remodeling of direct and indirect pathway neuronal activity in the nucleus accumbens during Pavlovian reward conditioning

Authors: *J. G. PARKER[1], J. D. MARSHALL[2], B. AHANONU[3], B. GREWE[4], J. ZHONG LI[4], M. D. EHLERS[1], M. J. SCHNITZER[5];
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Abstracts: Learning is thought to occur through selective alterations in neuronal excitability that contribute to adaptive responses to environmental stimuli. In the nucleus accumbens (Acb), the differential modulation of D1- and D2-dopamine receptor expressing medium spiny neurons (MSNs) by stimulus-elicited dopamine release is hypothesized to be critical for associative learning and goal-directed behavior. To date, efforts to validate this hypothesis have been limited by the inability to differentiate the two cell types using traditional recording techniques. To overcome this limitation, we conditionally expressed GCaMP6 in Acb D1- and D2-MSNs and then used a miniature fluorescence microscope to image the simultaneous Ca^{2+} dynamics of hundreds of individual neurons during acquisition, extinction, and reinstatement of an appetitive

Pavlovian association in freely-moving mice. Activity in both D1- and D2-MSNs was organized into spatiotemporally coordinated ensembles, which exhibited similar dynamics of activation in response to conditioned stimulus presentation. Although reward consumption was correlated with a dramatic decrease in neural activity, we observed transient and pathway-specific reactivation of neuronal ensembles in response to rewarded licking. During extinction or systemic D1R antagonism, conditioned responding diminished and task-related Acb network activity closely resembled the unlearned state. Taken together, our results reveal novel ensemble features of Acb D1- and D2-MSNs during dopamine-dependent learning.

Disclosures: **J.G. Parker:** A. Employment/Salary (full or part-time):; Pfizer. **J.D. Marshall:** None. **B. Ahanonu:** None. **B. Grewe:** None. **J. Zhong Li:** None. **M.D. Ehlers:** A. Employment/Salary (full or part-time):; Pfizer, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix, Inc.

Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.16/TT30

Topic: F.02. Animal Cognition and Behavior

Support: ANR Grant ANR-11-BSV4-006

Title: A model of negative automaintenance in pigeons: Dual learning and factored representations

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Abstracts: In this work, we apply a model that combines dual-learning systems and factored representations to experimental data about negative automaintenance in pigeons. In such a procedure, a key light is paired with the subsequent delivery of food where any peck at it results in the omission of reward. Various studies reported very distinct behaviours between pigeons. Some observed that pigeons persisted in pecking despite its negative consequence, while others

observed that pigeons quickly learned to refrain from it and maximized their cumulative rewards. Interestingly, in a previous work, we showed that the model already accounts for the variability of behaviours observed in a population of rats undergoing an autoshaping experiment, a similar procedure where the conditioned stimulus has no negative impact (Lesaint et al., 2014). More precisely, it accounts for the presence of goal-trackers and sign-trackers amongst rats (Flagel et al., 2011). We show that the model can similarity account for the variability of behaviours observed in pigeons, suggesting that the dichotomy between sign-tracking and goal-tracking might apply in pigeons. It also explains why the introduction of an irrelevant key light, on which pecking has no effect, allows pigeons that are unable to refrain from pecking to shifts their pecks towards it. Finally, it also reproduces the incapacity of a continuous irrelevant key light, which is never turned off, to attract such detrimental pecks when combined with the negative key light. We discuss the interest of the main mechanisms combined in the model (factored representations and dual-learning) in reproducing experiments about Pavlovian and instrumental conditioning, especially during their interaction. The model provides a tool to further investigate the neural mechanisms that lead to such observations and allows us to draw predictions that may be experimentally verified, for example about the dopaminergic patterns that should be expected to be recorded.

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Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.17/TT31

Topic: F.02. Animal Cognition and Behavior

Support: Henry Wellcome Fellowship 098830/Z/12/Z

Title: Value learning mechanisms for novel stimuli in orbitofrontal and anterior cingulate cortex

Authors: *S. KENNERLEY, L. T. HUNT, N. MALALASEKERA;
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Abstracts: Neurons whose activity reflects the value of choice alternatives are reliably isolated in studies of orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC). Such studies frequently use ‘overlearnt’ stimuli to indicate decision values to the subject, which have been seen by the subject in a large number of previous sessions. In the present study, we: (i)

investigated how neuronal activity in OFC and ACC varied as subjects learnt the value of, and made choices between, entirely novel stimuli, and (ii) compared value coding selectivity of the same neurons during choices of overlearnt stimuli. Each experimental session consisted of two phases. In the first phase ('learning' trials), subjects were presented with one of 10 novel cues. Each cue was followed by a secondary reinforcer that predicted either the probability or magnitude of reward, and then juice reward was delivered after a delay. During this phase, subjects' learning was probed using 'catch' trials in which they chose between two of the novel stimuli. In the second experimental phase ('choice' trials), subjects either chose between two novel stimuli, two overlearnt stimuli, or one novel and one overlearnt stimulus. Activity from 112 single neurons (57 ACC, 55 OFC) was investigated. Subjects learnt the value of the novel stimuli with remarkable speed and accuracy, such that their performance in catch trials at the end of the learning phase (10 exposures per cue, 100 trials total) often exceeded 90% accuracy. Moreover, subjects reliably used this information to choose reliably between novel and overlearnt stimuli in the choice phase. In spite of this, there was a clear distinction in neuronal responses of OFC (but not ACC) to novel vs. overlearnt stimuli in the choice phase. A similar proportion of ACC and OFC neurons reflected the value of the chosen option. However, whereas ACC chosen value neurons responded similarly irrespective of cue novelty, OFC chosen value neurons again differentiated between novel and overlearnt cues. This suggests that whereas ACC carries an abstract value code irrespective of cue identity, OFC carries a specific code that discriminates novel from familiar stimuli. The same "stimulus code" profile of OFC (but not ACC) activity also occurred during the learning phase of the experiment as during the choice phase. Intriguingly, this stimulus code persisted until the end of the secondary reinforcer epoch in early learning trials, but attenuated shortly after stimulus onset as learning progressed. This suggests a mechanism by which value might be ascribed to novel stimuli in orbitofrontal cortex during learning.

Disclosures: **S. Kennerley:** None. **L.T. Hunt:** None. **N. Malalasekera:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Program#/Poster: 650.18/TT32

Topic: F.02. Animal Cognition and Behavior

Title: Induction of associative olfactory memory by targeted activation of single olfactory neurons in *Drosophila* larvae

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Abstracts: Appetitive olfactory memory is formed in *Drosophila* by at least two sets of external stimuli, conditioned odor stimuli (CS) mediated by the olfactory receptor neurons (ORNs) and unconditioned reward stimuli (US) mediated by the octopaminergic (OA) neurons. Previous studies demonstrated that neural signaling of OA neurons is required for the acquisition of appetitive memory in both adult flies and larvae. It has also been shown that induced activation of OA neurons substitutes appetitive US. While these studies have uncovered the functional neural circuitries that mediate US information during the course of associative memory induction, the contribution of CS pathway in memory acquisition is yet to be addressed by targeted activation approaches. In particular, whether associative memory can be induced by targeted activation of only the olfactory CS and the OA-mediated US pathways remains to be elucidated. Moreover, whether the complex combinatorial signals mediated by the activation of multiple ORNs are necessary to induce distinctive associative memory has not been addressed so far. With its simple neuroanatomical design, the larval brain of *Drosophila melanogaster* can be utilized as an excellent model system for the elucidation of the neurocircuitry mechanism of memory. The architecture of the larval brain is a simple extension of the embryonic axonal plan, and the neuronal pathway of olfactory information is straightforward without redundancy. Using a single-odor paradigm, we described previously that sugar-reward training produces appetitive olfactory memory that persists medium term in the third instar larvae. In this study, we applied optogenetic and thermogenetic techniques to the analysis of the neurocircuitry mechanism of memory induction in the larval brain. We substituted sugar reward stimuli by thermogenetic activation of OA neurons with the dTrpA1 channel, and odor stimuli by optical activation of a specific class of ORNs with Channelrhodopsin-2 (ChR2). We showed that targeted activation of the converging memory circuitry with blue light and heat produces associative memory in the transgenic larvae. We also showed that memory thus produced is specific to the odorant determined by the type of the activated ORNs. Furthermore, we successfully demonstrated that this artificial olfactory memory persists for medium term, and as stable as natural memory produced by the activation of multiple ORNs using a real odorant.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI Grant #24530917

Title: Autoshaping the lever-press response and its subsequent continuous reinforcement in spontaneously hypertensive rats: Effects of distant placement of the lever

Authors: *T. SATO[1], J. GYOBA[2];
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Abstracts: In the autoshaping paradigm, animals engage in goal tracking first, followed by sign tracking. The progress to sign tracking could be strongly affected by the spatial location of the lever (i.e., "sign" for food delivery) and food cup (i.e., "goal" of the food reinforcement). It becomes more difficult for rats to find the sign if it is located away from the goal or out of their spatial attention span. Further, rats with shorter attention spans might show lower performance in the autoshaping paradigm when the sign is located away from the goal. The present study examined the acquisition of a lever-pressing response in a spontaneously hypertensive rat (SHR), a rodent model of human ADHD. Eight male SHRs and Wistar Kyoto (WKY) rats were used. Half were allocated to an autoshaping test in which a retractable lever was placed on the same wall as the food cup and the distance between them was short (adjacent placement). The others were allocated to a test in which the lever was placed on the wall opposite to the food cup (distant placement). After food cup training, four test sessions were conducted, each with 50 trials. At the beginning of each trial, one lever was introduced in the chamber and a cue light was simultaneously turned on. If a lever-pressing response appeared within 15 s since lever presentation, a 45-mg food pellet was presented in the food cup, the lever was retracted, and the cue light was turned off immediately after the criterion response. If the criterion response did not appear, the food pellet was presented, lever was retracted, and cue light was turned off 15 s after the lever presentation. Subsequently, using the rats in the distant placement condition, one or more sessions of continuous reinforcement, or an FR1 schedule, were performed. In this, three retractable levers were introduced on the wall opposite to the food cup. The test was considered complete when 30 food pellets were acquired by pressing these levers within 400 s. In the adjacent placement, both strains rapidly acquired the lever-press response in the first session; the SHRs showed a similar increase in the frequency of the lever-press as did the WKY rats. However, in the distant lever placement, the SHRs could not acquire the lever-press response through all four autoshaping sessions, while three out of the four WKY rats acquired it at least by the third session. Their performance in subsequent reinforcement sessions also indicated that acquisition of the lever-pressing response further deteriorated in the SHRs in this placement. In

the continuous reinforcement task, the SHRs required two or more sessions to achieve the criterion to complete it, while all WKY rats successfully completed it in the first session.

Disclosures: **T. Sato:** None. **J. Gyoba:** None.

Poster

650. Appetitive and Incentive Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH 093897

State of Connecticut

NIDA A036151

NSF GFRP

Title: Differential role of VTA cholinergic and glutamatergic receptors in conditioned reinforcement

Authors: ***R. WICKHAM**, W. SOLECKI, E. NUNES, N. ADDY;
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Abstracts: **Abstract** Stimuli which have been associated with rewards (cues) can become strong motivators and drivers of food-seeking and drug-seeking behavior. Dopamine (DA) cells in the ventral tegmental area (VTA) encode rewards and cues by switching from a tonic to burst-like firing pattern - leading to elevated nucleus accumbens (NAc) DA release. VTA nicotinic acetylcholine receptors (nAChRs), muscarinic acetylcholine receptors (mAChRs), and N-methyl-D-aspartate receptors (NMDARs) are critical for NAc phasic DA release. This increase in DA release has been shown to be important for the reinforcing properties of cues. Thus, we tested the role of nAChRs, mAChRs, and NMDARs in permitting a cue to act as a reinforcer. Rats received 10 days (25 pairings per day) of an 8-second lever cue presentation followed by delivery of a banana-flavored sucrose pellet. Rats either developed sign-tracking (approach to the cue), goal-tracking (approach to the food receptacle), or intermediate approach strategies during Pavlovian conditioning. The day after training, rats were tested on the acquisition of a novel response with conditioned reinforcement. We infused either saline, the NMDAR antagonist AP-5 (0.1 or 1μg),

the nAChR antagonist mecamylamine (MEC: 3 or 30µg), or the mAChR antagonist scopolamine (SCOP: 3 or 66.7µg) bilaterally into the VTA immediately before conditioned reinforcement testing. During the test, nosepoking in the port assigned as active (CR) produced the lever cue while nosepoking on the port assigned inactive (NCR) had no consequence. AP-5 robustly decreased CR, and not NCR responding, but did not block the subjects' ability to discriminate between CR and NCR. The 3µg MEC dose had no effect on either CR nor NCR, while the 30µg MEC dose nonspecifically decreased both CR and NCR responding. The 3µg SCOP dose had no effect on either CR nor NCR while the 66.7µg SCOP dose increased NCR and disrupted the subject's ability to discriminate between CR and NCR. These results provide new insight of the role of VTA NMDARs and AChRs in mediating the ability of natural reward associated cues to act as a conditioned reinforcer.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: University of Michigan Department of Psychiatry (U032826)

Department of Defense National Defense Science and Engineering Graduate Fellowship

Title: Ventral hippocampal lesions attenuate the acquisition of sign-tracking behavior in rats

Authors: *C. J. FITZPATRICK, J. D. MORROW;
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Abstracts: It is important to understand why some individuals can try potentially addictive drugs without developing addiction, while others try the same drug and are quickly rendered incapable of controlling their urges to repeat the experience. In order to model addiction vulnerability in animals, we use a Pavlovian conditioned approach procedure to identify individual rats, called "sign-trackers," that become attracted to and motivated by a cue that predicts reward. Other rats, called "goal-trackers," remain motivationally fixated on the reward itself. Sign-trackers are more susceptible to cue-induced reinstatement of cocaine self-administration and seek drug even in the face of adverse consequences, two hallmarks of addiction in humans. Thus, sign-tracking for

food reward predicts addiction-like behaviors and can be used as a proxy for addiction vulnerability. Sign-trackers release more dopamine in the nucleus accumbens than goal-trackers in response to reward cues. Sign-tracking has also been shown to be dopamine-dependent, while goal-tracking is not. Afferents from the ventral hippocampus are the most potent drivers of dopaminergic activity and thought to thereby enhance the motivational salience of relevant cues. A reduction of activity in the ventral hippocampus would therefore be expected to decrease dopaminergic activity in response to reward cues and consequently reduce sign-tracking behavior. In agreement with this hypothesis, we found that rats with excitotoxic lesions of the ventral hippocampus exhibited less sign-tracking and more goal-tracking behavior than sham-operated rats. This suggests that activity within the ventral hippocampus may promote sign-tracking behavior to the exclusion of goal-tracking behavior. Individual variation in the strength of ventral hippocampal influence over dopaminergic activity may therefore be one source of individual variation in susceptibility to addiction.

Disclosures: C.J. Fitzpatrick: None. **J.D. Morrow:** None.

Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.01/TT36

Topic: F.02. Animal Cognition and Behavior

Support: F32EY023526

R01MH55806

P30EY008126

P30HD015052

Title: Neuronal correlates of choosing and stopping in macaque frontal eye field

Authors: *P. MIDDLEBROOKS, J. D. SCHALL;
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Abstracts: Perceptual choice and response inhibition have each been a major focus of decision-making research for decades. To date no studies examined the relationship between them at the single neuron level. Neuronal correlates of response inhibition have been described in saccadic

stop or step tasks. Whereas visual neurons simply register current location of target, pre-saccadic movement neurons in the frontal eye field (FEF) and superior colliculus have different firing rate dynamics for no-stop, noncanceled stop, and canceled stop trials. During no-stop and noncanceled stop trials, firing rate increases to a constant threshold and a saccade is triggered. During canceled stop trials, firing rates increase but are modulated before reaching threshold. Critically, the modulation occurs before a point of no return, the stop signal reaction time (SSRT), indicating the neurons control whether a saccade is withheld. Neuronal correlates of perceptual choice have been described in saccadic choice response time (RT) tasks. Neuron activity in multiple brain areas such as LIP and FEF increase quickly (slowly) during easy (difficult) trials, followed by short (long) reaction times (RTs) We explored FEF neuron activity of monkeys performing a saccadic choice RT stop-signal task (Middlebrooks & Schall 2014 Atten, Percept & Psychophys). The choice stimulus was a cyan-magenta checkerboard. Saccade choice was specified by the fraction of cyan or magenta in the checkerboard, varied around discrimination threshold. On 25-40% of trials a visual stop signal replaced the central fixation spot after a variable stop-signal delay. On no-stop signal trials reinforcement was earned for a correct choice. On stop signal trials reinforcement was earned for inhibiting the saccade. We recorded FEF visual, visual-movement, and movement neurons. We observed a variety of task-related modulations, including the following. Visual neuron activity selected the eventual target but did not rise to a threshold immediately before a saccade during no-stop and noncanceled stop trials. During canceled stop trials visual neuron activity did not modulate before SSRT and thus could not play a role in withholding a response. Movement neuron activity rose to a threshold before a saccade during no-stop and noncaneled stop trials. The rate of rise varied with choice difficulty. During canceled stop trials movement neurons modulated before SSRT and thus could contribute to withholding a response. These behavioral and neural results, in conjunction with ongoing stochastic accumulator modeling, provide the opportunity to unify two major frameworks of decision-making, perceptual choice and response inhibition.

Disclosures: P. Middlebrooks: None. **J.D. Schall:** None.

Poster

651. Decision Making II

Location: Halls A-C

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Program#/Poster: 651.02/TT37

Topic: F.02. Animal Cognition and Behavior

Support: EC-FP7 BrainLeap Project - GA 306502

Title: The effect of stop signal perceptual manipulations on upper limb suppression process

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Abstracts: Primates may have occasionally to inhibit an impending action because of sudden changes in the environment. This ability has been studied by using the countermanding task, that permits to evaluate how long it takes to stop an impending movement in response to a stop signal(Stop signal reaction time, SSRT). To a first approximation SSRT can be considered composed by two different processing stages: a perceptual stage, in which the stop signal is perceived and a late inhibitory stage. An open question is how the information between these stages is transmitted: discretely, thus requiring that the previous stage is completed before the late stage starts; or continuously, so that some information can be transmitted to the late stage before the completion of the perceptual one.. To gain insight on this topic we first analyzed the behavior of a rhesus monkey that performed a delayed reach version of the countermanding task. Each trial started with the presentation of a central circular target (CT)and two small dots above it. The monkey had to hold the CT and after 0.4-0.8s a peripheral target (PT) appeared either to the left or to the right. Then, after a delay(0.8-1.2 s) the right small dot (RSD) above the CT changed color (from red to green, Go signal) and the monkey had to reach and touch the PT (no-stop trials). Unpredictably, in 34% of the trials (stop trials), after a variable time from the Go signal RSD changed color again thus instructing the monkey to refrain (stop signal). The stop signal color was selected from a sample of 5 different colors between blue and green, making the stop signal progressively similar to the go signal and more difficult to be perceived. In fact at the behavioral level the color of the stop signals strongly affected the ability to inhibit the response. The closer color of the stop signal to the green, the longer the SSRT and the more disrupted the ability to stop (SSRT:196, 284 ,301, and disrupted performance for the last two). While the monkey performed the task we recorded the neural activity from a 96 electrodes chronic array. A preliminary investigation shows that the lengthening of the SSRT is associated with different changes in activity: while some neurons show a change in the onset of the activity after different stop signals, others show a change in the rate of rise or decrease of activity. These data suggests that in the premotor area, probably both continuous and discrete mechanism of information transmission are at play during motor control.

Disclosures: P. Pani: None. **R. Montanari:** None. **F. Fabbrini:** None. **F. Giarrocco:** None. **V. Mione:** None. **E. Brunamonti:** None. **S. Ferraina:** None.

Poster

651. Decision Making II

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Program#/Poster: 651.03/TT38

Topic: F.02. Animal Cognition and Behavior

Support: EU FP7 grant 269921

Title: A model of perceptual discrimination under sequential sensory evidence

Authors: *E. HUGUES[1], C. STEIN NAVES DE BRITO[2], W. GERSTNER[2], R. ROMO[3], G. DECO[1,4];
[1]Univ. Pompeu Fabra, Barcelona, Spain; [2]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [3]Univ. Nacional Autónoma de México, Mexico, Mexico; [4]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

Abstracts: Perceptual discrimination may be interpreted as a decision between alternatives based on available sensory evidence. In many experiments, the different alternatives are presented simultaneously and encoded by quite distinct neuronal groups. In this case, proposed biophysical neural models consider that the decision results from the competition between decision-specific neuronal groups, each of these integrating distinct sensory evidence. Alternatively, evidence may be presented in a sequential manner, and the different stimuli may be encoded by the same neuronal group, as exemplified by experiments where monkeys are engaged in a vibrotactile discrimination task [1]. To achieve discrimination in this case, the nervous system needs to keep a trace of the previously presented stimuli. How the nervous system manages to achieve a good performance in such perceptual discrimination tasks is not understood. To address these questions, we propose a biophysical model inspired from electrophysiological data obtained in a vibrotactile discrimination task [1]. In this task, a monkey is presented two vibrotactile stimuli (with frequencies f1 and f2), separated by a delay period: the animal is instructed to decide whether f1 > f2 or f1 < f2. The diversity of partial differential (PD) neurons which encode both stimuli by keeping the memory of the first one during the delay period is shown to be reproduced by a spiking neuron network model with short-term facilitating synapses. To achieve the decision, we consider a decision making (DM) spiking neuron network, whose input is given by the heterogeneous population of PD neurons. To learn how to decide from this input, the strengths of the projecting synapses are learned using a reinforcement learning based rule applied after the decision. The task is found to be efficiently learned across trials, reproducing the experimental results. In this respect, the PD neurons heterogeneity is crucial to provide the necessary information to the DM network. More generally, we have shown that our proposed biophysical two-networks model, including both working memory and decision-making capabilities, provides a way by which a perceptual discrimination task under

sequential sensory evidence could be achieved by the nervous system. 1. Hernández et al. 2010, Neuron 66:300-314.

Disclosures: **E. Hugues:** None. **G. Deco:** None. **C. Stein Naves de Brito:** None. **W. Gerstner:** None. **R. Romo:** None.

Poster

651. Decision Making II

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Topic: F.02. Animal Cognition and Behavior

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CFI

FRSQ

EJLB Foundation

Fondation Fyssen to DT

GRSNC fellowship to DT

Title: Micro-stimulation of premotor and motor cortex delays the commitment to an action choice

Authors: ***D. THURA**, P. CISEK;
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Abstracts: Are decisions about actions determined in cognitive or motor regions? We have recently shown that when monkeys perform a task in which relevant sensory information for action selection varies over time and they are free to respond at any moment, neurons in dorsal premotor (PMd) and primary motor cortex (M1) appear to be involved in the processes of deliberation and commitment (Thura and Cisek, 2014). In particular, during deliberation many PMd and M1 neurons reflect the evolution of the sensory information along with a signal related to the growing urgency to respond. Furthermore, approximately 280ms prior to movement onset, the activity of these cells reveals the resolution of a competition between the choices, implicating them in the process of volitional commitment. Here, to test whether these cells are causally

involved in commitment, we perturb their activity using brief pulses of subthreshold micro-stimulation. One monkey performed a reaching decision task in which sensory evidence continuously evolves during the time course of a trial. In different blocks, the temporal properties of the task were varied to induce adjustments of monkey's speed-accuracy trade-off. On about 2/3 of trials, we stimulated sites in either PMd or M1 where we had recorded task-related neurons. Stimulation (57ms train of 0.2ms pulses at 330Hz with amplitude ranging from 20-70μA) was applied at different times during the deliberation process: either at trial onset, 800ms or 1400ms later. We found a time-dependant effect of stimulation in both PMd and M1 on the monkey's reactions times (RTs). When applied at trial onset, stimulation had almost no effect. However, when applied later, stimulation caused a significant increase of RTs depending on trial difficulty. A comparison between conditions where the monkey either favored decision speed or accuracy suggests that stimulation was more effective when the monkey was close to commitment. This effect was also most pronounced when stimulation preceded movement onset by about 300ms, suggesting that stimulation interfered with the normal process of volitional commitment, and not the process of movement initiation. In contrast, during a task in which movement initiation was externally instructed, we found that stimulation of PMd, but not M1, significantly shortened RTs. Together, our results suggest that PMd and M1 neurons are causally involved in the volitional commitment to an action choice. Thura D, Cisek P. Deliberation and commitment in the premotor and primary motor cortex during dynamic decision-making. Neuron. 81(6): 1401-1416 (2014).

Disclosures: D. Thura: None. **P. Cisek:** None.

Poster

651. Decision Making II

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Topic: F.02. Animal Cognition and Behavior

Support: NSF Graduate Research Fellowship Program

Title: Supplementary eye field activity during and after self-selection of abstract rules

Authors: *Z. M. ABZUG, M. A. SOMMER;
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Abstracts: Selection and implementation of abstract rules is a crucial part of human cognition. Previous work has shown that neurons in the frontal cortex of monkeys (Macaca mulatta), including Supplementary Eye Field (SEF), encode instructed rules through firing rate modulations (White & Wise, 1999). However, there is a dearth of work addressing whether or not neurons in frontal cortex encode self-selected rules, or if they differentiate between self-selected and instructed rules (i.e. agency selectivity). We previously designed and validated an oculomotor task, compatible with neurophysiological recordings in monkeys, constructed to answer this question and showed that one monkey was able to perform the task appropriately (Abzug & Sommer, SfN 2013). In the first stage of this two-stage task, the monkey was required to saccade to one of two colored peripheral targets. The color of each target was associated with a particular rule, and the targets could be either different colors (allowing the monkey to select between different rules) or the same color (forcing the monkey to select an instructed rule). After refixating a central location, the monkey then had to correctly apply the selected or instructed rule in a perceptual discrimination between two visual targets in order to receive liquid reward. Many neurons in SEF show complex and variable activity throughout the duration of this task. Consistent with previous reports, a subset of neurons distinguish between the two rules in various time epochs during the task. We have also found a sub-population of agency-selective cells that distinguish between self-selected and instructed trials. These cells may help fill an important metacognitive role by distinguishing between rules inherited through cognitive decision-making and rules inherited through simple sensory cueing.

Disclosures: Z.M. Abzug: None. **M.A. Sommer:** None.

Poster

651. Decision Making II

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Program#/Poster: 651.06/TT41

Topic: F.02. Animal Cognition and Behavior

Support: Finnish Foundation for Alcohol Studies

Title: Dopaminergic and opioidergic neuronal mechanisms in decision making under risk in rats: Studies using a novel operant task

Authors: V. OINIO[1,2], P. BÄCKSTRÖM[1], J. UHARI-VÄÄNÄNEN[1,2], A. RAASMAJA[2], *T. PIEPPONEN[2], K. KIIANMAA[1];

[1]Dept. of Alcohol, Drugs and Addiction, Natl. Inst. for Hlth. and Welfare, Helsinki, Finland; [2]Fac Pharm, Div. Pharmacol & Pharmacother, Helsinki, Finland

Abstracts: We present a novel method for studies on the role of different central neuronal processes of decision making in a gambling situation in rats. Based on existing decision making models, our model employs probabilistic discounting in a long run protocol, i.e. devaluing a reinforcer by decreasing the probability of obtaining it in a between-sessions manner. The model can be used to investigate how decision making under risk can be modulated with different pharmacological treatments. The aim of the present study was to investigate the role of dopaminergic and opioidergic neuronal mechanisms in decision making in rats. Male alcohol preferring AA (Alko Alcohol) rats where trained to self-administer sucrose pellets (45 mg) in an operant chamber. Depending on the lever they chose, the rats could obtain one or three sucrose pellets in a 15-minute two-lever choice task. The probability for obtaining three pellets was decreased over time (100%, 50%, 33%, 25%, and 20%, "large, risky reward"), whereas the probability for obtaining one pellet was kept constant at 100% ("small, certain reward"). Thus, in this model the probability at which the levers were equally advantageous was 33%. At higher probabilities the rational choice to obtain a maximum amount of pellets would have been the 3-pellet lever and at lower probabilities the one-pellet lever. Once the choice of lever at a designated probability level had stabilized, rats were administered d-amphetamine (0.1, 0.3, 1.0 mg/kg s.c.), morphine (0.3, 1.0, 3.0 mg/kg s.c.) or their vehicle. D-amphetamine induced some irrationality in lever choice when the probability was set at the level of 100% and 50%, but it had no effect at the level of 33%. When the probability was set at 25% and 20%, d-amphetamine significantly increased risky and irrational choice in a dose-dependent manner. Morphine had, however, no effect on choice of lever. The results suggest that increased dopaminergic activity impairs rats ability to make rational choices especially under risk, and that reward-related opioidergic processes are not involved in this process. They also support a role for dopaminergic mechanisms in decision making under risk, and show the feasibility of our model in studies on decision making under risk.

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Poster

651. Decision Making II

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Title: The impact of prior expectations in an auditory discrimination task

Authors: A. HERMOSO-MENDIZABAL[1], P. E. RUEDA-OROZCO[2], S. JARAMILLO[3], D. ROBBE[2], *J. DE LA ROCHA[1];
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Abstracts: Previous experience plays a critical role in how subjects acquire and interpret sensory information. Expected stimuli for instance produce faster responses and can be discriminated with greater accuracy. Little is known however about how the brain circuitry accumulates experience over different time scales to build expectations that combined with sensory information give rise to perception. In order to identify the neural basis of expectation and its impact on perceptual decisions we designed a variant of a standard two alternative forced-choice (2AFC) task that required the generation of an unbalanced prior that had to be updated trial-by-trial in order to maximize performance. Acoustic stimuli consisting in the superposition of two amplitude modulated tones of high and low frequency (31 kHz and 6.5 kHz) were presented to rats that were trained to associate each frequency to the delivery of water in the Right and Left ports. Stimuli were parameterized by the coherence c that set the relative weight of each tone: c = +1 or -1 represented the high and low tones in isolation, respectively, and c =0 a balanced superposition of the two. The two stimulus categories, i.e. sign(c) = ±1, were presented randomly using a two-state Markov chain. Transitions were parameterized such that the probability of repeating the previous stimulus category was 0.5+λ and the probability to switch was 0.5-λ. The absolute value of the coherence was picked among the values 0, 0.1, 0.4 and 1, randomly and independently of previous history. We presented blocks of ~100 trials with fixed λ=0.2 and -0.2. We termed these blocks the repetitive and switching environments, respectively, and cued them to the animals using different lighting. We found that animals learned the statistics of each environment and made use of them to increase reward rate. Specifically, after error trials animals did not show a choice bias that depended on recent trial history. After a correct trial however, the

behavior showed a bias towards the same or the opposite choice depending on whether they were in the repetitive or in the switching environments. Moreover, the magnitude of this bias increased with the number of correct past responses following the environment's sequence pattern (e.g. in the switching environment, the probability of choosing Right conditioned of trial history grew as P(R|Error) < P(R|L, Error)< P(R|L,R, Error) < P(R|L,R,L,Error) <⋯). The magnitude of the biases were greater in the repeating than in the switching environment. These results demonstrate that perceptual decisions are influenced by the statistics of the sensory environment.

Disclosures: **A. Hermoso-Mendizabal:** None. **P.E. Rueda-Orozco:** None. **S. Jaramillo:** None. **D. Robbe:** None. **J. de la Rocha:** None.

Poster

651. Decision Making II

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Program#/Poster: 651.08/TT43

Topic: F.02. Animal Cognition and Behavior

Title: A computational model of cognitive control for context effects on multi-attribute decisions

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Abstracts: Real-life decisions are often made among multiple alternatives with multiple attributes such as reward value, effort, and visual saliency. Daily life also entails multiple sequential decisions, leading to choice burstiness: i.e., bursts of long consecutive series of identical choices interspersed with multiple switches among choice options. This burstiness can be characterized by two key features of sequential choices: preference bias, defined as the skew of the distribution of choices among alternatives; and choice persistence, defined as the tendency to repeat a choice previously made. Although context effects in decision-making have been extensively studied, the underlying computational process of how cognitive control for context effects dynamically modulates preference bias and choice persistence in multi-attribute decisions is not fully understood. Here we propose a computational model of this cognitive control. Taking into account selective attention and motivation for preference bias and choice persistence, respectively, our model can capture the dynamic choice patterns of rhesus monkeys under different choice contexts. Our modeling results suggest that context effects based on attention and motivation distinctly influence preference bias and persistence, respectively, and provide

insight into the computational and neural mechanisms of context effects on sequential choice dynamics of long-term multi-attribute decisions.

Disclosures: **K. Jung:** None. **J. Jeong:** None. **J.D. Kralik:** None.

Poster

651. Decision Making II

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BMBF, 01GQ1003A

DFG FI848/3-1

Title: Touchscreen-paradigm for mice reveals cross-species evidence for an antagonistic relationship of cognitive flexibility and stability

Authors: ***A. VOGEL**[1], S. RICHTER[2], K. UELTZHÖFFER[3], C. MUZZILLO[4], M. A. VOGT[2], K. LANKISCH[2], D. J. N. ARMBRUSTER-GENÇ[3], M. A. RIVA[4], C. J. FIEBACH[3], B. VOLLMAYR[2], P. GASS[2];
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Abstracts: The abilities to either flexibly adjust behavior according to changing demands (cognitive flexibility) or to maintain it in the face of potential distractors (cognitive stability) are critical for adaptive behavior in many situations. Recently, a novel human paradigm has found individual differences of cognitive flexibility and stability to be related to common prefrontal networks. The aims of the present study were, first, to translate this paradigm from humans to mice and, second, to test conceptual predictions of a computational model of prefrontal working memory mechanisms, the *Dual State Theory*, which assumes an antagonistic relation between cognitive flexibility and stability. Mice were trained in a touchscreen-paradigm to discriminate visual cues. The task involved “ongoing” and cued “switch” trials. In addition distractor cues were interspersed to test the ability to resist distraction, and an ambiguous condition assessed the

spontaneous switching between two possible responses without explicit cues. While response times did not differ substantially between conditions, error rates (ER) increased from the "ongoing" baseline condition to the most complex condition, where subjects were required to switch between two responses in the presence of a distracting cue. Importantly, subjects switching more often spontaneously were found to be more distractible by task irrelevant cues, but also more flexible *in situ*ations, where switching was required. These results support a dichotomy of cognitive flexibility and stability as predicted by the *Dual State Theory*. Furthermore, they replicate critical aspects of the human paradigm, which indicates the translational potential of the testing procedure and supports the use of touchscreen procedures in preclinical animal research.

Disclosures: **A. Vogel:** None. **S. Richter:** None. **K. Ueltzhöffer:** None. **C. Muzzillo:** None. **M.A. Vogt:** None. **K. Lankisch:** None. **D.J.N. Armbruster-Genç:** None. **M.A. Riva:** None. **C.J. Fiebach:** None. **B. Vollmayr:** None. **P. Gass:** None.

Poster

651. Decision Making II

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Topic: F.02. Animal Cognition and Behavior

Title: Rats' preferences for differently priced liquid reinforcers are modulated by budget constraints in a two alternative forced choice decision making task

Authors: *M. VAN WINGERDEN, C. MARX, T. KALENSCHER;
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Abstracts: Classic analysis of consumer demand investigates the changes in purchases of a commodity as a function of its price. When using animal consumers, price is typically operationalized as a work required, such as a fixed-ratio (FR) schedule to obtain a reinforcer. If two alternative commodities are concurrently available, the elasticity and cross-elasticity of demand for both choice options can be calculated, using a set of different price ratio regimes. Changing prices affects the animals' choice distribution between the alternatives even when budgets are adjusted to allow reselection of the previously chosen bundle. Animals have been shown to exhibit budget sensitivity in their demand elasticity, however it remains unclear if the changes in budgets are reflected in the animals' dynamic preferences for both commodities, or whether the number of choices available under normal versus compensated budgets can account

for the differences in demand elasticity. If the latter is true, then an analysis of choice frequency using the Matching Law framework should not differentiate between normal and compensated budget conditions, as work requirements and reinforcer quantity remain stable between these conditions. We trained 8 male Long-Evans rats to make choices between chocolate and vanilla flavored soymilk by making nosepokes to spend their daily budget. The concurrent FR-schedules were set at 4:4 during baseline, and each rat went through four additional conditions: uncompensated price shifts to 5:3 and 3:5, and similar price shifts with budget compensation (5:3C and 3:5C). We found relatively inelastic demand ($0 > e > -1$) for the preferred commodity (chocolate soymilk) that was significantly less elastic than demand for vanilla. Demand for chocolate was less elastic under compensated the under uncompensated budgets. When we compared time-resolved preference estimates between compensated and uncompensated conditions, using equal-length sets of trials, we found significantly higher preferences for chocolate in the 5:3C condition. Importantly, this difference emerged before the budget would have run out, confirming that rats' preferences are dynamically sensitive to budget conditions. Accordingly, the behavioral data could only be fit in an extended Matching Law framework when allowing for an effect of budget condition on the sensitivity parameter. This parameter, which indexes under- or overmatching to non-unitary FR ratios was found to be significantly lower under compensated versus uncompensated budget conditions, confirming that compensating budgets reduces sensitivity to price changes in line with the observed reduced elasticity of demand.

Disclosures: **M. van Wingerden:** None. **C. Marx:** None. **T. Kalenscher:** None.

Poster

651. Decision Making II

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Howard Hughes Medical Institute

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Title: Exploration and uncertainty in the frontal eye field during value-guided choice

Authors: *B. A. EBITZ[1], E. ALBARRAN[2], A. SOLTANI[3], T. MOORE[2];
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Abstracts: Reward value can have profound effects on gaze, perhaps via its well-established modulation of neural activity throughout the oculomotor system. However, it remains unclear whether other factors critical for decision-making also affect gaze-related signals in the brain. In particular, the effects of exploratory states on saccadic target selection have yet to be elucidated. Moreover, although exploration is thought to increase information seeking via modulating attention, it is unclear if this state affects activity in neural structures that regulate attentional selection. In order to address this question, we recorded from neurons in the frontal eye field (FEF) of rhesus macaques while they performed a saccadic two-armed bandit task. The FEF is involved in both target selection and visual attention, and contain well-characterized classes of neurons differentially implicated in these two processes. The FEF is therefore an ideal structure in which to study the effects of exploratory states on visual selection. In the bandit task, the monkeys made decisions between saccades towards probabilistically rewarded targets. On each trial, the monkeys were presented with two targets. The monkeys made a saccade to one of the targets, at which point they were either rewarded or not, depending on the reward probability parameter of the selected target. The reward probabilities of the targets walked randomly and independently over time. In order to maximize reward intake, the monkey had to infer the current reward values via integrating reward information over past outcomes. The monkeys tracked reward history over multiple trials, outperforming simulated operators that used various alternative heuristic strategies. Therefore, we fit a model to the monkeys' choice behavior to infer the monkeys' subjective trial-by-trial estimate of the targets' underlying reward values. This model allowed us to quantify both the subjective reward value and the subjective uncertainty of each target while the monkeys made saccades. The monkeys' decisions were informed by the difference in subjective reward probability between the targets, but also by other factors not directly related to the probability of reward on the current trial, including value uncertainty. In addition, FEF responses were also dependent on value information beyond the immediate likelihood of receiving a reward for a given choice. In particular, FEF activity differentiated between exploitative or exploratory saccades to the same target. These preliminary results suggest that information-seeking saccades are represented uniquely in at least one structure implicated in attention and gaze control.

Disclosures: B.A. Ebitz: None. **E. Albarran:** None. **A. Soltani:** None. **T. Moore:** None.

Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.12/TT47

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA029613

Title: Reciprocal cooperation in rats playing iterated Prisoner's Dilemma

Authors: *R. I. WOOD[1], J. Y. KIM[2], G. R. LI[2];
[2]Cell & Neurobio., [1]Keck Sch. Med. USC, Los Angeles, CA

Abstracts: Humans and animals show cooperative behavior, but our understanding of the neurobiology of cooperation among unrelated individuals is limited. The classic laboratory model to test cooperation in humans is the Prisoner's Dilemma game, where two players receive varying payoffs for cooperation or defection. We tested rats with Prisoner's Dilemma using operant responses for food reward. An operant chamber was bisected by a metal screen, with a retractable lever and pellet dispenser on each side. Cage-mates were tested as pairs in 25 trials/day over 4 days. When the levers extended, each rat had 2 seconds to respond. Mutual cooperation (Reward) delivered 3 pellets to each rat, mutual defection (Punishment) provided no pellets, and unilateral defection (Temptation) gave 5 pellets while the cooperative partner (Sucker) received none. In 8 pairs of rats, cooperation was defined by withholding a response (Reward-). In 7 pairs, cooperation was defined by responding on the lever (Reward+). Rats in the Reward- and Reward+ groups received an average of 57.3±3.4 pellets/day, 54.0±3.8% from Reward trials and 46.0±3.8% from Temptation. Individual rats cooperated on 62.0±3.6% of all trials, resulting in 40.4±3.3% Reward trials, 43.1±1.6% Temptation/Sucker, and 16.5±3.0% Punishment. However, rats did not use either tit-for-tat or Pavlov response strategies. In each pair, the dominant rat made more responses (15.5±1.5 per day) than the subordinate (12.2±1.4 responses, $p<0.05$). Dominant rats earned more pellets than subordinates in Reward- pairs (62.7±5.3 vs 43.2±7.0), but received fewer pellets in Reward+ pairs (55.8±5.1 vs 68.6±6.8). In the same bisected chamber, we subsequently tested pairs of rats for reciprocity in 24 alternating trials/day. A response on the lever within 10 seconds delivered 3 pellets to the partner. Reciprocity was correlated in pairs of rats when tested either with cage-mates ($R2=0.67$) or in unfamiliar pairs ($R2=0.73$). However, rats made more responses for their cage-mate (6.3±0.4 responses/12 trials) than for an unfamiliar partner (5.3±0.4). When no partner was present, responses declined significantly to 3.8±0.4 per 12 trials after 5 days, but increased to 6.2±0.7 responses/12 trials on the first day when paired with an unfamiliar partner who never reciprocated (bad stooge). These results demonstrate that rats working for food reward show both reciprocity and reciprocal cooperation in an iterative Prisoner's Dilemma game. Their responses

depend on familiarity with their partner and dominance status. This can be a useful model to explore the neurobiology of cooperative behavior.

Disclosures: **R.I. Wood:** None. **J.Y. Kim:** None. **G.R. Li:** None.

Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.13/TT48

Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant 085739

Title: Environmental rearing effects on impulsivity, reward sensitivity, and behavioral flexibility

Authors: ***Z. WANG**[1], A. T. MARSHALL[2], K. KIRKPATRICK[2];
[2]Psychological Sci., [1]Kansas State Univ., Manhattan, KS

Abstracts: The goal of the current study was to test the effect of the early-life rearing environment, especially novel object and/or social enrichment, on impulsive choice behavior, behavioral flexibility, action inhibition, and reward sensitivity in rats. Four rearing conditions were implemented (n = 6): isolated, isolated with novel objects, socially paired, and socially paired with novel objects. Subsequently, an impulsive choice task was delivered, in which the rats were given a choice between a 10-s smaller-sooner (SS) versus a 30-s larger-later (LL) reward. The SS reward was one pellet throughout the task, whereas the LL reward was one, two or three pellet(s) manipulated across three phases. All rats were then tested on a set-switching task to assess behavioral flexibility. Afterwards, a differential reinforcement of low rate (DRL) 30-s procedure was delivered to measure the rats' ability to inhibit action. Finally, the rats experienced a reward sensitivity task in which they were given a choice between a smaller lever with one pellet reward on a VI-30 schedule and a simultaneously presented larger lever with one, two, or three pellet(s) changing across three phases on a concurrent VI-30 schedule. A buffer task was implemented between the tasks mentioned to minimize carry-over effects. The rats were sensitive to changes in the LL magnitude of reward, and rats from different rearing conditions showed differential acquisition efficiency in the behavioral flexibility task. These results suggest that novelty and social enrichment may affect impulsivity, reward sensitivity, and the flexibility in rule-governed learning.

Disclosures: **Z. Wang:** None. **A.T. Marshall:** None. **K. Kirkpatrick:** None.

Poster

651. Decision Making II

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.14/TT49

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant 085739

Title: The role of timing processes in three different impulsive choice procedures

Authors: ***J. R. PETERSON**, C. HILL, K. KIRKPATRICK;
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Abstracts: Measures of impulsive behavior are often used interchangeably in studies examining choice behavior. Moreover, individual differences are generally overlooked or eliminated by considering only group means and effects. This study compared three paradigms commonly used to assess the effect of delay manipulations in impulsive choice behavior and to determine the validity and reliability of each measure. Measures of both timing and choice behavior were collected for each procedure. Delay tolerance, or the ability to wait longer for a larger reward, is indicative of self control and lower impulsivity. Individual differences in timing measures within the choice paradigms and a progressive interval task were examined. Forty-eight male Sprague-Dawley rats were randomly assigned to one of three groups (n =16). The SS outcome was always a 5-s delay for 1 pellet and the LL was always 2 pellets but the delay was altered. In the first systematic procedure (Green & Estle, 2003) the LL delay incremented across phases, whereas in the second systematic procedure (Evenden & Ryan, 1996) the LL delays incremented within each session: 5, 15, 30, 60 s. In the adjusting procedure (Mazur, 1987), the LL delay increased or decreased by 1 s as a function of the rats' most recent choice. After 20 sessions, the rats were tested on one of the other two choice procedures and then were re-tested on the second procedure and finally retested on the original procedure for complete counter balancing. Next, a progressive interval schedule was used for all rats to assess delay tolerance and its potential relationship with timing and choice behavior. These data suggest that, while different procedures may yield somewhat different measures of impulsive choice and timing, individual differences are relatively stable across procedures.

Disclosures: **J.R. Peterson:** None. **C. Hill:** None. **K. Kirkpatrick:** None.

Poster

651. Decision Making II

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Program#/Poster: 651.15/TT50

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant EY022411

Title: A computational analysis of the influence of elapsed time on reward-modulated perceptual decisions

Authors: *Y. FAN[1], J. I. GOLD[2], L. DING[2];
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Abstracts: Perceptual decisions can be influenced by many non-sensory factors, including stimulus prior probabilities and expected reward outcomes. In a drift-diffusion model (DDM) of perceptual decision making, sensory evidence is accumulated over time into a decision variable (DV). When the DV reaches a threshold, a decision corresponding to that threshold is made. Under certain conditions, decisions biased by unequal prior probabilities or reward expectations can be well accounted for by adding a static bias term to the DV. Recently, however, Hanks and colleagues (2011) found that decision biases that result from unequal prior probabilities may be better accounted for using a dynamic bias that increases over time. According to this idea, elapsed time helps to calibrate the relative influence of sensory and non-sensory factors: the more time that has elapsed before reaching a decision threshold implies weaker sensory evidence and thus an increasing reliance on the priors. In this study, we tested whether a dynamic-bias DDM also performs better than a static-bias DDM for unequal reward-biased decisions. We trained a monkey to discriminate the net motion direction of a field of randomly moving dots. Motion strength was determined by the percentage of the dots moving in the same direction. The monkey indicated its choice by making a saccade to the corresponding choice target at a self-controlled time. Correct choices were rewarded with juice. In some blocks of trials, leftward correct choices were rewarded with more juice than rightward correct choices. In alternate blocks, the reward contingency was reversed. The monkey's performance was influenced by both motion strength and reward magnitude. We constructed two DDM-based bias models, with static and dynamic reward biases, respectively, and fitted them to choice and reaction-time data using maximum likelihood methods. As a control, we also constructed a DDM-based momentary-evidence model, in which reward asymmetry modulates moment-to-moment evidence. Our preliminary fitting results (n=50 sessions of data from one monkey) show that

both bias models are superior to the momentary-evidence model, but do not reveal consistent differences in the goodness of fits between the two bias models. We thus did not find evidence of a dynamic bias that increases with elapsed time in this unequal reward-biased perceptual decision. It remains to be tested whether this negative finding extends to other individual subjects and whether the discrepancy reflects differences in neural implementation of prior probability and reward expectation information. Reference: Hanks et al., J.Neurosci. 31(17):6339-52, 2011.

Disclosures: Y. Fan: None. **J.I. Gold:** None. **L. Ding:** None.

Poster

651. Decision Making II

Location: Halls A-C

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Program#/Poster: 651.16/TT51

Topic: F.02. Animal Cognition and Behavior

Title: The role of different dopaminergic populations in *Drosophila* choice behavior

Authors: C. ROHRSEN, *B. BREMBS;
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Abstracts: Dopamine (DA) appears to be an important neurotransmitter involved in assigning value in vertebrates and invertebrates. In *Drosophila*, DA neurons are known to play an essential role in associative learning. The valence of learning depends on the DA cluster involved: whereas the protocerebral anterior medial cluster (PAM) mediates appetitive learning, the protocerebral posterior lateral cluster 1 (PPL1) mediates aversive learning. Studies in the fruit fly have focused in these two clusters because they are the DA clusters projecting to the mushroom body, an essential brain structure for olfactory classical conditioning. Assuming that neurons mediating appetitive and aversive valence in conditioning experiments ought to influence choice behavior also directly, we decided to identify DA neurons, projecting and non-projecting to the mushroom bodies, which are directly involved in choice behavior. For assessing the role of different DA clusters in mediating valence we took advantage of flies expressing Channelrhodopsin (ChR) under different DA promoters. These flies were tested in a T-maze so that if an individual fly approached one arm illuminated by the ChR excitable-wavelength, a given cluster is activated whereas when approaching the other arm with non-excitable light, no neurons are activated. In contrast to our expectations, we found that the flies preferred activity in most, but not all of the different DAergic subpopulations we were able to test. Interestingly, the

pattern of preference we obtained did not correlate very well with the pattern of preference obtained using classical conditioning protocols. We hypothesize that the DA neurons function rather differently when modulating actively ongoing behavior as opposed to when the animals are passively perceiving stimuli in a classical conditioning experiment.

Disclosures: C. Rohrsen: None. **B. Brembs:** None.

Poster

651. Decision Making II

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Topic: F.02. Animal Cognition and Behavior

Support: DAAD Postdoc Fellowship

Title: Behavioral flexibility in *Drosophila* phototaxis

Authors: *E. A. GOROSTIZA, B. BREMBS;
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Abstracts: Animals exhibit innate preferences for different stimulus modalities and intensities, which likely reflect evolutionary responses to specific ecological needs. Insects such as *Drosophila* move towards a light source when startled. Given the robustness of this response, positive phototaxis has been categorized as an example of hard-wired input-output behaviors. However, classic experiments performed by McEwen in 1918 and Benzer in 1967 demonstrated that wing defects, caused by mutation or damage, profoundly affect phototaxis preferences in walking *Drosophila* [1,2]. The fact that manipulating an unrelated organ (wings), affects positive phototaxis stands against the hard-wired conception of this behavior, showing that it contains a certain element of flexibility. We hypothesize that phototaxis is not just an automated response, but that there may be a central decision-making stage influenced by a number of environmental and physiological variables, such as flying ability, that are continually monitored. To test our hypothesis we evaluated flies in two different phototactic paradigms (Benzer Countercurrent Apparatus and T-Maze) after altering their flying ability using a range of mechanical and genetic manipulations. Here we show that flies unable to fly exhibit a negative phototactic behavior. This reversal is not learned, as neither learning mutants nor transgenic flies deficient in various learning paradigms show any deficit, and the effect is immediate. The effect is neither due to injury, as injuries not affecting flight ability do not affect phototaxis. Genetic manipulations

preventing the flies from flying but leaving wing-morphology intact also affect phototaxis. Finally, if flying ability is temporarily compromised and then restored, the phototactic behavior changes concomitantly, demonstrating the reversibility of the phototactic effect. These results reveal the flexibility of this taxis, and the existence of an evaluation step prior to behavioral performance. 1. McEwen, R. S. The reactions to light and to gravity in *Drosophila* and its mutants. *J. Exptl. Zool.* **25,** 49-106 (1918). 2. Benzer, S. BEHAVIORAL MUTANTS OF *Drosophila* ISOLATED BY COUNTERCURRENT DISTRIBUTION. *Proc. Natl. Acad. Sci. U. S. A.* **58,** 1112-9 (1967).

Disclosures: E.A. Gorostiza: None. **B. Brembs:** None.

Poster

651. Decision Making II

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Topic: F.02. Animal Cognition and Behavior

Support: Global Common Operating Environment Program of the Japanese Ministry of Education, Culture, Sports, Science and Technology in the form of the "Basic and Translational Research Center for Global Brain Science" at Tohoku University

Title: Dopaminergic and serotonergic modulation of anterior insular and orbitofrontal cortex in risky decision making

Authors: *H. ISHII[1], S. OHARA[1], Y. KAIZU[1], P. N. TOBLER[2], K.-I. TSUTSUI[1], T. IIJIMA[1]; [1]Div. of Sys. Neurosci., Tohoku Univ., Miyagi-Ken Sendai-Si, Japan; [2]Dept. of Econ., Univ. of Zurich, Zurich, Switzerland

Abstracts: To obtain desired outcomes we sometimes need to take risks although excessive risk taking also can lead to ruin. However, dysfunction of two neuromodulators, dopamine and serotonin can cause abnormal risky decision making (Cools et al, 2011; Takahashi, 2012; Rogers, 2011). Previous animal model studies using systemic manipulations of dopamine and serotonin have shown that these neuromodulators are involved in risky decision making. Amphetamine increases risk preference of rats, an effect that is blocked by co-administration of either D1 or D2 receptor antagonists (St Onge and Floresco, 2009). Transgenic mice lacking GABAA receptors in dopamine neurons show higher risk preference than controls (Parker et al, 2011). Moreover, serotonin-depleted monkeys and rats show higher risk preference (Long et al,

2009; Koot et al, 2012). However, how dopamine and serotonin work in their target regions remains unclear. The present study investigated the role of dopamine and serotonin in the rat anterior insular cortex (AIC) and orbitofrontal cortex (OFC), which make different contributions to risky decision making (Ishii et al, 2012). We examined the effects of local injection of either the D1 (SCH23390), D2 (eticlopride), 5-HT1A (WAY100635) or 5-HT2A (M100907) receptor antagonists into the AIC or OFC on risk preference in a gambling task. The gambling task required the rats to choose between a risky option (4 drops or no water, 50-50 chance, random order) and a sure option (2 drops). Intra-AIC injection of the D2R and 5-HT1AR blockers increased risk preference, whereas intra-OFC injection of the 5-HT1AR blocker decreased risk preference. Risk preference was not altered by intra-AIC injection of D1R and 5-HT2AR blockers or by intra-OFC injection of D1R, D2R, and 5-HT2AR blockers. Furthermore, intra-AIC injection of the D2R blocker increased risk preference particularly after winning in a previous risky choice, whereas intra-AIC injection of the 5-HT1AR blocker increased risk preference after losing. These results suggest that different dopamine and serotonin receptor subtypes in the AIC and OFC play different and experience-dependent roles in risky decision making.

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Poster

651. Decision Making II

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Title: Reduced temporal discounting by methylphenidate in non-human primates

Authors: A. Z. RAJALA[1], R. L. JENISON[2], *L. C. POPULIN[3];
[1]Neurosci. Training Program, [2]Dept. of Psychology, [3]Dept of Neurosci., Univ. Wisconsin, MADISON, WI

Abstracts: When given a choice, humans and animals prefer a smaller reward immediately over a larger reward that is delayed in time, a phenomenon known as temporal discounting. Impulsivity, the tendency to act without forethought, is associated with impaired dopamine (DA) transmission and excessive discounting of larger delayed rewards, which has been documented in patients with attention deficit hyperactivity disorder combined type (ADHD-C). Delayed reward value is encoded in the discharges of DA neurons and DA neurotransmission is crucial for reward processing. Methylphenidate (MPH), which blocks the DA transporter and increases extracellular DA in the basal ganglia and prefrontal cortex, is a primary treatment for ADHD and, at low doses, ameliorates impulsivity in both humans and animals. We tested the hypothesis that low doses of MPH will reduce temporal discounting in both impulsive and calm subjects, shifting preference towards larger delayed rewards. Two male rhesus monkeys, one impulsive and one calm, were tested in a reward preference task that required them to choose between a smaller reward right away (SS) or a larger reward after a delay (LL). The task required fixating a red dot straight ahead, after which two images appear to the right and left of the fixation representing the SS and LL. When the fixation dot was turned off, the subject made an eye movement towards one of the images indicating his choice. Eight fractal images represented each of the 4 SS and LL conditions. The SS delay was constant at 0 sec with 4 reward magnitudes (0.2, 0.3, 0.4, and 0.5 mL) while the LL reward magnitude was constant at 0.59 mL with 4 delay times (2, 4, 8, and 16 sec). A set of 16 blocks of 14 trials each contained all possible combinations of SS and LL and were presented in either ascending or descending order. The inter-trial time was adjusted to have constant trial length within each block. Methylphenidate was administered orally, dissolved in 0.5 mL juice 45 min prior to testing; juice alone was delivered on control days. Both monkeys exhibited hyperbolic temporal discounting estimated from a single logit probability choice model. The impulsive subject had steeper discounting and chose the SS significantly more often compared to the calm. Low doses of MPH shifted the choices of both subjects toward the LL and reduced discounting of LL reward value. Furthermore, MPH increased the randomness of choice significantly in a dose dependent manner, and decreased overall reaction time (RT) for both monkeys. The RT function has an inverted U-shape, which differentially shifts under MPH. These results suggest that MPH could improve impulsive behavior by altering the computation of reward value.

Disclosures: **A.Z. Rajala:** None. **R.L. Jenison:** None. **L.C. Populin:** None.

Poster

651. Decision Making II

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Program#/Poster: 651.20/TT55

Topic: F.02. Animal Cognition and Behavior

Support: DA10588

Title: Genetic influences on delay discounting

Authors: *L. MILITELLO[1], J. B. RICHARDS[1], A. A. PALMER[2];
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Abstracts: Delay discounting (DD) was tested using a sequential choice procedure that simulates contingencies of reinforcement similar to those that animals encounter while foraging for important resources in patchy environments. Rats chose between an immediate but small amount of water by staying in a rapidly depleting patch and a larger amount of water that required the animal to invest the time needed to change to a new full patch. Longer delays to a new patch make staying in the old depleting patch a better choice, while shorter delays to new patches make staying in the old depleting patch longer a worse choice. Greater DD is indicated by longer stays in the depleting patch while consuming smaller amounts of water. Optimal foraging theory predicts that evolutionary processes will select genes which enable the animal to maximize the long term intake amount. Performance on DD tasks has been used as a measure of impulsivity and there is evidence that more rapid discounting is associated with drug abuse in humans and drug self-administration in animals. In a previous paper (Richards et al, 2013), we reported strong heritability for this behavior as measured by strong between-strain differences in DD using this procedure. Here we extend those results with comprehensive testing over a 6 month time period of two of the previously studied rat strains: August Copenhagen Irish (ACI/SegHsd) and Dahl Salt Sensitive (SS/JrHsd). The rats were tested on a variety of discounting procedures in which changing patches involves either long delays to the new patch, low probabilities of finding a new patch or both. In all cases the SS rats discounted less than the ACI rats. In relatively rich testing contexts (shorter delays and higher probabilities), the decision strategy of the SS rats produced larger overall rates of water consumption. In poor testing contexts (longer delays and lower probabilities), however, the decision strategy of the ACI rats produced larger overall rates of water consumption. Strain differences in domestic rats produced consistent biases in decision-making which may direct individual animals away from (or toward) optimality. Domestic rats, however, may have alleles that are quite different from those that would be found in a natural population and it is unclear if similar genetic effects would be observed in wild rats that are exposed to selective pressure. These results suggest that genetically

determined biases in decision-making may underlie personality differences described as 'impulsivity' in humans.

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Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.21/TT56

Topic: F.02. Animal Cognition and Behavior

Title: Expected information signals in the posterior cingulate cortex

Authors: *D. L. BARACK[1], J.-F. GARIEPY[2], M. L. PLATT[2];
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Abstracts: Animals must balance learning about the environment with collecting rewards. The neural mechanisms that encode rewards and their predictive cues are well-understood. The neural mechanisms that encode information about the environment independently of reward value, by contrast, remain unexplored. Rewards collected while foraging carry information about the state of the environment, useful for guiding future decisions, and animals may anticipate the information learned from these decisions. We hypothesize that foraging animals maximize this expected information, defined as the amount of information learned from an outcome multiplied by the probability of that outcome, with independent neuronal encoding of expected information and expected value. We test these ideas with a novel behavioral paradigm that decorrelates expected value and expected information in a single task. In our experiment, two different fixed rewards randomly assigned to two of six locations in the environment. The rewarded targets' locations vary from trial to trial and are uncorrelated, though every target had to be selected on each trial in order to continue to the next trial. The amount of information about the reward sequence on every trial decreased over the course of the trial, while the expected value of upcoming actions could decrease or increase, depending on that trial's history of received rewards. Response times correlated with expected value and expected information over the course of the trial, with faster responses for higher value or greater information, as well as slower response for later choices in the trial (multilinear regression, $p < 0.05$). Cells in the posterior cingulate cortex (CGp), an area known to be involved in strategic choice, policy selection and adaptive behavior, preferentially encode expected information over expected value. We collected 33 cells from CGp from one animal running our task. During a whole choice epoch, from 500 ms

before feedback to 250 ms after, CGp cells encode the choice number in a trial (GLM, $p < 0.05$, 13/33 cells) and expected information (16/33), but not the expected value (0/33) of a choice. Our data suggest that an animal's choices are influenced by both expected value and expected information when exploring an environment with an unknown spatial distribution of rewards, and that expected information dominates CGp cell encoding, possibly serving as a substrate for the influence of expected information on decision behavior.

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Poster

651. Decision Making II

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Program#/Poster: 651.22/TT57

Topic: F.02. Animal Cognition and Behavior

Title: How indirect pathway works - Stable object value coding by pallidal neurons

Authors: *H. F. KIM, O. HIKOSAKA;
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Abstracts: The output of the striatum is mediated by direct and indirect pathways which are thought to control behavior in opposite manners: direct pathway for GO and indirect pathway for NOGO. However, an important question remains unsolved, especially for the indirect pathway: What information is used for NOGO? A key structure of the indirect pathway is globus pallidus external segment (GPe), but little is known about its information processing. GO-NOGO mechanism would be critical when animals choose high-valued objects (GO) among low-valued objects (NOGO). Our studies have shown that tail of caudate nucleus (CDt) is necessary for choosing high-valued objects based on long-term experience (Kim & Hikosaka, 2013). Here we examined how GPe neurons process such stable value information. After injecting CTB in the CDt, we found that anterogradly labeled axon terminals were localized in the caudal-ventral region of the GPe (GPe(cv)) (indirect pathway) in addition to the caudal-dorsal-lateral region of the substantia nigra pars reticulata (SNr(cdl)) (direct pathway). We then recorded activity of single GPe(cv) neurons while high-valued and low-valued fractal objects were presented to monkey. The monkey had experienced each of these objects in association with a water reward or no reward consistently for many days, and thus had developed a strong preference of looking at reward-associated (high-valued) objects (GO) and avoiding non-reward-associated (low-valued) objects (NOGO), even when no reward outcome was presented. In GPe(cv) we found

many neurons that responded to these fractal objects. Among 31 visually responsive GPe(cv) neurons, 18 neurons (58.1%) responded to the objects differentially by their stable values. Importantly, a majority of them (n=13) were inhibited more strongly by low-valued objects than high-valued objects. Since GPe has inhibitory connections to the basal ganglia output regions, an inhibitory response of GPe(cv) neurons would be converted to a disinhibition of SNr(cdl) neurons. Indeed, SNr(cdl) neurons are excited by low-valued objects (Yasuda et al., 2012). These results suggest that the object-sensitive indirect pathway, CDt-GPe(cv)-SNr(cdl) circuit, transmits stable value information which would be used to suppress saccades to low-valued objects.

Disclosures: H.F. Kim: None. **O. Hikosaka:** None.

Poster

651. Decision Making II

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Topic: F.02. Animal Cognition and Behavior

Support: NIAAA Intramural Research Program

Title: A 3-choice visual discrimination learning and reversal task for mice

Authors: *K. KAUGARS, H. BERGSTROM, M. REGER, L. HALLADAY, E. BUSCH, C. PICKENS, M. BACHU, A. HOLMES;
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Abstracts: Alcohol use disorders and other addictions are characterized by cognitive abnormalities. Pairwise discrimination and reversal paradigms have proven valuable to studies of the neural mechanisms underlying rewarded learning and cognitive flexibility. One limitation of procedures that use 2 stimuli is that, following reversal of learned stimulus-reward contingencies, it is unclear whether errors committed are due to a failure to inhibit responding to the previously rewarded stimulus, or an inability to form the new stimulus-reward association. To better disambiguate perseveration and learning during reversal, we tested the performance of mice in a 3-stimulus task. In this task, the mouse is presented with 3 stimuli during discrimination, one of which is rewarded, and trained to criterion (75%) levels of accuracy. On reversal, the same 3 stimuli are presented: the previously rewarded stimulus is now unrewarded (‘previous’), one of the previously non-rewarded stimuli is now rewarded (‘new’) and the other non-rewarded

stimulus remains unrewarded ('never'). Responses to the 'never' stimulus represent learning errors, while responses to 'previous' will primarily (though not exclusively) reflect perseverative errors. To investigate the neural circuits mediating performance in the task, C57BL/6J mice received bilateral excitotoxic lesions of the PL prior to reversal testing and were compared to sham controls for learning and perseverative errors. Additionally, mice with deletions of the GluN2A (brain-wide) or GluN2B (in cortical pyramidal neurons) NMDA receptor subunits were phenotyped for discrimination and reversal. These experiments could provide new insight into the role of the prelimbic cortex and NMDA receptors in rewarded learning and cognitive flexibility, with potential implications for understanding the neurobiology of cognitive abnormalities in psychiatric disorders and addictions. Research funded by the NIAAA Intramural Research Program.

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Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.24/TT59

Topic: F.02. Animal Cognition and Behavior

Support: Brain Research Foundation

Title: Short- and long-term effects of adolescent methylphenidate exposure on decision-making in rats

Authors: *L. R. AMODEO, M. S. MCMURRAY, C. R. SHORT, J. D. ROITMAN; Psychology, Univ. of Illinois, Chicago, Chicago, IL

Abstracts: Adolescence is transitional stages hallmarked by decisions that disproportionately favor immediate rewarding outcomes. This lack of forethought can be consequently dangerous. While an increase in behaviors that are impulsive and risky are normal in this stage of development, failure to inhibit inappropriate behaviors, such as experimentation with alcohol or illicit drugs, can lead to detrimental consequences. Differences in the developmental stage of prefrontal cortical and subcortical systems in adolescents might explain the relative increase in maladaptive decisions during this period. Specifically, the orbitofrontal region of prefrontal cortex (OFC) has been shown to play a central role in processing value expectation in a Delay

Discounting task. Recent evidence suggests that dopaminergic innervation of the OFC plays an important role in mediating delay discounting choices and pharmacological treatment with methylphenidate (MPH; an ADHD medication) decreases impulsive behavior in adolescents. For this study, adolescent rats were injected with MPH (2, 5, 10 mg/kg/day) twice daily from PD35 to PD52. During this period, rats performed a progressive delay discounting task, in which rats were given the choice between two levers: one leading to a smaller sooner (SS) reward and the other to a larger later (LL) reward. On each day the delay to the LL reward increased (5, 10, 15, 20, 30, 50s). In contrast to prior studies in hyperactive rats, we found that treatment with MPH did not decrease naturally impulsive behavior in normal (non-hyperactive) adolescent rats. However, continual administration of MPH during this crucial stage in development caused deficiencies in behavioral flexibility in normal rats. After discontinuation of MPH, risk-preference was subsequently assessed in adulthood. Rats performed a risk task in which they chose between small-certain and large-risky options. Probability payoff for the large-risky option (16%, 33%, 67%) varied every second day. Preliminary data indicated that the high dose of MPH (10 mg/kg) during adolescence increased risk preference in adulthood. These results provide an interesting platform for future research studying the involvement of OFC in impulsivity and risky decision-making.

Disclosures: L.R. Amodeo: None. **M.S. McMurray:** None. **C.R. Short:** None. **J.D. Roitman:** None.

Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.25/TT60

Topic: F.02. Animal Cognition and Behavior

Support: Marie Curie IEF to CREW: “Reversible Cognition” 273790

LabEx Cortex: ANR-11-LABX-0042

Fondation Neurodis

Fondation de France

Fondation CERAL

ANR

Title: Frontal beta oscillations are differentially modulated by time-on-task, cognitive control, and pauses in work

Authors: *C. R. WILSON[1,2], F. M. STOLL[1,2], M. C. M. FARAUT[1,2], K. KNOBLAUCH[1,2], J. VEZOLI[3], E. PROCYK[1,2];
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Abstracts: Beta oscillations are associated with top-down control mechanisms, but little is known about their contribution to cognitive functions. 15-30Hz oscillations are widespread in the brain, with a variety of characteristics, but it remains unclear whether they reflect a unitary or multiple mechanisms. We investigated whether frontal beta oscillations are differentially modulated by cognitive control and time-on-task. Vigilance and attention decrements over time, referred to as the time-on-task effect, have been shown to alter cognitive performance and some EEG patterns in humans. Two macaque monkeys learned the Problem Solving Task (PST), using visual feedback to search by trial and error amongst several targets for one that was associated with juice reward (the search phase, SEA), and then repeating this correct response a number of times (the repetition phase, REP). A change signal then instructed them to begin a new problem by searching again. The contrast between SEA and REP phases provides an index of cognitive control (Procyk & Goldman-Rakic 2006). Cognitive and execution performance were studied continuously through the session, during which the monkeys worked for a fixed number of problems, thus permitting time-on-task measures. Monkeys were chronically implanted with at least 22 electrodes resting on the dura mater to provide electroencephalographic (ECoG) recordings. We characterized 15-30Hz beta oscillations in the delay period of each trial prior to stimulus onset. To test for multiple modulating influences on the beta power, we used mixed effects modelling. We showed that beta power is modulated by the cognitive control demands of the task. Critically, beta oscillations in this same period showed a significant within-session change, seemingly tracking time-on-task. The separate modulations of beta oscillations by cognitive control and time-on-task did not interact. Importantly, when the monkey spontaneously paused for several minutes during the session, these beta power modulations were reset, and the time-on-task effect was re-initialised. The magnitude of this reset depended on multiple behavioural factors. This striking effect of spontaneous pauses in work strongly suggests that such pauses should be systematically taken into account in neural measures of time-on-task and fatigue. Comparisons of time-on-task changes in beta with behavioural performance suggest that modulation in beta oscillations within the session reflect a stabilization of cognitive but not execution performance throughout the session. Hence, frontal beta oscillations associated with cognitive performance are modulated by multiple separate mechanisms.

Disclosures: **C.R. Wilson:** None. **F.M. Stoll:** None. **M.C.M. Faraut:** None. **K. Knoblauch:** None. **J. Vezoli:** None. **E. Procyk:** None.

Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.26/TT61

Topic: F.02. Animal Cognition and Behavior

Support: Marie Curie IEF to CREW "Reversible Cognition" 273790

LabEx Cortex: ANR-11-LABX-0042

Fondation Neurodis

Title: From learning-set to task-set in macaque monkeys: Behaviour and neural correlates

Authors: *M. C. FARAUT, C. R. E. WILSON, E. PROCYK;
INSERM U846, Stem Cell and Brain Res. Inst., Bron, France

Abstracts: Learning from the environment in order to reach our goals efficiently, and reacting flexibly when the environment changes, are important capacities of primate behaviour that enable efficient decision making. An important process is task-set, referring to the ability to link together information that is relevant for doing a task and to modify it in a flexible manner when it ceases to be optimal. However, questions remain about how we acquire these abilities, how this flexibility is implemented, and what the neural correlates of such processes are. We tested cognitive flexibility in macaque monkeys with chronic electrophysiological recordings, using a test of their ability to adapt to changing task information in an environment that doesn't always give the right answer. 3 monkeys learned a test of Task-Set Manipulation, a monkey equivalent of the task of Collins & Koechlin (2012).The task consisted of 2 concurrent stimulus-target associations in a noisy feedback environment (10% trap feedback: an incorrect feedback was given after a correct response and a correct feedback after an incorrect response). Once the monkey reached a performance criterion, the associations were changed in an unpredictable manner. This task enables us to contrast phases of exploration and exploitation of the environment by the monkey, which require different levels of cognitive control. In order to understand the basic processes of this complex behaviour, we trained the monkeys in 2 steps. First monkeys acquired a learning set for a version of the task in which shifts between problems were signaled by changes of stimuli. A learning set is the ability to learn to learn. Learning becomes more and more efficient as more problems are solved in a task. We show that monkeys formed a stable learning set and adapted their responses to the stochastic environment. Second monkeys learned the Task-Set version of the task, in which shifts between problems were not cued any more, and the 2 stimuli always remained the same. Thus, an efficient way to perform

this task is to link the 2 stimulus-target associations into a task-set and modify this task-set when it is changed. Monkeys were able to transfer their learning set to this task, showing striking performance from the start. We show some evidence that monkeys created task-sets and modified them when contingencies changed. Finally, monkeys were implanted with chronic electrocorticography electrodes covering prefrontal and parietal cortex. We investigated task-related changes in cortical oscillatory dynamics and event-related signals.

Disclosures: **M.C. Faraut:** None. **C.R.E. Wilson:** None. **E. Procyk:** None.

Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.27/TT62

Topic: F.02. Animal Cognition and Behavior

Title: Demonstrating a stress-free way to administer drugs during behavioural testing: Modafinil restores attentional deficits in rats with lesions of the subthalamic nucleus

Authors: E. E. BOWMAN, S. XIA, D. S. TAIT, *V. J. BROWN;
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Abstracts: Choice of route of drug administration is influenced by the chemical and pharmacokinetic/pharmacodynamics properties of the compound. Modafinil - a putative cognitive enhancer - is a large molecule which does not easily dissolve in water and therefore solvents or detergents are required: modafinil can be administered to rats by i.p. injection in a vehicle of DMSO (soluble to 75 mM). However, when testing for behavioral effects of cognitive enhancers, the additional factors of timing of drug administration as well as the impact of the stress of handling during administration become important considerations. In our experience using the attentional set-shifting task, interrupting testing to administer compounds by gavage or injection can be so disruptive to performance that collection of useful behavioral data is often compromised. Furthermore, the solvents in the vehicles may also be neuroactive. In this study, we sought to test the effects of modafinil on performance in an attentional set-shifting task. We tested 14 rats, 6 controls and 8 of which had lesions of the subthalamic nucleus (STN) and a stable impairment, established over 5 testing sessions, in forming an attentional set. A modified version of the set-shifting task was used, with 9 stages, to maximise the opportunity to form an attentional set, including a series of novel acquisitions, reversal and probe stages. To minimize stress of administration during testing, the drug was given to the rats in flavored sugar-free jellies

which they ate. Individual modafinil jellies were made for each rat, with drug concentration of 30mg/kg. The rats were given jellies without modafinil several times in their home-cage in the days preceding testing to overcome neophobia. On the day of the test, a modafinil (or non-drug) jelly was given 30mins before the start of testing and a second jelly was given 60mins later. The interval between two jellies was chosen based on the established half-life of modafinil. All rats were tested once with and once without modafinil, with order of testing counterbalanced. Non-drug jellies were without behavioral effects, but jellies with modafinil significantly improved performance of the STN lesioned rats on the final ID stage, restoring the ID/ED difference to the same level as controls, from which they no longer differed. While modafinil had a significant cognitive benefit in STN lesioned rats, it was without effect in the control group. Using jellies had several advantages, including: that it overcame the difficulties of getting the drug into solution; stressful handling and injections were avoided; testing was not disrupted; and the tester was blind to drug condition.

Disclosures: **E.E. Bowman:** None. **D.S. Tait:** None. **V.J. Brown:** None. **S. Xia:** None.

Poster

651. Decision Making II

Location: Halls A-C

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Program#/Poster: 651.28/TT63

Topic: F.03. Motivation and Emotion

Support: Fondation de France 2008 005902

ANR 09 MNPS 028-01

Title: Implication of motor effort and reward size encoding in the monkey basal ganglia for decision making

Authors: S. NOUGARET[1], R. ABITBOL[2], C. BAUNEZ[1], M. PESSIGLIONE[2], *S. RAVEL[1]; [1]CNRS-AMU, Inst. De Neurosciences De La Timone, Marseille, France; [2]Inserm U1125, Univ. Pierre et Marie Curie (UPMC - Paris 6), Motivation, Brain & Behavior lab, Brain & Spine Institute, Pitié-Salpêtrière Hosp., Paris, France

Abstracts: Motivation to perform an action can be defined by the subjective value of this action, and quantified by the ratio between its cost and its benefit. It requires convergence and integration of limbic, motor and associative information to adapt behaviors accordingly. The

basal ganglia (BG) are known for their implication in processes involving these different types of information. The striatum and the subthalamic nucleus (STN), considered as the two input structures of this subcortical system, are functionally divided into 3 different areas, sensorimotor, associative and limbic, based on the distribution of the cortical inputs. The external segment of the globus pallidus (GPe) is well positioned to play a key role in the integration of information from the different striatal and subthalamic territories before their transmission to the BG output structures and, consequently to encode information about motivation to perform an action. We recorded 532 neurons in the striatum, STN and GPe of two monkeys (N=307, 93, 132, respectively), in a visuomotor task requiring a motor effort to get a reward. Four distinct associations of visual stimuli determine four cost (force to develop on a lever: small or high)/benefit (reward size: small or large) ratios for the animal. Behavioral data showed that animals discriminate the different conditions of the task and modulate their behavior accordingly, making more errors in conditions in which a high pressing force is required and less errors when the upcoming reward is large. Single-unit activities were analyzed using iterative multiple linear regressions. We first examined the influence of the internal motivational state of the animals (cumulative reward received, cumulative effort developed and number of trials performed in the session) on the baseline neuronal activity. Secondly, we investigated how the baseline activity and the effort and reward information were combined to explain the response evoked by visual stimuli. Finally, we studied the influence of the evoked response on the decision of the animals to perform or not the action. A striking result across structures was the strong impact of baseline activity, which mediated the effects of internal motivational state on behavioral outputs. Our preliminary analyses also revealed dissociations between structures, with a particular influence of the evoked response in the STN and in the limbic part of the GPe on the propensity to perform the task.

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Poster

651. Decision Making II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 651.29/TT64

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01-EY019039

Title: Self-control signals in the supplementary eye field of monkeys during a temptation task

Authors: *J. HWANG[1], E. E. EMERIC[1], V. STUPHORN[1,2];
[1]Zanvyl Krieger Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstracts: Self-control is the ability to resist rewards that are tempting, but lead to suboptimal long-term outcomes. It has been suggested that the intertemporal choice task can be used as a measure of self-control, because the task requires subjects to choose between a smaller, sooner reward (S) and a larger, later reward (L). However, it has been difficult to behaviorally estimate self-control in this task, because self-control is required only for a short period just before a choice is made and there are too many other factors that affect the choices of the subjects. Therefore, we developed a new temptation task that provides a measure of resistance to persistent temptation and trained monkey subjects with it. In every trial, two annulus-shaped targets (S and L) were presented after a fixation period. Each annulus had a colored section, whose length and color indicated the delay and amount of rewards, respectively. The subjects had to make a saccade to one of the targets and fixate it, until they received the indicated reward amount. In no-temptation trials, the unchosen target disappeared upon the choice of either target. However, in temptation trials, the unchosen target did not disappear and the subjects were allowed to change their initial choice as long as the time of the chosen target did not run out. Since temptation and no-temptation trials were randomly interleaved with no cue, the subjects' initial choice functions were identical in both conditions and therefore indicated their true preferences regardless of the conditions. Nevertheless, the subjects sometimes switched targets in temptation trials. This typically involved a switch from an initial delayed L target to an immediate S target (L-S switch). The opposite switch (S-L switch) almost never occurred. These results indicate that an L-S switch is the result of a failure in resisting temptation of the immediate reward and not the correction of a mistake in the initial choice. To investigate the neural correlates of self-control, we recorded single-unit activity from the supplementary eye field (SEF) while the subjects performed the temptation task. We found that some SEF neurons were more active when the subjects succeeded in exerting self-control and stayed with the initial delayed large reward target (L-L stay) than when they failed in exerting self-control and switched to the immediate small reward target (L-S switch). Next, we inactivated SEF by cooling it down. This caused a significant shift in the choice functions so that the subjects chose S more often at the initial choice and also switched from L to S at a shorter L delay. These results suggest that SEF has a causal role in exerting self-control.

Disclosures: J. Hwang: None. **E.E. Emeric:** None. **V. Stuphorn:** None.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.01/TT65

Topic: F.02. Animal Cognition and Behavior

Support: Israel Science Foundation (ISF) grant

Title: Comparing monkey and human multi-item memory

Authors: *S. HOCHSTEIN[1], V. YAKOVLEV[2];
[1]Hebrew Univ. Jerusalem, Jerusalem, Israel; [2]Hebrew Univ., Jerusalem, Israel

Abstracts: Are the memory capabilities of humans and monkeys similar or does one species have superior abilities? In particular, does language afford better memory facilities? To answer these questions, we compared memory capabilities of human and monkey participants in a delay-match-to-multiple-item memory task. In each trial, a series of samples was presented and the task was to detect and respond to a repetition of any previous item seen in the same trial. Two difficulties are included that are not present in standard delay-match-to-sample tasks: First, the repetition can be for any image in the trial, not only for the first image, so that participants must remember all the images seen. Secondly, repetition of an image that appeared in a previous trial is not considered a valid repetition, and should be ignored. Thus, participants need not only remember all the images of the trial, they must also remember if the image was seen in the current trial. In general, we used novel images that had not been seen before, introducing a small number of catch images that had appeared in previous trials, which should be ignored. Performance Hit rate is similar for monkeys and humans, about 90% except for the longest trials. The False Positive (FP) rate is very different, however, about 80% for monkeys and 30% for humans, for images from the preceding trial. When monkeys are intensively trained with a limited set of images, they necessarily develop a reset mechanism that allows them to reject the frequent presentation of images seen in earlier trials. Interestingly, they are able to transfer this reset capability to task performance with novel (and catch) images, reducing the FP rate to below 20%. Thus, there is a surprising similarity between human performance and monkey performance following intensive training with a limited set of images, forcing acquisition of a reset mechanism. This similarity is found even for human performance without such prior training. We conclude that human participants have an inherent reset mechanism before visiting our laboratory.

Disclosures: S. Hochstein: A. Employment/Salary (full or part-time):; Hebrew University, Jerusalem. 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 from Israel Science

Foundation (ISF). **V. Yakovlev:** A. Employment/Salary (full or part-time):; Hebrew University, Jerusalem.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.02/TT66

Topic: F.02. Animal Cognition and Behavior

Support: Pioneer Grant 2013008915

NRF Grant 2012R1A2A2A02011838

Title: Cav2.1 P/Q type calcium channel in CA1 region contributes to properties of the place field as well as neuronal firing pattern of the pyramidal cells

Authors: *D. JUNG[1,2], H. SHIN[3], J. CHO[1,4];
[1]Seongbok-Gu, Korea Institude of Sci. and Technology(Kist), Seoul, Korea, Republic of; [2]Univ. of Sci. and technology, Daejeon, Korea, Republic of; [3]Inst. for Basic Sci., Daejeon, Korea, Republic of; [4]Univ. of Sci. and Technol. (UST), Daejeon, Korea, Republic of

Abstracts: The hippocampus has a critical role in learning and memory and has been especially known to process the spatial information in respect to place-dependent firing of the pyramidal neurons. The hippocampus has distinct firing modes (tonic and burst firing) and burst firing is thought to transmit signals and participates more efficiently in formation of hippocampal-dependent memories. It is evidenced that Voltage gated calcium channels (VGCCs) in the hippocampus modulate the action potentials and the distinct properties of different types of VGCCs result in different structures of firing pattern. However, electrophysiological and behavioral effects of Cav2.1 P/Q type calcium channel in the hippocampus have not been well studied in spite of its abundant expression. Therefore, we examined the role of Cav2.1 P/Q type calcium channel by measuring unit signals of behaving mice with CA1 specific deletion of Cav2.1 P/Q type calcium channel (CA1-Cav2.1 KO). The mice were introduced to an open-field arena with a local cue attached for 2 consecutive sessions with a 30 min interval in order to investigate firing pattern and spatial representation of place cell during exploring the same environment. In the firing pattern between the groups in each session showed the same tendency in terms of burst firing: The ratio of burst and burst length were modulated in the CA1-Cav2.1 KO mice compared to the control. In addition, the spatial information in the CA1-cav2.1 KO

group was decreased in both sessions, suggesting that deletion of Cav2.1 calcium channel reduced the ability to encode the location information using a local cue. Moreover, the CA1-Cav2.1 KO mice showed reduction in the similarity index of the place field between the two sessions, indicating retention of memory about the same environment was impaired in the KO mice. Together, this study suggests that the Cav2.1 P/Q type calcium channel in CA1 region modulates not only the intrinsic neuronal discharging but also spatial representation of place cells.

Disclosures: D. Jung: None. **J. Cho:** None. **H. Shin:** None.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.03/TT67

Topic: F.02. Animal Cognition and Behavior

Title: Diet-induced obesity induces insulin resistance and hyperactivity

Authors: *S. BLYTHE, S. MARWITZ, L. WOODIE;
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Abstracts: The prevalence of obesity in children and adolescents has increased rapidly over the past 30 years, as has the incidence of attention deficit hyperactivity disorder (ADHD). In 2012, it was found that overweight children have a twofold higher chance of developing ADHD than their normal weight counterparts. Previous work from our lab and others has documented learning and memory impairments linked to consumption of a Western-style diet in rats, but the relationship between diet and ADHD-like behaviors has yet to be explored using animal models. Therefore, the purpose of this study was to explore the role of diet in the etiology of attention and hyperactivity disorders using a rat model of diet-induced obesity. Male, Sprague Dawley rats (p40) were fed either a control diet (n=8) or a Western-style diet (n=9) for ten weeks, and the physiological and behavioral effects were examined. Tail blood samples were collected to measure fasting blood glucose and insulin levels in order to assess insulin insensitivity. Rats also performed several behavioral tasks, including the novel object task, attentional set-shifting task, and open field test. Rats exposed to a Western-style diet (67.4 ± 5.03 pmol/L) had significantly higher fasting insulin levels than controls (28.8 ± 3.64 pmol/L), but both groups had similar glucose levels. The quantitative insulin sensitivity check index (QUICKI) indicated the development of insulin resistance in Western-style diet rats. Furthermore, control diet animals

were able to discriminate between old and novel objects (exploration ratio: 0.72 ± 0.11), whereas the Western-style diet animals were significantly impaired in object recognition (0.56 ± 0.08). However, regardless of dietary condition, rats were able to perform the attentional set-shifting paradigm. Finally, performance on the open field test indicated that Western-style diet induced pronounced hyperactivity. Western-style animals spent considerably more time moving (280 ± 12 seconds) compared with control animals (200 ± 12 seconds). While Western-style diet impairs episodic memory and induces hyperactivity, attentional set-shifting capabilities are unaffected. With the increasing prevalence of both obesity and ADHD, understanding the potential links between the two conditions is clinically important.

Disclosures: S. Blythe: None. **S. Marwitz:** None. **L. Woodie:** None.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.04/TT68

Topic: F.02. Animal Cognition and Behavior

Title: Effects of ginkgo biloba supplementation on locomotor activity and anxiety levels in aged female rats

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[1]Dept. of Physiol. Fac. of Med. Selcu, Konya, Turkey; [2]Dept. of Physiol., Fac. of Med. Selcuk Univ., Konya, Turkey

Abstracts: The aim of study was to investigate the effects of ginkgo biloba supplementation on locomotor activity and anxiety levels in aged female rats. The study protocol was approved by the Local Ethics Committee. The principle of laboratory animal care of the National Institute of Health guideline was followed in all these experiments. Twenty-eight female Wistar rats were divided into the four groups according to their age (young vs. aged) and treatment (ginkgo biloba vs. vehicle) status. Supplements were given once daily for a period of 30 days, beginning 28 days prior to and 2 days during the behavioral tests. Locomotor activity was assessed in open field (OF) and anxiety levels were measured in elevated plus maze (EPM). All behavioral tests were recorded online and analyzed offline with analytical software. Both locomotor activity and exploratory behavior were lower and anxiety levels were higher in the aged rats compared to the young rats ($P<0.05$). In conclusion, aging affects spatial and emotional memory in different aspects and ginkgo biloba supplementation has limited protective effects in aged female rats.

Disclosures: **N. Okudan:** None. **M. Belviranli:** None.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.05/TT69

Topic: F.02. Animal Cognition and Behavior

Title: The role of the supramammillary area in spatial learning and memory

Authors: *H. SHIM[1], H.-J. PARK[2], H. LEE[2], I. SHIM[2];
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Abstracts: The supramammillary area (SuM) of the hypothalamus, although small in size, has wide spread connection with numerous brain structures. It is known that the SuM can control the frequency of the hippocampal theta rhythm, which plays a role in the cognitive functions of the hippocampal formation. In order to examine the role of the specific cells of the SuM in learning and memory, selective cholinergic neurotoxic or excitotoxic lesioned rats of the SuM were tested for spatial memory on the Morris water maze (MWM) test. After the behavior tests, the expression of acetylcholine esterase (AChE) in the hippocampus was studied using the immunohistochemistry. In the MWM test, both lesion of the SuM with 192 IgG-saporin and ibotenic acid produced the impairment of spatial learning and memory. In the immunohistochemistry, the SuM-lesioned rat model by selective cholinergic neurotoxin showed decrease in the AChE expression in the hippocampal CA3. These findings suggest that cholinergic cells of the SuM area play a critical role in the process of consolidation of memory.

Disclosures: **H. Shim:** None. **H. Park:** None. **H. Lee:** None. **I. Shim:** None.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.06/TT70

Topic: F.02. Animal Cognition and Behavior

Support: Facultad de Medicina. HERMES 16355

Title: Behavioral and neurobiochemical comparative study between males and females that have been separated from their mother during nursing

Authors: L. CORREDOR-VELANDIA, *Z. DUENAS;
Ciencias Fisiológicas, Univ. Nacional De Colombia, Bogota DC, Colombia

Abstracts: Early experiences affect brain development and the behavior of individuals. Clinical studies assessing the consequences of adverse early experiences such as child abuse, maternal neglect and psychosocial stress, suggest that these factors may increase susceptibility to developing several psychopathologies, particularly during adolescence and adult ages. A study was made searching for possible differences in the expression of GFAP and BDNF proteins in adult Wistar rats that were submitted to maternal separation during nursing, as well as the relationship of this with the assessment of spatial learning made with Barnes maze, on the prelimbic cortex, cingulate cortex, hippocampus and amygdala, areas well known for their role in memory processes. Wistar rats with reversed light-dark cycle with water and food ad libitum were separated from their mother from postnatal day 1 and until day 21, in two periods of 180 minutes. During the period of 60 to 65 days, the performance of rats on the Barnes maze was assessed and immunohistochemistry for GFAP and BDNF was made. Spatial learning measured with latency, errors, path length and exploration of the escape hole were significantly different in the groups studied. Positive cells were counted in the mentioned areas finding that maternal separation during nursing leads to a reduction of the amount of positive cells for both proteins as well as a differential significant effect between separated males and females.

Disclosures: L. Corredor-velandia: None. **Z. Duenas:** None.

Poster

652. Learning and Memory: Physiology II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 652.07/TT71

Topic: F.02. Animal Cognition and Behavior

Support: UFOP

CAPES

FAPEMIG

CNPq

Title: High fat diet-induced obesity promotes anxiety-like behavior in Wistar rats

Authors: *S. I. NORONHA, A. R. R. ABREU, G. S. V. CAMPOS, A. M. A. DE SOUZA, D. A. CHIANCA JR, R. C. A. MENEZES;
Dept. of Biol. Sci., Federal Univ. Of Ouro Preto, Ouro Preto, Brazil

Abstracts: It has been shown that obesity potentiates the cardiovascular response to stress in rats due to an inefficient GABAA-mediated inhibition within the dorsomedial hypothalamus. Changes in GABAergic inhibition within the hypothalamus have been linked to generalized anxiety disorder and panic disorder. Thus, the aim of our study was to evaluate the effect produced by a high fat diet 45% w/w fat (HFD) on the behavior of rats. We investigated the anxiety-like and panic-like behaviors in these animals by using the Elevated-T-Maze (ETM), an apparatus that permits determining behavioral changes related to escape, inhibitory avoidance and memory. Male Wistar rats (100 ± 10g) were fed with CD (n=16) and HFD (n=17) for 9 weeks. These groups were submitted to an ETM during 3 subsequently days. On the first day animals were exposed to an open arm of the ETM for 30 min. On the second day, the animals were placed, initially, at the distal end of the enclosed arm of the ETM, facing the intersection of the arms. The time taken by the rat to leave this arm with four paws was recorded (avoidance 1). This measurement was repeated in two subsequent trials (avoidance 2 and 3) at 30s intervals. After 30s from avoidance training, rats were placed at the end of the open arm, and the time taken to leave this arm with four paws was recorded 3 times, (escape 1, 2 and 3), with 30s intervals between it. On the third day animals were replaced once on the enclosed arms and after 30s on the open arm. A cutoff time of 300s was established for the avoidance and escape latencies. Our results showed that obesity facilitate ETM avoidance, an anxiogenic response (diet effect [$F(1,124=16,57, p<0,05$]). When compared to control (125 ± 34 s) the obese (231±22 s, Bonferroni post hoc test; $p<0.05$) animals spent more time in the enclosed arm during the avoidance 3. The third day of experiment confirm this results, showing that the control rats (135±33 s) spent less time in the enclosed arms than the obese rats (248±24 s, Bonferroni post hoc test; $p<0.01$). No differences were observed on escape tests. These results indicate that obese animals fed with a high-fat-diet exhibit anxiety-like behavior but not panic-like behavior.

Disclosures: S.I. Noronha: None. **A.R.R. Abreu:** None. **G.S.V. Campos:** None. **A.M.A. de Souza:** None. **D.A. Chianca Jr:** None. **R.C.A. Menezes:** None.

Poster

652. Learning and Memory: Physiology II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Training Grant T32 AG000114

NIA Grant RO1 AG28488

University of Michigan Nathan Shock Center

Title: Over-expression of the L-type voltage-gated calcium channel CaV1.3 leads to alterations in neuronal function associated with aging

Authors: *S. J. MOORE[1], J. N. SLATER[1], G. G. MURPHY[1,2];
[1]Mol. & Behavioral Neurosci Inst., [2]Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstracts: Cognitive impairments in the aged population are usually associated with neurodegenerative disorders like Alzheimer's disease, but in fact they often occur in the absence of any overt pathology. However, the neurobiological mechanisms that mediate this "normal" age-related cognitive decline are not fully understood. Previous work in rodent models has shown that aging is associated with an up-regulation of L-type voltage-gated calcium channels (LVGCCs) and that this increase is correlated with age-related memory impairments in a hippocampus-dependent learning and memory task. We have generated a novel transgenic mouse line in which the αCamKII promotor drives exogenous expression of an HA-tagged subtype of LVGCCs (CaV1.3) to directly examine the relative contribution of increased LVGCCs to cognitive function. We have shown that young CaV1.3 HA+ mice exhibit impairments in hippocampus-dependent learning and memory tasks, such as the Morris water maze and contextual fear conditioning. We now investigate molecular mechanisms that may contribute to these cognitive deficits by examining electrophysiological measures of neuronal function, including alterations in intrinsic excitability and synaptic plasticity. Taken together, our results suggest that an age-dependent up-regulation of the LVGCC CaV1.3 contributes to cognitive deficits in learning and memory and thus may represent a novel therapeutic target for the amelioration of age-related cognitive decline.

Disclosures: S.J. Moore: None. **J.N. Slater:** None. **G.G. Murphy:** None.

Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.09/TT73

Topic: F.02. Animal Cognition and Behavior

Support: EU GOAL LEADERS 270108

Title: Sustained firing patterns in rat perirhinal cortex chunk large segments of spatial trajectories in a visual discrimination task

Authors: *J. J. BOS[1], M. VINCK[2], A. B. MOURIK-DONGA[1], J. C. JACKSON[3], M. P. WITTER[4], C. M. A. PENNARTZ[1];
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Abstracts: The perirhinal cortex (PRh) is widely associated with higher-order sensory processing and object recognition. It is generally classified as part of the “what” pathway of the medial temporal lobe memory system. However, the role of this structure in spatial coding remains unclear. In the present study, in addition to units tuned to sensory events, we found units showing sustained activations and deactivations for large segments of a figure-8 maze. These units showed differential responses for leftward vs. rightward trials. Three rats were trained on a spatial, visual discrimination task. A target and a distracter stimulus were presented simultaneously on two screens flanking the two arms of the figure-8 maze. Rewards could be obtained at the end of the arm corresponding to the target image. Tactile cues at the beginning of the arms indicated reward amount. The rats performed ~80 trials with an average performance of 75% correct per session. Using 36 tetrode drives we recorded spikes and local field potentials from four brain areas simultaneously: PRh, dorsal CA1, barrel cortex and visual cortex. In total we recorded >1500 well-isolated single units. The sustained, left-right discriminating responses found in PRh were dissimilar from responses found in the other areas. For instance, place fields found in area CA1 were much more focal than PRh patterns. In contrast, PRh units showed consistent, sustained differential firing throughout the side arms. Examples were found of both in- and decreases in firing rate on a given arm, compared to baseline or the other arm. The units appear to chunk the environment and/or the corresponding segment of the behavioral task. The left-right discriminating responses as found in PRh could not be explained by differences in task variables such as presented images, textures, correct/incorrect trials or running speed. These

results show an involvement of PRh in chunking the environment and the task segments associated with the animal's trajectory. As such, these results argue in favor of a less narrow definition of PRh as being restricted to the "what" pathway, emphasizing a unitizing or "chunking" function in spatial behaviors.

Disclosures: **J.J. Bos:** None. **M. Vinck:** None. **A.B. Mourik-Donga:** None. **J.C. Jackson:** None. **M.P. Witter:** None. **C.M.A. Pennartz:** None.

Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.10/TT74

Topic: F.02. Animal Cognition and Behavior

Support: NWO Grant 823.02.020

Title: Corticosterone impairs adaptation in an associative place-reward learning task

Authors: ***S. I. RUSU**[1], M. JOËLS[2], C. M. PENNARTZ[1];
[1]Neurosciences, Swammerdam Inst. For Life Sci., Amsterdam, Netherlands; [2]Dept. of Neurosci. and Pharmacol., Brain Ctr. Rudolf Magnus, Utrecht, Netherlands

Abstracts: Within a normal physiological range, acute stress-induced responses may be beneficial in coping with challenging situations. However, they can become maladaptive in extreme circumstances or following prolonged stress exposure, impairing both memory and behavioral flexibility. Stress is a major risk factor in a multitude of psycho-pathological conditions such as PTSD, depression and anxiety, with effects ranging from over-consolidation of emotional memories to debilitating memory loss and impaired executive function. A key player in the brain's response to stressors in rodents is the hormone corticosterone (CORT), which binds, amongst others, to receptors in the hippocampus (HPC) and prefrontal cortex (PFC). Navigational strategies employed by rodents are often driven by associations made between spatial information and specific reward contingencies, recruiting therefore both HPC and associated areas. The HPC, ventral striatum, amygdala (AMY) and PFC are core structures involved in encoding and updating spatial information, emotional management and the control of cognitive processes such as flexible decision-making and behavioural inhibition. Recent electrophysiological data support a robust interaction between HPC, PFC and AMY during anxiety-related behaviors, as suggested by both an increased local field potential synchrony and

the synaptic modulation of perceived anxiety. However, it remains unclear how motivational and spatial memory systems are altered by stress hormones during complex behavior. In this study we trained a total of 22 male rats on an hexagonal track fitted with six spatially symmetric reward ports. Of these, 21 were subjected to a partial extinction procedure across 5 sessions, with each daily session immediately followed by systemic administration of either CORT or saline. We showed that, during the first three sessions, performance is significantly impaired by CORT ($p = 0.042$), suggesting that post-task hormone treatment retards adaptation. Furthermore, 4 rats were implanted with a hyperdrive containing 14 independently moveable tetrodes directed at the HPC and the orbitofrontal cortex. By repeatedly changing contingencies every 3rd session on at least 4 reward sites, we estimated how associative place-reward information is updated following post-task CORT administration. Preliminary analysis of local field potential data recorded from the CA1 subfield shows a decrease in hippocampal ripple activity during post-task slow wave sleep epochs, consistent with an adverse effect of CORT on mnemonic function.

Disclosures: S.I. Rusu: None. **M. Joëls:** None. **C.M. Pennartz:** None.

Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.11/TT75

Topic: F.02. Animal Cognition and Behavior

Support: FWF Grant SFB-F44

Title: Impact of L-type voltage gated calcium channels on neurogenesis and cognition in adult and aged mice

Authors: J. MARSCHALLINGER[1], P. ROTHENEICHNER[2], C. SCHMUCKERMAIR[3], A. SAH[3], N. SINGEWALD[3], S. COUILLARD-DESPRES[2], *L. J. AIGNER[1];
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Abstracts: L-type voltage gated Calcium channels (LTCCs) are widely expressed within the CNS and play an important modulatory role in brain function. Aberrant activity of brain LTCCs is involved in various CNS pathologies (e.g. PD, AD), and modulation of LTCCs may demonstrate a therapeutic approach to treat these disorders. However, the LTCC channel subtypes Cav1.2 and Cav1.3 are also expressed within the adult neurogenic brain regions

(dentate gyrus, subventricular zone), and modification of these channels may have profound impact on neurogenesis. Hence, this study examined the involvement of LTCCs in adult neurogenesis. Expression patterns of Cav1.2 and Cav1.3 within the neurogenic regions of adult mice were examined immunohistologically, and the impact of Cav1.3 inhibition on neurogenesis and a possible relevance for cognitive performance was analysed in adult and aged Cav1.3-/- mice. Cav1.2 and Cav1.3 expression was predominately found in mature granular neurons in the dentate gyrus. Cav1.3 was further observed in neural stem cells and astrocytes, but was nearly absent in neuronal progenitors. Cav1.3-/- mice showed increased cell proliferation, reduced cell survival, and a reduced number of neural stem cells in the dentate gyrus. In addition, Cav1.3-/- mice exhibited a reduced surface area of GFAP+ astrocytes in the hippocampus compared to wildtype controls. Addressing possible effects of Cav1.3 knockout on cognition, the Object Location Memory Test revealed a significant impairment in Memory performance of young adult (3 months) Cav1.3 -/- mice compared to age-matched wildtype mice. The expression of Cav1.2 and Cav1.3 within the neurogenic niches, together with the observed alterations of adult neurogenesis and of memory skills in Cav1.3-/- mice, let strongly suggest an involvement of LTCCs in adult neurogenic processes. Thus, modulation of LTCC activities may have a crucial impact on neurogenic responses, which should be considered for future therapeutic administration of LTCCs.

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Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.12/TT76

Topic: F.02. Animal Cognition and Behavior

Title: Ginkgo biloba improves spatial memory and decreases oxidative damage in aged female rats

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Abstracts: The purpose of the present study was to investigate the effects of ginkgo biloba supplementation on cognitive impairment evaluated by Morris Water Maze (MWM) as well as

the oxidative stress in brain tissue induced by aging in female rats. The study protocol was approved by the Ethics Committee of the Experimental Medicine Research and Application Center. Twenty-eight Wistar rats were divided into the four groups according to their age (young vs. aged) and treatment (ginkgo biloba vs. vehicle) status. Ginkgo biloba or vehicle (corn oil) were given once daily for a period of 30 days, beginning 25 days prior to and 5 days during the behavioral tests at a dose of 100 mg.kg-1.day-1. Behavioral assessment was performed in MWM. All behavioral tests were recorded online and analyzed offline with analytical software. At the end of the behavioral test, brain tissues were taken for the analysis of malondialdeyde (MDA), glutathione (GSH) and 8-hydroxydeoxyguanosine (8-OHdG) levels and superoxide dismutase (SOD) activities. During the training session, ginkgo biloba supplementation decreased latency to reach to the platform and the total distance traveled ($P<0.05$). During the probe trial, ginkgo biloba supplementation increased the number of platform crossings ($P<0.05$). In addition to the behavioral testing, biochemical results showed that GSH levels decreased and 8-OHdG levels increased in brain tissue of aged rats ($P<0.05$). It may be concluded that, ginkgo biloba supplementation improves cognitive functions by decreasing the oxidative stress in brain tissue of aged female rats.

Disclosures: **M. Belviranli:** None. **N. Okudan:** None.

Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.13/TT77

Topic: F.02. Animal Cognition and Behavior

Support: DARPA N66001-14-C-4016

NIDA DA023573

NIDA DA026487

NIDA DA006634

Title: Evaluating neurophysiological representations of hippocampal-dependent reversal learning with multifractal analysis

Authors: ***D. FETTERHOFF**[1], R. A. KRAFT[2], I. OPRIS[3], A. J. SWEATT[3], C. A. SEXTON[3], S. A. DEADWYLER[3], R. E. HAMPSON[3];

[1]Neurosci., [2]Biomed. Engin., Wake Forest Univ., Winston-Salem, NC; [3]Physiol. & Pharmacol., Wake Forest Univ. Hlth. Sci., Winston-Salem, NC

Abstracts: Successful performance on the delayed nonmatch-to-sample (DNMS) working memory task requires rats to combine spatial and nonspatial information. However, the DNMS paradigm is limited by a two-choice decision. Therefore, to further examine cognitive and behavioral flexibility as well as neural encoding of additional behavioral events by the same neurons, a reversal learning paradigm was implemented. Rats initially learned to perform a delayed match-to-sample (DMS) task before the operant rule was "reversed" to nonmatch. Hippocampal neuronal spike trains were recorded using chronic bilateral microelectrode arrays implanted in CA3 and CA1 sub-regions and neuronal discharge patterns were investigated with peri-event histogram analysis and Wavelet Leaders-based Multifractal Analysis. Behaviorally, all rats tested successfully learned the reversal over a variable period of 3 to 12 days. Distinct changes in the neuronal representation of the task occurred during the operant rule change which depended on each individual neuron's firing correlates of the pre-reversal phase. Neurons with stronger involvement in reversal learning also exhibited larger multifractal complexity compared to neurons without task correlates. On average, multifractal complexity decreased immediately after the rule change, but returned to pre-reversal levels when animals acquired the newly informed behavior, with a few neurons exhibiting opposite polarity trends. In this test of behavioral flexibility and re-learning, single neuron alterations in multifractal complexity correlated with the neuronal firing rate relationship to behavioral performance. This presentation will discuss implications of multifractal analysis for cognitive neuroscience and neural encoding and compare with other measures of interspike-interval variability.

Disclosures: D. Fetterhoff: None. **R.A. Kraft:** None. **I. Opris:** None. **A.J. Sweatt:** None. **C.A. Sexton:** None. **S.A. Deadwyler:** None. **R.E. Hampson:** None.

Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.14/TT78

Topic: F.02. Animal Cognition and Behavior

Support: UFOP

CAPES

FAPEMIG

CNPq

Title: Food restriction induces anxiety-like behaviour in female Fischer rats

Authors: *G. S. CAMPOS[1], A. M. A. DE SOUZA[2], S. I. S. R. NORONHA[2], A. R. R. ABREU[2], D. A. CHIANCA JR[2], R. A. C. MENEZES[2];
[1]Dept. of Biol. Sci., [2]Dept. of Sci. Biol., Federal Univ. of Ouro Preto, Ouro Preto, Brazil

Abstracts: Studies have indicate a strong association between food restriction (FR) and the development of anxiety disorders (generalized anxiety and panic), relating these behavioral alterations to malnutrition caused by FR. Many hypotheses are being studied to explain this mechanism, however, still unclear whether anxiety disorders could be complete or partial consequence of malnutrition caused by reducing caloric and nutrient intake. Thereby, the aim of this study is evaluate whether food restriction can alter behavioral responses (anxiety and panic) compared to control animals. For this, female Fischer rats (210 ± 10g) were divided into control and food restriction (FR) and housed individually. For 15 days the control animals received ad libitum and FR animals received 40% of the average intake of the control group. On the 14th day, the animals were exposed to the Elevated T-Maze (ETM), an apparatus that permits determining behavioral changes related to escape, inhibitory avoidance and memory, to perform behavioral tests of anxiety (3 trials: avoidance 1, 2 and 3), the time taken by the rat to leave the enclosed arm with four paws and panic (3 trials: escape 1, 2 and 3), the time taken by the rat to leave the open arm with four paws. The test was repeated the next day (ND) (anxiety: 1 trial; panic: 1 trial) to confirm the results of the previous day. A cutoff time of 300s was established for the avoidance and escape latencies Our result demonstrates that when comparing the trials of control animals in the avoidance test, we found differences between all trials (9.9 ± 2.8 s, 126.0 ± 43.9 s, 254.9 ± 24.66 s, $n = 10$, $p < 0.005$) and also between avoidance 1 and ND (213.5 ± 44.08 s, $n = 10$, $p < 0.05$). Among the FR, we found a significant increase avoidance between 1 and 3 (109.2 ± 60.4 s, 300.0 ± 0.0 s, $n = 6$, $p < 0.05$) and avoidance 1 and ND (109.2 ± 60.4 s, 300.0 ± 0.0 s, $n = 6$, $p < 0.05$) by one-way ANOVA followed by the Tukey's post hoc test. When comparing the intergroup trial, we observed that the control animals spent less time in the first trial shorter that submited to FR (C: 9.9 ± 2.8 s, $n = 10$ vs FR: 109.2 ± 60.4 s, $n = 6$, $p = 0.0480$) while trials 2, 3 and ND are similar between groups. No differences were found in the panic test. These results suggest that food restriction promotes anxiety -like behavior, but not observed panic-like responses.

Disclosures: **G.S. Campos:** None. **A.M.A. De Souza:** None. **S.I.S.R. Noronha:** None. **A.R.R. Abreu:** None. **D.A. Chianca Jr:** None. **R.A.C. Menezes:** None.

Poster

652. Learning and Memory: Physiology II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DA023573

NIH grant DA026487

NIH grant DA06634

Title: Hippocampal encoding of unfamiliar events

Authors: *S. A. DEADWYLER, A. J. SWEATT, I. OPRIS, F. M. MILLER, D. FETTERHOFF, C. A. SEXTON, R. E. HAMPSON;
Wake Forest Sch. of Med., Winston-Salem, NC

Abstracts: Recordings from well trained rodents performing a touchscreen image delayed match to sample discrimination task show a unique type of encoding related to visual features of the image sets that were changed on a weekly (5 day) basis. Performance was shown to improve as a function of the number of random exposures to each combination of Sample (n=5) and Distracter (n=5) images (total of 25 different Sample-Distracter combinations). Hippocampal neural activity was recorded with microwire multielectrode arrays bilaterally implanted in CA3 and CA1, and recorded with respect to firing in sample and match phases of the task. Cell responses were varied according to specific combinations of Sample and Distracter as well as a function of correct or incorrect performance. However, much of the variance with respect to performance was related to two major factors: 1) asynchronous discharge related to match screen onset in which the distracter and sample image were both presented in different spatial positions or 2) eliciting a firing pattern appropriate for a different image combination: i.e. same Sample but and a different Distracter, or same Distracter and different Sample image. The major differentiation in firing on correct vs. incorrect trials was the synchrony of oscillations after image presentation but prior to the occurrence of the match response. These firing tendencies reflect inherent hippocampal encoding patterns for different images and may well reflect categorical processing to facilitate improvement in performance related to occurrences of unfamiliar events.

Disclosures: S.A. Deadwyler: None. **A.J. Sweatt:** None. **I. Opris:** None. **F.M. Miller:** None. **D. Fetterhoff:** None. **C.A. Sexton:** None. **R.E. Hampson:** None.

Poster

652. Learning and Memory: Physiology II

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Program#/Poster: 652.16/TT80

Topic: F.02. Animal Cognition and Behavior

Support: CNRS UMR7241

NIMH 60670

DeparT Anesthesiology, U Mich

Title: Locus coeruleus activity time-locked to hippocampal rythmns during sleep

Authors: G. POE[1], *S. J. SARA[2];
[1]Anesthesiol., Univ. of Michigan, Ann Arbor, MI; [2]Collège de France, Paris, France

Abstracts: Locus coeruleus (LC) involvement in memory formation, retrieval and other cognitive processes is well established. Noradrenaline (NA) provided to the forebrain by the LC facilitates LTP and prevents depotentiation. There is an increase in LC activity during nonREM sleep specifically following learning, when replay is thought to reinforce memory. The LC is silent during REM sleep and this silence is hypothesized to allow depotentiation within neural networks consolidating memories. During nonREM sleep, LC activity is phase locked to cortical slow oscillations(1). The LC also falls silent just prior to the onset of cortical sleep spindles (10-15 Hz activity) that, in themselves, have been well correlated with sleep dependent memory consolidation. In order to further understand the action of NA in off-line memory consolidation during sleep, we investigated the precise temporal relationship between LC unit activity and the predominant hippocampal and prefrontal cortical EEG frequencies during REM and nonREM sleep in the freely behaving, learning, sleeping rat. Rats were implanted under electrophysiological control with movable micro electrodes in the LC for unit recording, and with fixed tungsten wire electrodes to record, simultaneously, LFPs in dorsal CA1 and medial prefrontal cortex. They were submitted to various appetitive, nonstressful learning situations, followed by a 2-3 hour recording session, during which the rat slept most of the time. Firing of LC neurons significantly reduced spindles, beginning immediately for hippocampal spindles and with a 120ms delay for cortical spindles. The spindle power nadir, revealed by root mean square (RMS), occurred at 1 sec post LC spiking for hippocampus and slightly later in cortex. LC activity was time locked to hippocampal delta (0-4 Hz) oscillations and reset theta and gamma waves. RMS analysis showed that LC neurons spiked 0.5 to 1 seconds before the nadir and onset of rebound in power for gamma, theta and slow wave oscillations, and at the nadir/inflection

point for delta power. These results lend strong support to the notion, derived mainly from *in vitro* or anesthetized preparations, that LC activity plays an intimate role in shaping cortical and hippocampal oscillatory activity that promotes off-line memory consolidation during sleep. We are currently investigating the effect of manipulation of LC activity on sleep-related hippocampal rythmns and consequences for memory consolidation. (1) Eschenko et al Cerebral Cortex, 2012

Disclosures: G. Poe: None. **S.J. Sara:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

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Program#/Poster: 653.01/TT81

Topic: F.02. Animal Cognition and Behavior

Support: R01NS061973

Title: Peripheral injection of macrophages ameliorates learning deficits in aged mice by a phagocytosis-dependent mechanism

Authors: *N. C. DERECKI, I. SMIRNOV, J. KIPNIS;
Neurosci., Univ. of Virginia, Charlottesville, VA

Abstracts: Age-associated cognitive decline is paralleled by immune system decline. Along these lines, past work from our lab has shown that normal learning and memory is associated with a robustly functioning immune system. We showed that an influx of CNS-directed T cells, acting in the meningeal spaces surrounding the brain, were critical to performance in the Morris water maze (MWM) via production of interleukin (IL)-4, an anti-inflammatory cytokine. Influx of anti-inflammatory T cells was associated with a subsequent anti-inflammatory skew of meningeal myeloid cells, suggesting a possible connection between meningeal myeloid cell skew and learning and memory. Intriguingly, when we examined aged mice, isolated meningeal cells expressed high levels of pro-inflammatory cytokines. Furthermore, meninges of aged mice showed substantial reduction in IL-4 producing T cells. These findings suggested a mechanistic connection between immune decline and cognitive impairment in aged mice. Here, we show that aged (18-22 mo) mice, normally impaired in MWM as compared to young counterparts, display significantly improved performance (compared to PBS-injected controls) following a single i.v. injection of anti-inflammatory skewed macrophages. Interestingly, injected macrophages were completely cleared from mice by the end of the MWM task; yet, meningeal macrophages

isolated from cell injected mice displayed a marked anti-inflammatory phenotype as compared to controls. Along these lines, it is known that phagocytosis of apoptotic cells is sufficient to guide resident macrophages to anti-inflammatory phenotype. Thus, we reasoned that phagocytosis of injected cells might underlie the observed anti-inflammatory skew by resident cells. Indeed, our studies indicate that apoptotic macrophages promote anti-inflammatory skew in phagocytosing macrophages. When phagocytosis is blocked using Annexin V, which masks phosphatidyserine residues on apoptotic cells, anti-inflammatory skew of phagocytosing macrophages is abolished. These results suggest that age-associated immune system decline may indeed contribute to cognitive decline, and that boost of phagocytic activity might represent a possible method of ameliorating cognitive decline by immune manipulation.

Disclosures: **N.C. Derecki:** None. **I. Smirnov:** None. **J. Kipnis:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.02/TT82

Topic: F.02. Animal Cognition and Behavior

Title: Spatial memory deficits in old homing pigeons (Columba livia): A novel experimental model for age-related cognitive decline

Authors: *V. J. COPPOLA[1,2], V. P. BINGMAN[1,2];
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Abstracts: The mammalian hippocampus is particularly susceptible to age-related degeneration that, like hippocampal lesions, results in spatial-cognitive deficits. Lesions to the avian hippocampal formation (HF) also result in spatial-cognitive deficits, but it is currently unknown what, if any, affect aging has on HF-dependent spatial cognition. Therefore, this study investigated the possibility of an age-related spatial working memory deficit in old homing pigeons. Young adult (1 - 2 years) and old (10 - 13 years) pigeons were tested on a spatial, delayed non-match-to-sample task in a modified radial arm maze. Compared to young adults, old pigeons required more choices to task completion (i.e., they committed more errors) and were less accurate with their first four choices. Additionally, old pigeons were much more likely to adopt a stereotyped sampling strategy, a behavior typical of hippocampal-lesioned animals. These results suggest that birds, like mammals, experience age-related memory impairment, and

are a critical first step in promoting the homing pigeon as an additional model species for cognitive aging research. Future directions include a broader behavioral assay, as well as investigations of potential neural correlates of age-related cognitive decline in birds.

Disclosures: V.J. Coppola: None. **V.P. Bingman:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.03/TT83

Topic: F.02. Animal Cognition and Behavior

Support: "Train the Brain" grant from Fondazione Pisa

Title: Environmental enrichment induces hippocampal plasticity in the aged brain through a reduction in ccl11/eotaxin

Authors: *M. MAINARDI[1,2], M. SCALI[2], G. SCABIA[4], M. MAFFEI[4,3], L. MAFFEI[2,5]; [1]Inst. of Physiol., Sacred Heart Catholic Univ., Rome, Italy; [2]Neurosci. Inst., [3]Inst. of Clin. Physiol., CNR - Natl. Res. Council, Pisa, Italy; [4]Dept. of Clin. and Exptl. Med. - Obesity Ctr., Univ. Hosp., Pisa, Italy; [5]Accademia Nazionale dei Lincei, Rome, Italy

Abstracts: Increasing physical and mental exercise can represent an effective strategy to improve brain health during aging. However, the precise details explaining this beneficial action are still poorly understood. To explore this problem, we exploited environmental enrichment (EE) on aged mice. EE is based on rearing mice in large social groups, with free access to voluntary physical exercise and cognitive stimulation, provided by novel objects to be explored. It therefore represents an accurate way to model an active life style. Moreover, EE has been shown to be highly effective in inducing neural plasticity in a variety of experimental models, ranging from sensory cortices to the hippocampus and subcortical brain areas. Notably, EE has been shown to positively affect the physiological status of the aged brain, a phenomenon that is accompanied by the induction of neural plasticity. It has been recently demonstrated that the plasmatic levels of the chemokine ccl11/eotaxin increase during the aging process. Moreover, ccl11 has been clearly shown to be a negative regulator of plasticity. Thus, a higher concentration of ccl11 could account for the lower cognitive performance displayed by aged animals in comparison to younger subjects. Based on this evidence, we reasoned that a reduction in ccl11 plasmatic concentration could be induced by exposure to EE during aging. To address

this issue, aged mice were reared in EE for one month and we found that this manipulation induces a significant decrease in plasmatic ccl11. Thus, we hypothesized that lower ccl11 levels could be the crucial mediator of the beneficial effects of EE on the aged brain. To demonstrate this point, we treated aged mice with ccl11 injections while exposed to EE (EE-ccl11 group), in order to counteract the decrease in its plasmatic levels. The cognitive performance of this experimental group was then compared with vehicle-injected mice either exposed to EE (EE-veh group) or maintained in standard rearing (SC-veh group), by testing on the Morris Water Maze. We found that ccl11 injection correlates with ablation of the improvement in short- and long-term memory retention that is otherwise caused by EE. Taken together, our findings point to the decrease in ccl11 plasmatic levels as a key phenomenon responsible for the improved neural plasticity and cognitive performance observed in aged mice exposed to EE.

Disclosures: **M. Mainardi:** None. **M. Scali:** None. **G. Scabia:** None. **M. Maffei:** None. **L. Maffei:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.04/TT84

Topic: F.02. Animal Cognition and Behavior

Support: NSFC Grant 81273489

BK2012582

12KJA180008

Title: Effects and mechanisms of ADDLs on AMPA receptors trafficking and cognitive deficits

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Abstracts: It is widely accepted that ADDLs (Aβ-derived diffusible ligands) play a prominent role in triggering the early cognitive deficits that constitute Alzhimer's Disease (AD). However, the mechanisms for its effects on synaptic plasticity are not fully understood. Regulation of a-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor trafficking in and out of excitatory synapses is important for controlling the strength of excitatory synapses in long-term potentiation (LTP), long-term depression (LTD) and other forms of synaptic plasticity. So

targeting AMPA receptors trafficking and its regulation is a new strategy for AD early treatment. We used primary hippocampal cultures and APP/PS1 transgenetic mice to test the hypothesis that ADDLs influence synaptic plasticity by modulating AMPA receptor subunit GluA1 trafficking. Three hours exposure of ADDLs decreased surface expression of GluA1-containing AMPA receptors by decreasing their rate of externalization at extrasynaptic sites. LTP was also inhibited in hippocampal slice. This was mediated mainly by protein kinase A (PKA), which could phosphorylate S845 of GluA1. *l*-Stepholidine (l-SPD), which belongs to the tetrahydroprotoberberines elicits D1 agonistic activity while acts as D2 receptor antagonist. *l*-SPD not only rescued the phosphorylation of GluA1 and surface expression in hippocampal cultures but also protected the LTP in hippocampal slice induced by ADDLs. PKA agonist SpcAMP and D1 agonist SKF81297 had similar effects, while PKA antagonist RpcAMP or D1 receptor antagonist SCH23390 diminished the effects of *l*-SPD on GluA1 trafficking and LTP. Furthermore, *l*-SPD partialy rescued both the hippocampal-dependent memory and surface expression of GluA1 in hippocampus in APP/PS1 mice. These results demonstrated that D1-PKA singal pathway plays an important role in early dysfunction of synaptic plasticity in AD. Elucidating the molecular mechanisms that ADDLs affect AMPA receptors surface expression and disrupt synaptic plasticity for triggerring memory malfunction and cognitive deficits will provide new strategy for early treatment of AD and shed light on new target for drug discovery.
Key Words: ADDLs, Alzhimer's Disease, AMPA receptors, Learning and memory, Synaptic plasticity, Trafficking

Disclosures: J. Hao: None. **N. Sun:** None. **L. Lei:** None. **X. Li:** None. **K. Sun:** None. **C. Gao:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

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Program#/Poster: 653.05/TT85

Topic: F.02. Animal Cognition and Behavior

Support: John G. Kulhavi Professorship

Field Neurosciences Institute

Jeff Lichon Spinal Cord Injury Foundation Scholarship

Central Michigan University College of Medicine

Title: Behavioral phenotypes in the 5xFAD mouse model of Alzheimer's disease and their relation to amyloid-beta plaque load

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Abstracts: Alzheimer's disease (AD) is a progressive, neurodegenerative disorder affecting millions of aging adults. It is characterized by the trademark physiology of intracellular tau tangles, amyloid beta plaque formation, cortical inflammation, and neuronal atrophy, which are each thought to contribute to the loss of cognitive function. The 5xFAD mouse model of AD replicates aggressive plaque formation as early as two months of age, though there is much disagreement among studies regarding the magnitude of its effect on behavior. The present study explored a variety of behavioral tests in the 5xFAD mouse at three time points, and compared findings with final histological measures in an attempt to validate previous observations within the model. 5xFAD mice demonstrated progressive motor deficits when compared to wild type (WT) control, as well as decreased anxiety as measured by the elevated plus maze, but no significant changes in learning and memory behavior were observed when assessed by water T-maze, Y-maze for spontaneous alternation, or an operant alternation paradigm. Histological analysis of AD and WT brains revealed dramatically elevated amyloid beta-42 (Aβ-42) levels in the cortex, hippocampus, lateral septum, and olfactory bulb regions, as well as increased neural inflammation in the cortical region, with trends toward increased inflammation in the hippocampal regions, though cortical density was not affected. These findings suggest that cognitive deficits in 5xFAD mice are as robust as expected, despite evidence for an increased plaque load. These results provide further support for other findings showing a dissociation between plaque load and learning deficits.

Disclosures: J.J. Matyas: None. **A. Rechenberg:** None. **S.A. Lowrance:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.06/TT86

Topic: F.02. Animal Cognition and Behavior

Title: Effect of aging and repeated testing on performance of mice in the continuous alternation test

Authors: *L. VER DONCK[1], H. DUYTSCHAEVER[2], R. WILLEMS[2], L. MERTENS[2]; [1]Neurosci., Janssen Res. & Development, A Div. of Jans, Beerse, Belgium; [2]Neurosci., Janssen Res. & Development, A Div. of Janssen Pharmaceutica NV, Beerse, Belgium

Abstracts: Spontaneous alternation (SA) is an innate tendency of mice to avoid visits to previously explored locations. It can therefore serve as a measure of spatial exploratory behavior, defined as a visit to the other of the two goal arms of a triangular maze from the arm visited before. In order to alternate or avoid a revisit, a mouse must use spatial orientation. The information about the previous arm-visit is stored in the spatial working memory. The SA-test is therefore suited to assess the effects of cognition enhancing and impairing experimental manipulations. OBJECTIVE: To evaluate the effect of aging and of repeat testing on SA-performance of mice. METHODS: The setup consisted of 3 square plastic boxes (15x15x15cm) each with a ∞3cm hole in one wall; the latter were placed to face each other thus creating a triangular centre. Mice were placed in the centre and explored the setup for 8 min. Sessions were recorded by a video camera and images were analysed using EthoVision XT to determine distance traveled by the mouse, number of entries into the boxes (visits), number of alternations from one box to another and % spontaneous alternations (%SA: mouse enters the three boxes sequentially one after the other, relative to total number of visits). Six groups (n=16 each) of male 2 month old C57BL6J mice (Janvier) were housed in IVC cages. Five groups started their experimental series at either 3, 6, 9, 12 or 15 months of age respectively (effect of age), followed by testing once a week for 4 weeks (weekly repeat testing). Group 6 was tested in SA once every 3 months (3-monthly repeat testing). RESULTS: Naive mice showed reduced performance from 6 months onward on all parameters, while the group with 3-month repeat testing showed reduced performance at each age tested. Performance of the latter was less than naive animals at ages ≥6 month. Weekly repeated testing at each age was decreased for all parameters measured, except for %SA which remained constant. CONCLUSION: Aging and repeated testing decreased performance of mice on most parameters in the continuous alternation test, but %SA remained constant throughout.

Disclosures: L. Ver Donck: A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV. **H. Duytschaever:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV. **R. Willems:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV. **L. Mertens:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.07/TT87

Topic: F.02. Animal Cognition and Behavior

Support: J.O. and J.R. Wicking Trust

Title: Effects of environmental enrichment in a mouse model of Alzheimer's pathology

Authors: *K. E. STUART[1], A. KING[2], M. J. SUMMERS[3], J. C. VICKERS[2];
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Abstracts: Objective Environmental enrichment (EE) has been proposed to reduce the risk of developing dementia in conditions such as Alzheimer's disease (AD). The current project investigates the potential of different forms of later-life EE to ameliorate cognitive deterioration in ageing in wildtype (WT) mice as well as in a transgenic model (APP/PS1) of early-stage AD. **Method** Male transgenic (APPswe, PSEN1dE9) and WT mice entered differential housing from 6 – 12 months of age. Mice entered standard (SH) or EE housing. EE housing comprised a cage double the size of the SH cage, with various enrichment objects. A sub-set of EE mice (EE+) received additional stimulation by spending several hours a week in a larger cage with novel objects. Working memory (Y maze) was tested at 6 months, before entering differential housing, and at 9 and 12 months. At 12 months, mice were also tested for their long-term memory function on the Barnes maze. **Results** At 6 months, APP/PS1 mice demonstrated inferior Y maze performance compared to WT mice ($n = 75$, $p < .001$). After 3 months of differential housing, WT performance declined to the level of TG mice, and a combined effect of housing and genotype on Y maze performance was detected ($p = .01$). Post-hoc tests demonstrated that WT mice exhibited similar levels of performance regardless of housing condition, however TG EE mice demonstrated improved Y maze performance compared to SH mice ($p < .01$). After 6 months of differential housing, this effect dissipated ($p = .76$). Significantly longer latency on the Barnes maze was demonstrated by TG mice ($p = .01$). Housing condition did not predict long-term memory function on the Barnes maze in TG mice, however, WT animals exposed to EE made less errors on the Barnes maze than those in SH ($p = .03$). **Conclusions** The results indicate that EE confers differential beneficial effects to cognitive function. The results also suggest there is no additive effect of more complex, novel stimulation on memory function above that of standard EE. Results from cognitive testing data will be correlated with pathological brain alterations, including β-Amyloid plaque load and synaptic alterations.

Disclosures: **K.E. Stuart:** None. **A. King:** None. **M.J. Summers:** None. **J.C. Vickers:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.08/TT88

Topic: F.02. Animal Cognition and Behavior

Support: CNRS

Université de Strasbourg

Neurex

Marie Curie Grant European Reserach Council

Title: Whole life environmental enrichment impacts hippocampal oscillatory activity in CA1 of aged rats

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Abstracts: Whereas a prominent cognitive decline is commonly described with aging, some old subjects perform as well as young. According to the "cognitive reserve" hypothesis, some individual characteristics either innate and/or related to life experience (e.g. level of education, professional and leisure activities) limit cognitive alterations in aged subjects. In rats, lifelong environmental enrichment attenuates deleterious effects of aging on spatial memory, suggesting that it may allow the constitution of such a reserve. To assess whether preserved hippocampal oscillatory activity contributes to the enrichment-induced maintenance of spatial memory in aging, local field potentials were recorded throughout the dorsoventral axis of CA1 in adult rats submitted to standard environmental condition and in old rats reared until 24 months in enriched or standard environment. During paradoxical sleep, in the *stratum radiatum* there was no difference in theta (4-12 Hz) and slow and fast gamma (25-50 and 60-140 Hz respectively) power between the three groups. However, theta-gamma coupling, a mechanism thought to play an important role in cognitive functions, was different in old rats. Whereas theta-slow gamma coupling was lower in older animals whatever their rearing conditions, theta-fast gamma coupling was lower specifically in aged enriched animals. Moreover, they also presented a

significantly lower theta-fast gamma coupling during active wake state and spatial novelty exposition. These results show that environmental enrichment during the whole life may modify the hippocampal oscillatory activity, particularly the theta-fast gamma coupling, suggesting that aged enriched rats process notably spatial information in a different way. How this may explain their better spatial capabilities needs to be explored.

Disclosures: **F. Fuchs:** None. **A. Barbelivien:** None. **K. Herbeaux:** None. **C. Kelche:** None. **C. Mathis:** None. **M. Majchrzak:** None. **R. Goutagny:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

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Program#/Poster: 653.09/TT89

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 08-43175

NSF IOS 13-18490

NIA P30 AG034464

Alzheimer's Association

Title: Senile or sage? Improved memory and sensitivity to cognitive priming accompany aging in male rats

Authors: D. L. KOROL[1], L. A. NEWMAN[1], *P. E. GOLD[2];
[1]Biol., [2]Dept of Biol., Syracuse Univ., Syracuse, NY

Abstracts: Aged rats exhibit impairments in several cognitive tasks, particularly those designed to be solved using spatial cues or to require memory across delays. Findings like these suggest that decreased hippocampal functions develop with age. Interestingly, in young adult rats, diminished hippocampal function can result in enhanced learning of tasks associated with non-hippocampal learning and memory systems, as seen in demonstrations of competition across multiple memory systems. Here we examined the possibility that striatum-sensitive learning and memory improve with age, such that aged rats might be superior to young rats when trained on striatum-sensitive tasks. Young adult (3-mo-old) and aged (24-mo-old) Fischer-344 rats were trained on two versions of the same maze, a place version that is hippocampus-sensitive and a

response version that is striatum-sensitive. These mazes have the same motivational (food) and locomotor (arm entry) requirements and differ mainly in the rule used to solve the maze, i.e. leave the start arm and go to a place in the room for place learning or turn in a specific direction for response learning. The findings revealed a slight impairment in place learning in the aged compared to the young rats. However, the aged rats were significantly better than young rats at learning the response maze, suggesting that cognitive decline with age is not a unitary process. Moreover, these results support the idea that aging brains are quite plastic. We recently found that cognitive priming with a spatial working memory task, spontaneous alternation, enhanced later maze learning in young adult rats. Here we tested whether cognitive priming with spontaneous alternation one hour prior to learning would enhance subsequent learning in old rats. Spontaneous alternation testing administered to 24-mo-old rats one hour before place learning improved their rate of learning to that of young adult rats. This suggests that any hippocampal dysfunction in the aged rats is not irreversible and that the hippocampus may retain sufficient plasticity to handle spatial learning even into senescence. Based on our recent findings in young adult rats, we are currently testing whether BDNF signaling and lactate delivery to neurons from astrocytic glycogen are mediators of the age-related shifts in learning abilities across memory systems and the priming by cognitive activity.

Disclosures: **D.L. Korol:** None. **P.E. Gold:** None. **L.A. Newman:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.10/TT90

Topic: F.02. Animal Cognition and Behavior

Title: Evaluating aged mice in two touchscreen tests that differ in visual demands: Impaired cognitive function or declining visual abilities?

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Abstracts: Aging is associated with cognitive decline mediated by changes in hippocampal and prefrontal function, decreases in hippocampal LTP, and changes to cell size and density in neuronal populations. Accordingly, aged rodents have been proposed as a model of cognitive impairment for research into potential symptomatic treatments of Alzheimer's disease (AD). However, a short-coming of this approach is that aging is also associated with impaired vision

that may impact performance on cognitive tasks. We have recently established that rats with impaired vision can complete a hippocampus-dependent touchscreen-based Automated Spatial Search Task (ASST; inspired by the CANTAB Spatial Working Memory Test) that requires no or only limited visual acuity, despite showing clear impairments on acquisition and reversal of a visually more demanding visual discrimination (VD). Here we test aged mice on the acquisition of VD and performance of ASST to evaluate age-related cognitive decline, while also investigating the effects of age on visual acuity. Two cohorts of male mice (C57BL/6N, aged 6-8 or 18-20 months) were trained on touchscreen (MedAssociates; K Limbic Software, Conclusive Solutions) VD or ASST. In VD, the mice learned to discriminate between two complex visual stimuli displayed simultaneously on a touchscreen, one of which was correct (S+; rewarded), and the other incorrect (S-). In ASST, mice learned to find the location of a rewarded area on the screen (S+). The location of S+ was not indicated otherwise, but remained in the same location for 10 trials and then shifted to a new area, requiring the mouse to search for and then maintain the memory for the new location (4 locations per session). Visual acuity was assessed using an animal's ability to detect a moving vertical grey scale sine wave and measured in terms of cycles per degree (Virtual Optomotor System, Cerebral Mechanics). Aged mice were impaired in VD acquisition (reduced % correct responses and increased response latencies). Moreover, aged mice showed impaired spatial performance in ASST, requiring more trials to find a rewarded location, while rate of responding was unaltered. There was also an age-dependent decline in visual acuity, but that was not correlated with performance in AST or VD in the old mice, suggesting that the age-related decline in VD performance was at least in part unrelated to visual acuity. Thus, both touchscreen tests may be of utility to assess age-related cognitive deficits in C57BL/6N mice.

Disclosures: **N. Buscher:** A. Employment/Salary (full or part-time):; All authors work for Janssen Research and Development. **P. van Dorsselaer:** A. Employment/Salary (full or part-time):; All authors work for Janssen Research and Development. **T. Steckler:** A. Employment/Salary (full or part-time):; All authors work for Janssen Research and Development. **J.C. Talpos:** A. Employment/Salary (full or part-time):; All authors work for Janssen Research and Development..

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.11/TT91

Topic: F.02. Animal Cognition and Behavior

Support: UNT Health Science Center Faculty Seed Grant 2011-2012

P01 AG022550

Title: Curcumin as a dietary intervention improves functional outcomes in middle- aged and senescent male and female c57bl/6 mice

Authors: *M. SARKER[1], S. F. FRANKS[2], N. SUMIEN[3], F. FILIPETTO[2], M. J. FORSTER[3]; [2]Family Med., [3]Pharmacol. and Neurosci., [1]UNT Hlth. Sci. Ctr., Fort Worth, TX

Abstracts: Curcumin, a component of the Indian spice turmeric, has antioxidant, anti-inflammatory and anti-angiogenic effects that are hypothesized to benefit impaired cognitive and psychomotor performance related to normal aging. The results reported here are from an ongoing study of dietary curcumin supplementation alone and in combination with caloric restriction, testing functional and biochemical outcomes in late middle age (15 months) and senescent (20 months) C57BL/6J male and female mice after 16 weeks of treatment. Mice were assigned in groups to receive: (i) base diet ad libitum (AL), (ii) weight stable caloric restriction (WSCR), (iii) curcumin in the base diet (7200 mg/kg diet (CURCAL) or (iv) curcumin plus WSCR. Beginning 8 weeks after the initiation of the treatments, all mice received a battery of behavioral tests for psychomotor and cognitive function (Sumien et al., 2006) that included a test of spatial memory performance and one for cognitive flexibility. Both initial learning and cognitive flexibility, tested for by a discriminated avoidance, serial reversal task, was significantly better for mice under CR, CURAL and CURCR compared to AL, when measured by the number of trials required to reach criterion. In addition, the food intake of mice in the CURAL group was higher when compared to AL, though there was not a significant difference in body weight between CURAL and AL after 16 weeks of treatment which could be attributed to improved metabolic activity in CURAL mice. The results indicate dietary intake of curcumin has positive effects on frontal cortical functions in both genders, in mice of middle and more advanced age. Sumien et al. Age (Dordr). 2006 Sep;28(3):265-82

Disclosures: **M. Sarker:** None. **S.F. Franks:** None. **N. Sumien:** None. **F. Filipetto:** None. **M.J. Forster:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.12/TT92

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

CAPES

FAPERJ

Title: Investigation of the neuroprotective potential of bone marrow mesenchymal stem cells in an *in vitro* model of Alzheimer´s disease

Authors: *M. GODOY, L. M. SARAIVA, A. VASCONCELOS-DOS-SANTOS, H. J. V. BEIRAL, C. V. BRAGA, C. A. A. SILVA, R. B. LEAL, L. R. P. CARVALHO, A. P. C. LIMA, A. VIEYRA, F. G. DE FELICE, S. T. FERREIRA, R. MENDEZ-OTERO;
Univ. Federal Do Rio De Janeiro, Rio De Janeiro, Brazil

Abstracts: Alzheimer's disease (AD) is a neurodegenerative disease with high prevalence and morbidity, for which there are no effective therapies. Soluble oligomers of the amyloid-β peptide (Aβ) or ADDLs (Aβ-derived diffusible ligands) are the main neurotoxins involved in the early synaptic dysfunction and oxidative stress associated with the disease. The therapeutic potential of bone marrow mesenchymal stem cells (MSCs) has been investigated in several models of neurological diseases and the main mechanism of action of these cells is based on paracrine signaling, through the release of trophic or neuroprotective factors. The aim of the current study was to evaluate the neuroprotective actions of MSCs against the deleterious effects caused by exposure of rat hippocampal neurons to Aβ oligomers. We have also investigated the interaction of ADDLs with MSCs and possible mechanisms of neuroprotection. We established a model of indirect coculture of neurons and MSCs and our results indicate that the ADDLs do not alter the viability (LIVE/DEAD), proliferation (Ki67 expression) and respiration of MSCs (Oroboros oxygraph) *in vitro*. However, presence of MSCs in coculture protected neurons against oxidative stress generated by exposure to ADDLs (preventing the increase of 3 times in the levels of reactive species) or hydrogen peroxide (reducing by half the increase of ~ 8 x in the levels of reactive species), and preserved the integrity of synapses, evaluated by the expression of pre and post synaptic proteins. We also found a reduction in the concentration of exogenously added ADDLs in the culture medium after increasing periods of incubation with MSCs. In addition, we found that ADDLs are internalized by MSCs (reducing about 10 times their concentration in the medium, after 24 hours), which could partially explain the neuroprotection actions of MSCs. These data suggest that mesenchymal stem cells may constitute a novel therapeutic alternative for the treatment of Alzheimer's disease.

Disclosures: **M. Godoy:** None. **L.M. Saraiva:** None. **A. Vasconcelos-dos-Santos:** None. **H.J.V. Beiral:** None. **A.P.C. Lima:** None. **A. Vieyra:** None. **F.G. De Felice:** None. **S.T.**

Ferreira: None. **R. Mendez-Otero:** None. **C.V. Braga:** None. **C.A.A. Silva:** None. **R.B. Leal:** None. **L.R.P. Carvalho:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.13/UU1

Topic: F.02. Animal Cognition and Behavior

Support: UNAM 10113

CONACyT 155242

PAPIIT IN209413

PAPIIT 212013

Title: The inhibition of histone deacetylase rescues synaptic plasticity and memory function in aged mice

Authors: *G. RAMIREZ MEJIA[1,2], P. MORENO-CASTILLA[2], L. RODRIGUEZ-DURAN[2], M. L. ESCOBAR[2], F. BERMUDEZ-RATTONI[2];
[1]Inst. De Fisiologia Celular, Div. De Neuro, Mexico DF, Mexico; [2]UNAM, Mexico City DF 04510, Mexico

Abstracts: Aging is an irreversible process that affects heterogeneously all body's cells leading them to morphological and functional deterioration. Brain aging is marked by a gradual decline in cognitive function, linked to deterioration of synaptic function in brain regions crucial for memory formation and consolidation. Epigenetic regulation, including histone acetylation plays an important role in many physiological conditions, including aging. Increasing histone acetylation by inhibition of histone deacetylase (HDAC) enhances gene transcription and improves hippocampal long-term potentiation (LTP) and memory function in several experimental models, but the effect of HDAC inhibitors in non-hippocampal plasticity and memory has not yet described. The aim of this work was to determine whether the histone deacetylase inhibitor MS-275 could restore memory performance and cortical synaptic function affected by aging. We used B6129SF2/J young and aged mice. For synaptic function evaluation, we used LTP in anesthetized animals receiving intracerebral injections of MS-275 at IC prior to LTP induction in the basolateral amygdala-insular cortex (BLA-IC) projection. High frequency

stimulation was applied into the BLA, responses were then measured at IC. The effect of the HDAC inhibitors in memory performance was evaluated in aged animals administered with MS-275 or vehicle, bilaterally in the IC prior to acquisition of conditioned taste aversion (CTA) or prior to object recognition memory (ORM). We found that aged mice presented *in vivo* synaptic dysfunction in the BLA-IC pathway and deficiencies in memory performance of CTA and ORM tasks. Cortical acute administration of MS-275 in aged mice reestablished the induction of LTP and improved memory function. Our findings suggest that chromatin histone acetylation modifications by HDAC inhibitors could be a promising strategy to improve cognitive alterations during brain aging.

Disclosures: G. Ramirez Mejia: None. **P. Moreno-Castilla:** None. **F. Bermudez-Rattoni:** None. **L. Rodriguez-Duran:** None. **M.L. Escobar:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.14/UU2

Topic: F.02. Animal Cognition and Behavior

Support: NIH AG 038070

The Nathan Shock Center of Excellence in the Basic Biology of Aging at the Jackson Laboratory

Title: Age-related changes in the behavior and neuronal morphology of Diversity Outbred mice

Authors: *L. C. ANDERSON, L. C. HOERTZ, W. N. FERM, Jr., E. J. CHESLER;
The Jackson Lab., Bar Harbor, ME

Abstracts: The Diversity Outbred (DO) mouse population is a genetic mapping resource with high precision, genetic polymorphism, and phenotypic diversity. The DO provides a powerful tool for identifying genetic loci that influence behavioral and neuroanatomical changes that occur in the aging process. In a cross-sectional design we assayed 200 male and female DO mice of three age groups (~6, ~12, and ~18 months). Behavioral assays consisted of the Open Field (OF), hippocampal dependent T-Maze, Novel Object Recognition (NOR), and the Tail Suspension Test (TST). Behavioral measures are correlated with measures of spine density and type from pyramidal neurons in the CA1 hippocampal subfield. General linear models were used to test

effects of age, sex, and age x sex interactions for each behavior. In the OF older mice showed lower anxiety than younger mice as measured by fecal boli, latency to periphery of arena, and center time. For the NOE and NOR we observed a significant decrease with age on distance traveled and time spent exploring objects. Time spent exploring the objects on Day 1 was correlated with the time spent with the objects on Day 2 but was not predictive of novel object preference. Chi^2 analysis showed an age effect on the number of poorly performing mice (lower quartile of all groups combined) on novel object recognition among 18 month old males but not females. No difference was found among the top performers (in the first quartile), indicating that the proportion of older mice of both sexes displaying spared cognition are indistinguishable from younger mice. T-Maze behavior varied across age as measured by the percent of correct transitions and distance traveled. There was no age or sex effect on TST. The correlation between dendritic spine density of CA1 pyramidal neurons and cognitive performance on the T-maze varied in magnitude across ages. There were no significant correlations of spine type or density on the TST or the cognitive aspects of the NOR. The behavioral results reflect age-related changes in activity, anxiety, and cognition related phenotypes. Future quantification will provide data on neuronal arborization. By combining these data with neurological, physiological, and genetic information obtained from the same mice we will be able to uncover genetic mechanisms and pathways of age related behavioral variation.

Disclosures: L.C. Anderson: None. **E.J. Chesler:** None. **L.C. Hoertz:** None. **W.N. Ferm:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.15/UU3

Topic: F.02. Animal Cognition and Behavior

Support: RISE Grant GM 060665 CUNY Collaborative Grant and RCMI Grant RR03037

Title: The effect of parity on olfactory acuity and spine density in the piriform cortex

Authors: *S. BELGRAVE[1], M. FRANKFURT[3], V. LUINE[2];
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Abstracts: Cognitive decline as a result of aging can be attenuated in females that have been through the motherhood experience, parity. Previous studies show multiparous females exhibit preservation of spatial memory ability past the time that females actively care for young. The attenuation of cognitive decline is accompanied by morphological changes because apical spine density on pyramidal cells in the CA1 region of the hippocampus is the same as that of young adult females. Given that spatial memory, an important maternal behavior, is preserved by parity we hypothesized that parity may induce long lasting effects in other behaviors and brain areas related to maternal behavior. Olfaction is a necessary component of the maternal ability to identify offspring and is interconnected with the limbic system, which form part of the memory and emotion centers in the brain, making it a prime candidate for study in the context of parity. Previous studies have found that anxious behavior is also attenuated by parity, but other studies have found no differences. In this study we utilized three groups of female F344 rats (young virgins, retired breeders and middle-aged virgins). Olfactory ability was tested using an olfactory acuity task and an olfactory habituation/dis-habituation task. The anxiety measures used were a latency to approach a novel object task, open field and plasma corticosterone level measurement after an acute stress. No significant difference was found in the latency to approach the object task and the olfactory acuity task. Middle aged females exhibited rearing ($p< .05$) and wall climbing ($p<.05$) behaviors significantly more than other groups. There was significant difference between retired breeders and aged females ($p< .05$) in corticosterone levels. For the habituation/dis-habituation task all groups are able to do the task successfully, but on the test trial, the retired breeder group did not spend as much time with the novel scent as the other two groups. These results suggest that the retired breeders have difficulty distinguishing between the habituation odor and new odors. This result was unexpected in comparison with effects of parity on spatial memory. Spine density of the semi-lunar cells in layer II/III of the piriform cortex was assessed using Golgi impregnation. No significant differences were found in spine density. Though parity mitigates the spatial memory decline that accompanies age, as well as some anxious behaviors the preservation benefits do not seem to extend to olfactory acuity behaviors and the semi-lunar cells of the piriform cortex.

Disclosures: **S. Belgrave:** None. **M. Frankfurt:** None. **V. Luine:** None.

Poster

653. Learning and Memory: Aging I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 653.16/UU4

Topic: F.02. Animal Cognition and Behavior

Support: DANA Foundation

Stavros Niarchos Foundation

R37 AG06647, NIH

Alzheimer's Drug Discovery Foundation

Title: Glutamatergic regulation prevents age-related spatial memory decline through dendritic spine clustering

Authors: *A. C. PEREIRA[1], H. LAMBERT[2], Y. GROSSMAN[3], W. JANSSEN[3], B. MCEWEN[2], J. MORRISON[3];
[1]Neuroscience/ McEwen laboratory, Rockefeller Univ., New York, NY; [2]Rockefeller Univ., NYC, NY; [3]Mount Sinai Sch. of Med., NYC, NY

Abstracts: The devastating cognitive decline that accompanies Alzheimer's Disease (AD) results primarily from degeneration of neurons that furnish glutamatergic corticocortical connections that subserve memory and cognition. While neuron death is minimal in the absence of AD, age-related cognitive decline does occur in animals as well as humans, and it decreases quality of life and independence for elderly people. Age-related cognitive decline has been linked to specific synaptic alterations that impair function in regions such as hippocampus and prefrontal cortex. These synaptic alterations are likely reversible, such that maintenance of synaptic health in the face of aging is a critically important therapeutic goal. Here we show that riluzole can protect against some of the synaptic alterations in hippocampus that are linked to age-related memory loss in rats. Riluzole increases glutamate uptake through glial transporters and is thought to decrease glutamate spillover to extrasynaptic NMDA receptors while increasing synaptic glutamatergic activity. Treated aged rats were protected against age-related hippocampal cognitive decline displayed in non-treated aged animals. Memory performance correlated with density of thin spines on apical dendrites in CA1, though not with mushroom spines. Furthermore, riluzole treated rats had an increase in clustering of thin spines that correlated with memory performance and was specific to the apical dendrites of CA1. Clustering of synaptic inputs along dendritic branches has been shown in electrophysiological studies and computational models to allow non-linear summation of synaptic strength. These findings further elucidate mechanistic neuroplastic changes in glutamatergic neural circuits with aging and advance therapeutic development to prevent and treat age-related cognitive decline.

Disclosures: A.C. Pereira: None. **H. Lambert:** None. **Y. Grossman:** None. **W. Janssen:** None. **B. McEwen:** None. **J. Morrison:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.01/UU5

Topic: F.03. Motivation and Emotion

Support: CAPES/PROEX

FAPESP

CNPq

Title: The role of specific receptors for corticotropin-releasing factor CRF1 and CRF2 from basolateral and central nuclei of amygdala in tonic immobility behavior in guinea pigs

Authors: B. B. DE PAULA[1], J. R. M. M. COELHO[2], R. L. SPINIELI[1], *C. R. LEITE-PANISSI[2];
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Abstracts: The behavioral response of Tonic Immobility (TI) occurs under extreme dangerous and inescapable situations, such as the predator attack. This response is characterized by loss of righting reflex and the relative lack of responsiveness to environmental stimuli. Consistent studies have demonstrated the involvement of different brain areas to modulate this defensive behavior, including the periaqueductal gray matter, hypothalamus and amygdala. Whereas the amygdala in particular, studies have shown the involvement of receptors for corticotrophin-releasing factor (CRF) of the central (CeA) and basolateral (BLA) nuclei of amygdala in TI modulating. Indeed, in recent decades, several evidences suggest that CRF is closely correlated with emotional behavior associated with fear and anxiety. While it is clear the involvement of CRF receptors in the modulation of fear, and specifically in the TI modulation, it is still unclear the involvement of different subtypes of CRF receptor in this response. Hence, the aim of this study was evaluated the involvement of specific receptors for corticotrophin-releasing factor, CRF1 and CRF2, in TI behavior in guinea pigs (Cavia porcellus). Male guinea pigs (CEUA 12.1.1393.53.0, n=105) received unilateral administration of CP-376395 (CP37) and Astressin 2B (ASTR2B), both at doses of 0.8 μg/0.2 μl, either intra-basolateral (BLA) or intra-central (CeA) nucleus of amygdala. As well, these drugs were administered at the doses of 0.4 μg/0.2 μg before the administration of CRF at dose of 0.2 μg/0.2 μl in the same nuclei. The results showed that CP37 in BLA or CeA reduced the duration of TI per se (BLA-$F_{6,27}=6.987$, $p=0.003$; CeA-$F_{6,27}=8.629$, $p=<0.001$) and antagonized the increase of TI induced by CRF administration into

same site (BLA-F6,27=6.026, p=0.005; CeA- F5,23=8.482, p=0.002). Again, ASTR2B reduced the TI response either in BLA or CeA per se (BLA-F4,19=10.800, p=0.001; CeA- F4,19=4.575, p=0.023) and blocked the increase of TI duration induced by CRF (BLA- F6,27=3.360, p=0.033; CeA- F5,23=8.430, p=0.002). In addition, the drugs used in this study did not alter motor activity. These findings suggest that the antagonism of CRF1 and CRF2 receptors in BLA and CeA can reduce the duration of TI behavior, an innate fear behavior. Financial **Support:** CAPES/PROEX, FAPESP, CNPq.

Disclosures: **B.B. De Paula:** None. **J.R.M.M. Coelho:** None. **R.L. Spinieli:** None. **C.R. Leite-Panissi:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.02/UU6

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA034010

NIDA-IRP

Title: The dorsal raphe nucleus is integral to negative prediction errors in Pavlovian fear

Authors: *M. A. MCDANNALD[1], G. SCHOENBAUM[2], B. A. BERG[2];
[1]Dept. of Psychology, Boston Col., Chestnut Hill, MA; [2]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstracts: Prediction errors are central to modern learning theories. While brain regions contributing to reward prediction errors have been uncovered, the sources of prediction errors in Pavlovian fear remain largely unknown. Here we used probabilistic and deterministic reinforcement procedures, followed by extinction, to examine the contribution of the dorsal raphe nucleus to negative prediction errors in Pavlovian fear. Rats with dorsal raphe lesions were able to acquire fear and reduce fear to a non-reinforced, deterministic cue. However, dorsal raphe lesions impaired the reduction of fear to a probabilistic cue and fear extinction to a deterministic cue - both of which involve the use of negative prediction errors. The results point to an integral role for the dorsal raphe nucleus in negative prediction error signaling in Pavlovian fear.

Disclosures: **M.A. McDannald:** None. **G. Schoenbaum:** None. **B.A. Berg:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.03/UU7

Topic: F.03. Motivation and Emotion

Support: R21 HD070662-01

Title: Increased Egr-1 expression in the prefrontal cortex correlates with context-shock association in the context pre-exposure facilitation effect (CPFE) in adult rats

Authors: *T. CHAKRABORTY, A. ASOK, M. E. STANTON, J. B. ROSEN;
Psychology, Univ. of Delaware, Newark, DE

Abstracts: The context pre-exposure facilitation effect (CPFE) is a fear-conditioning paradigm that temporally separates the context acquisition and context-shock association phases of learning and can be used to investigate differential contributions of brain regions in each of these phases of learning. We previously found differential expression of *Egr-1 (Zif268)*, an inducible transcription factor associated with learning and synaptic plasticity, in the prefrontal cortex (PFC), dorsal hippocampus (dHC), but not the lateral nucleus of the amygdala (LA) during the CPFE in adolescent rats (Asok, et. al., 2013; Schreiber et. al., under review). In the present study we extended this work to adults. Adult male Long Evans rats were trained in the CPFE over 3 days. On the first day, animals were either left in their homecage (HC) as controls or preexposed to context A (Pre) or context B (Alt). Brains were collected from a third of the rats 30 min post-exposure. Another third of the rats were run in the context-shock association phase 24h after preexposure. Pre and Alt rats were given an immediate shock in Context A, and an additional novel context (NC) group was placed in context A but not shocked. Rats were sacrificed 30min post-training and their brains were collected. The last cohort of rats was tested for fear conditioned freezing in context A during a retrieval phase 24 h later. Pre rats displayed contextually conditioned freezing, but the Alt rats did not, indicating fear conditioning in Pre, but not Alt rats. *Egr-1* expression after the context preexposure and context-shock training phases was assayed by *in situ* hybridization in the PFC, dHC, and LA. After pre-exposure, increased *Egr-1* expression was found in all regions of the PFC in both the Pre and Alt groups compared to HC. No differences were found in the dHC or LA. After training, the Pre, Alt, and NC groups once again expressed higher *Egr-1* levels compared to HC. Importantly, a differential increase in *Egr-1* was found in the Pre group compared to the Alt and NC groups in the PFC. Alt and Pre groups also expressed increased *Egr-1* expression compared to the HC and NC groups in the dHC. The results indicate that differential activation of *Egr-1* in the PFC correlates with fear

learning during the context-shock association phase. Animals that were not preexposed to the conditioned context (Alt) exhibited the immediate shock deficit and had less expression of *Egr-1* in the PFC. The data from adult rats are similar to previous findings with juvenile rats. They suggest that the PFC, and *Egr-1* may play a role in contextual fear conditioning when a prior incidentally learned context is subsequently associated with an aversive US.

Disclosures: T. Chakraborty: None. **A. Asok:** None. **M.E. Stanton:** None. **J.B. Rosen:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.04/UU8

Topic: F.03. Motivation and Emotion

Support: NSERC Grant 288348

OGS

Title: Effects of CB1 receptor agonism and antagonism on fear and stress responses in adult male rats

Authors: *J. J. SIMONE[1], M. R. GREEN[2], T. E. HODGES[2], C. M. MCCORMICK[2];
[1]Biol. Sci., [2]Psychology, Brock Univ., St. Catharines, ON, Canada

Abstracts: The endogenous cannabinoid system (ECS) is a retrograde messenger system involved in the regulation of a wide variety of behavioural and physiological processes. ECS signalling occurs through two main receptor subtypes, cannabinoid receptor type 1 (CB1) and type 2 (CB2). Studies in rodents have revealed a role of the ECS in fear and stress responses; increasing ECS signalling decreases, whereas inhibiting ECS signalling increases fear and stress responses. Many studies administering systemic injections, however, use non-specific CB1/CB2 agonists and, as such, the specific contributions of each receptor to fear and stress responses are not well known. Using the highly selective CB1 receptor agonist ACEA and inverse agonist AM251, we investigated the effects of CB1 receptor activity on fear and stress responses in adult male rats. CB1 receptor effects on unconditioned fear were assessed using elevated plus maze, and open field testing, whereas conditioned fear was analyzed using a classical auditory fear conditioning paradigm. As CB1 is known to elicit changes in intracellular signalling cascades implicated in fear behaviours, we performed an additional study investigating the effects of

ACEA on hippocampal ERK1/2 and Akt signalling. When tested on the elevated plus maze both ACEA (0.5 mg/kg) and AM251 (3 mg/kg) decreased time spent on the open arm relative to vehicle ($p = 0.07$ and 0.004, respectively). When tested on an open field, ACEA (0.1 and 0.5 mg/kg) and AM251 significantly decreased the time spent in the center of the field compared to vehicle (all $p < 0.04$). Although neither AM251 nor ACEA affected freezing to a conditioned fear, AM251 impaired, and ACEA enhanced, fear extinction. After exposure to the conditioned stimulus, corticosterone concentrations were higher in rats given ACEA (0.5 mg/kg) and AM251 than those given ACEA (0.1 mg/kg) or vehicle. To determine the effects of ACEA on ERK1/2 and Akt signalling, rats were injected and brains harvested at various post-injection time points. ACEA (0.5 mg/kg) decreased pERK1/2 expression in the dorsal hippocampus 30 minutes post-injection ($p = 0.013$), and increased pERK1/2 expression in the ventral hippocampus 60 minutes post-injection ($p = 0.048$). There was no effect of ACEA on pAkt expression in either region at any of the time points examined. These results are consistent with reports of fear-enhancing effects of the inverse agonist, and add to evidence that relatively low doses of CB1 agonists can have both fear-enhancing and fear-reducing effects, depending on the measure. The results suggest ERK1/2 signalling may be involved in the behavioural effects of ACEA.

Disclosures: J.J. Simone: None. **M.R. Green:** None. **T.E. Hodges:** None. **C.M. McCormick:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

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Program#/Poster: 654.05/UU9

Topic: F.03. Motivation and Emotion

Support: Fondecyt Grant 1130042

Anillo ACT-66

Title: Activity of the interoceptive insular cortex during expression of conditioned fear

Authors: P. CASANOVA, M. AGUILAR, M. RODRIGUEZ, *F. TORREALBA;
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Abstracts: The insula is the cortical component of the interoceptive pathway. This cortex has been involved in regulating internal states and emotional responses. A growing body of evidence

suggests that learned fear may be related to the activity of the interoceptive Insular Cortex. Preliminary data from our laboratory show that the inactivation of the primary interoceptive Insular Cortex (pIC) of the rat markedly reduced fear expression to an auditory conditioned stimulus (CS). In the present study we tested the activity of single neurons from the pIC during expression of auditory conditioned fear in freely moving rats. The rats were implanted with an array of 6 tetrodes under general anesthesia and left in their homecages for a week. On experimental day 1, the rats received 5 auditory CS (5 kHz, 20 sec, 80 dB) that co-terminated with a mild footshock (0.5 sec, 0.5 mA). On day 2, the rats were placed in a different box located in a different room, and received 15 CS in the absence of footshock, while we recorded from the pIC. We calculated firing rate 20 sec before (baseline) and 20 sec after CS onset for the first 3 CS, and z-scored the data. Then we obtained average change in z-score for each cell during CS compared to baseline. CS elicited significant changes (Z-Score > 1.96) in 19.2 % of recorded 99 neurons: 10.1 % showed an increase and 9.1 % a decrease in their firing rate. These data suggest that the electrical activity of neurons from the pIC is associated to fear expression.

Disclosures: P. Casanova: None. **M. Aguilar:** None. **M. Rodriguez:** None. **F. Torrealba:** None.

Poster

654. Motivation and Emotions: Fear and Pain

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Program#/Poster: 654.06/UU10

Topic: F.03. Motivation and Emotion

Support: CIHR MOP 89758

NSERC RGPIN 261739-2008

Title: Changes in the orexin (hypocretin) system in rats following footshock exposure

Authors: H. WANG[1], S. LI[1], G. LIU[1,2], *G. J. KIROUAC[3];
[1]Oral Biol., Univ. of Manitoba, Winnipeg, MB, Canada; [2]Intl. School, Guangdong Food and Drug Vocational Col., Guangzhou, China; [3]Oral Biol. and Psychiatry, Fac. of Dent., Winnipeg, MB, Canada

Abstracts: Orexins (hypocretins) are peptides produced by neurons located exclusively in the hypothalamus. Orexin neurons project to many areas of the brain known to be important for

regulating arousal levels. A number of studies have also shown that orexin neurons are activated by stressful conditions and that these neurons play a role in the physiological and behavioral responses to stress. Post-traumatic stress disorder (PTSD) is an anxiety disorder triggered by traumatizing and stressful events. Similarly, rats exposed to an acute episode of moderately intense footshocks display long lasting fear and anxiety. Work in our laboratory has demonstrated that a subset of shocked rats which showed a high level of acute fear to a novel tone the day after the footshock experience (high responders; HR), showed high levels of anxiety that last for several weeks. Previous work in our laboratory has also shown that systemic treatment of HR with an orexin antagonist has an anxiolytic effect in these rats. The present study examined if the mRNA levels for prepro-orexin (ppOX), orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R) are increased in rats exposed to footshocks. On day 1, shocked rats were exposed to footshocks (5 × 2 sec episodes of 1.5 mA with an inter shock period of 10-50 s presented randomly over 3 min), whereas nonshocked (NS) rats were placed in the shock chamber for the same amount of time. On day 2, rats were placed in a novel chamber and the time that each rat spent immobile during the presentation of a novel tone was scored. Based on the immobility to the novel tone, shocked rats were assigned to the HR (rats spent > 60% of the time immobile) or low responders (LR; rats spent < 40% of the time immobile) groups. On day 14, the rats were anesthetized and the posterior hypothalamus, dorsal midline thalamus and locus coeruleus/parabrachial region were removed for analysis using real-time RT-PCR. The level of ppOX mRNA was increased in the hypothalamus of HR compared to the LR and NS. In addition, the level of OX1R mRNA was found to be increased in the hypothalamus of HR compared to NS. No difference was observed in OX2R mRNA levels in the hypothalamus between the 3 groups. There was no difference between groups in the level of OX1R and OX2R in samples of the midline thalamus and locus coeruleus/parabrachial region. Protein expression levels for the OX1R and OX2R are being quantified using Western blot and will be presented at the meeting. The results of the present study indicate that changes in orexin and OX1R synthesis occur in a subset of rats exposed to footshocks. These results may provide a better understanding of the brain mechanisms contributing to hyperarousal and anxiety in PTSD.

Disclosures: **H. Wang:** None. **S. Li:** None. **G.J. Kirouac:** None. **G. Liu:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.07/UU11

Topic: F.03. Motivation and Emotion

Support: VA Merit Review

VA Career Scientist

Title: Predator Exposure, but not footshock, produces a noradrenergic-dependent flashbulb memory in rats

Authors: *L. A. BULLARD[1], C. R. PARK[1,2], D. M. DIAMOND[1,2];
[1]Psychology, Univ. of South Florida, Tampa, FL; [2]Res. and Develop., VA Hosp., Tampa, FL

Abstracts: A primary component of flashbulb memories in people is the enhancement of memory for incidental events occurring close in time with an arousing experience. We theorized that a flashbulb memory-like effect should occur in rats that are given a stressful experience. We have assessed the effects of two kinds of arousing stimuli, footshock or predator (cat) exposure, on a rat's memory for an incidental (emotionally neutral) event.We hypothesized that either form of arousing stimulus (shock or cat) would enhance incidental memory, but only when the arousing event occurred close in time to the neutral experience. Adult male SD rats were habituated to an open field (OF) on 3 consecutive days. On Day 4 (training), the rats were placed in the OF which contained two identical objects. All behavioral manipulations occurred prior to or after OF exposure on Day 4. Memory retrieval was assessed on Day 5, when the rats were returned to the OF, which contained an identical (familiar) object and a novel object. Increased time spent around the novel, relative to the familiar, object was an index of enhanced memory. We have found that 2 min of cat exposure, either immediately before or after training (on Day 4), enhanced novel object memory on Day 5. The memory enhancement effect occurred only if the onset of cat exposure occurred within 2 min, but not 30 min, before or after training. We also found that the 2 min cat-induced enhancement of memory was mimicked by injection of epinephrine (0.1 mg/kg, ip) immediately before or after training, and was blocked by propranolol (beta antagonist). Administration of CPP (5.0 mg/kg, ip) blocked the memory enhancement produced by 2 min cat exposure. In contrast, we found that footshock had no effect on novel object memory, despite generating fear memory (freezing) to the context in which the shock occurred. That is, memory for the shock, itself, was indicated by freezing behavior to the context, but this stimulus did not enhance memory for the incidental stimuli occurring close in time to the shock. These findings indicate that predator exposure exerts a qualitatively different effect on memory processing than footshock, suggesting that a life-threatening experience gains greater access to trauma memory processes than does a painful stimulus. These findings may prove to be relevant toward enhancing our understanding of the neurobiology of traumatic memory processing.

Disclosures: L.A. Bullard: None. **C.R. Park:** None. **D.M. Diamond:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

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Program#/Poster: 654.08/UU12

Topic: F.03. Motivation and Emotion

Support: NIMHD MD007599 to Hunter College

NIDCD DC009910 & NIH-MH091451 to RMS

Title: Amygdala PKM zeta increases with functional emergence of amygdala-dependent fear learning in rat pups

Authors: *P. A. SERRANO[1], L. MICHELSON[2], R. M. SULLIVAN[2];
[1]Dept of Psychology, Hunter Col. and City Univ. of New York, NEW YORK, NY; [2]Child & Adolescent Psychiatry, Nathan Kline Institute, New York Univ., New York, NY

Abstracts: During infancy rapid learning associated with attachment and orientation to a caregiver is essential to survival. This developmental period also prevents the acquisition of avoidance learning. In rodent models this developmental time window occurs prior to post-natal day 10 (PND 10), during which pups display heightened preference learning accompanied by decreased aversion learning. PND 10 rats presented with odor-shock pairings fail to avoid the odor associated with shock, and show a preference for the paired odor. Older pups (PND-12) given odor-shock pairings develop an aversion to the odor at subsequent test. Even though pups at any age find the shock itself aversive, as measured by vocalizations and overall activity. One key developmental mechanism that appears to direct the change from a preference for the odor associated with shock to an aversion, involves the activation of the amygdala by corticosterone. Corticosterone is low in pups during the sensitive period and increases at PND 10. We investigate synaptic markers which may be important for establishing the avoidance memory and that are likely activated by corticosterone. Recently, protein kinase M zeta (PKMζ), which is important for late-phase LTP and long-term memory, is also upregulated during stress (Sebastian et al 2013, PLoS One, vol 8, e79077). Therefore, we investigated the role of PKMζ in avoidance vs preference learning in rat pups using the paired odor-0.5mA shock fear-conditioning paradigm. PND 8 and PND 12 pups were given either paired (simultaneous odor and shock) or unpaired (shock 2 min after odor) training and tested 24hr later on a Y maze with one arm containing the conditioned stimulus (CS) odor and the other a familiar odor. Immediately after Y maze test, pups were sacrificed and amygdalae were harvested. The tissues were separated into cytosolic and synaptic cellular fractions. Each fraction was analyzed by Western blots. Pups in

the unpaired condition showed no preference for either arm. PND 8 paired pups in the paired condition preferred the CS odor and PND 12 pups avoided the CS odor ($p<0.01$). PND 12 in the paired condition had higher cytosolic PKMζ in the amygdala compared to unpaired pups ($p<0.05$) with no change in synaptic PKMζ. PN8 paired did not show any changes in cytosolic or synaptic PKMζ. Thus, increased PKMζ expression following the sensitive period plays a role in the activation process of the amygdala and the formation of aversive memories.

Disclosures: **P.A. Serrano:** None. **L. Michelson:** None. **R.M. Sullivan:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.09/UU13

Topic: F.03. Motivation and Emotion

Support: FAPESP

PADC/FCFar-UNESP

CNPq

Title: Lack of neuroendocrine and behavioral habituation after repeated predatory threat in mice

Authors: ***K. S. GOMES**[1], A. C. CIPRIANO[1,2], R. L. NUNES-DE-SOUZA[1];
[1]Univ. Estadual Paulista, Araraquara, Brazil; [2]Joint Grad. Program in Physiological Sci., Ufscar/UNESP, Brazil

Abstracts: Predator exposure is a naturalistic stressor of high ethological relevance. As such, determining mechanisms underlying predator-related stress responses are valuable to understand how endogenous stress-related disease processes are initiated and perpetuated. Here we evaluate the impact of chronic predator exposure on the behavior and corticosterone levels of mice. Male Swiss mice (n=7-9) were repeatedly exposed to a toy or to a predator on the rat exposure test (RET) paradigm for seven days. Independent groups were sacrificed 5, 20 or 50 minutes after beginning of the exposure and trunk blood was collected for corticosterone quantification. The RET comprises a prey-predator based model that provides a home chamber connected via tunnel to a surface area in which a wire mesh prevents the predator (rat) from approaching or contacting the mouse subject. During a 10-min session in the RET, mice were exposed to a rat and the frequency of entries and time (in seconds) spent on each compartment of the apparatus, risk

assessment behavior (stretched attend postures, SAP), immobility, defensive burying (from the home chamber towards tunnel), as well as contact and climbing the wire mesh were scored. Compared to the toy-exposed group, mice repeatedly exposed to a rat exhibited a higher corticosterone level after 5 minutes (21 vs. 12 μg/dL, $p<0.05$) but not after 20 or 50 minutes from beginning of stress ($p>0.05$). Furthermore, the behavioral analysis revealed that the mice exposed to a rat spent more time on distal compartments of the RET apparatus compared to the control group, as well as exhibited more risk assessment behavior (time and frequency) and immobility time and less time in contact or climbing the wire mesh ($p<0.05$). The results show that, unlike other chronic homotypic stress regimens (e.g. repeated restraint stress), repeated rat exposure facilitated corticosterone secretion after the 7th day and indicate that predator stress engages a unique pattern of neuroendocrine activation that may contribute to long-lasting changes in brain stress responsivity. Moreover, the behavioral data also show that there is no habituation to the predatory threat after successive exposures. Further experiments are being conducted to determine the pattern of neural fos activation in areas of the brain defense system.

Disclosures: K.S. Gomes: None. **A.C. Cipriano:** None. **R.L. Nunes-de-Souza:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: MUI Start ST2013042001

FWF P25851

FWF P22830

Title: Role of Neurokinin B-expressing neurons in the basolateral amygdala in fear and fear extinction

Authors: *R. O. TASAN[1], G. LACH[1], J. WOOD[1], D. VERMA[2], S. WEGER[3], R. HEILBRONN[3], G. SPERK[1];
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Abstracts: Anxiety disorders constitute a major burden for the society and are characterized by pathological expression of anxiety and fear. Among the different brain areas involved in the encoding and modulation of fear memories the amygdala plays an exceptionally important role. Intrinsic and extrinsic amygdala connections are predominantly mediated by glutamate and GABA, but the resulting behavioral response is fundamentally shaped by various neuromodulators, including different neuropeptides. Despite extensive investigations, the role of many of these neuropeptides is still poorly understood. Among the neuropeptide family of tachykinins, substance P is promoting anxiety-related behavior, whereas the role of neurokinin B (NKB) that is abundantly expressed in different amygdala nuclei is not clear yet. Thus, we wanted to investigate the role of NKB and NKB-expressing neurons in the amygdala in fear conditioning and extinction. To test the specific role of NKB-expressing neurons in the basolateral amygdala (BLA), Tac2-Cre mice were locally injected into the BLA with a Cre-dependent rAAV-vector expressing tetanus-light-chain (TeLC) or rAAV-hM3Dq for specific and local permanent silencing or for transiently activating NKB neurons, respectively, followed by Pavlovian fear conditioning two weeks later. Pavlovian fear conditioning was used as a simple form of associative learning by pairing a tone with a mild electric food shock. Fear extinction was performed the following day by repetitive exposure to the tone without foot-shock. Inhibition of NKB-expressing neurons in the BLA did not affect fear acquisition or context fear but resulted in reduced expression of tone-induced freezing and facilitated extinction learning. This change was also observed in extinction recall, demonstrating a persistent effect of NKB neuron inhibition in the BLA. Interestingly, inhibition of all GABA-ergic neurons in VGAT-Cre mice resulted in increased fear and delayed extinction, indicating a highly specific function of NKB neurons in the BLA. Together our data indicate that inhibition of NKB-expressing neurons in the BLA reduces the expression of fear while facilitating fear extinction. Further studies will clarify the exact mechanism and the contribution of NKB itself in this process.

Disclosures: **R.O. Tasan:** None. **G. Lach:** None. **J. Wood:** None. **G. Sperk:** None. **D. Verma:** None. **S. Weger:** None. **R. Heilbronn:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: FAPESP (2011/19472-4)

Title: Protein synthesis inhibitor (anisomycin) microinjected into medial prefrontal cortex, amygdala or dorsal hippocampus disrupts the consolidation of step-down inhibitory avoidance in mice

Authors: *L. CANTO DE SOUZA[1], R. MATTIOLI[2];
[1]Univ. de Sao Paulo, Araraquara, Brazil; [2]Univ. Federal de São Carlos, São Carlos, Brazil

Abstracts: Several studies using inhibitory avoidance models have demonstrated the importance of limbic structures as the amygdala, dorsal hippocampus and medial prefrontal cortex on emotional memory. On the other hand, there are few studies assessing the involvement of these limbic structures on the emotional memory of mice exposed to step-down inhibitory avoidance. Therefore we aimed to investigate the role of the medial prefrontal cortex, amygdala and dorsal hippocampus, through bilaterally microinfusions of anisomycin (ANI-40µg/µl; a protein synthesis inhibitor), on the modulation of emotional memory in mice subjected to the step-down model. We observed that microinfusions of anisomycin into the medial prefrontal cortex, amygdala and dorsal hippocampus prevent the increase in latency observed in control groups during the test. We suggest that protein synthesis in the medial prefrontal cortex, amygdala and dorsal hippocampus of mice is independently necessary for the consolidation of emotional memory of mice in the step-down inhibitory avoidance model, and that step-down inhibitory avoidance task is also an interesting model to infer emotional memory in mice.

Disclosures: L. Canto De Souza: None. **R. Mattioli:** None.

Poster

654. Motivation and Emotions: Fear and Pain

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Topic: F.03. Motivation and Emotion

Support: NIH/NIDA-IRP

Title: Release of glutamate from ventral tegmental inputs into the lateral habenula elicits aversive conditioning

Authors: *D. H. ROOT[1], C. A. MEJIAS-APONTE[2], M. MORALES[2];
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Abstracts: Ventral tegmental area (VTA) activity is involved in the detection and prediction of rewarding stimuli. However, VTA has been shown to also be activated by aversive stimuli. Though the circuits involved in VTA participation in aversion are unclear, activation of glutamatergic inputs to VTA from lateral habenula (LHb) elicits conditioned aversion. Here we show that a glutamatergic input to LHb from VTA also elicits conditioned aversion. Channelrhodopsin2-eYFP (ChR2) or eYFP was expressed in VTA vesicular glutamate transporter 2 neurons of VGluT2::Cre mice. Bilateral optical fibers or cannulae were implanted dorsal to LHb. Using a place conditioning procedure, mice traversed a three chamber maze over several sessions. During training, when mice entered a randomly assigned Paired chamber, light was continuously delivered (473 nm, 5-10 mW, 10 ms duration, 50 ms period) until mice exited the Paired chamber. Light was not delivered in any other chamber. During training, ChR2-eYFP mice but not eYFP mice, spent more time in the Unpaired chamber in which light activation of ChR2 mesohabenular axon terminals did not occur. In the absence of light stimulation, ChR2-eYFP mice demonstrated a conditioned place aversion to the Paired chamber that was previously associated with light delivery. Following the reverse of Paired and Unpaired chamber light-delivery contingencies ChR2-eYFP mice, but not eYFP mice, spent more time in the chamber that did not result in light delivery. During a Reversal Test in which light was not delivered, ChR2-eYFP mice spent more time in the chamber that was not associated with light delivery, demonstrating the formation of a new conditioned place aversion. eYFP mice never formed conditioned place preference or aversion. To determine if LHb glutamate receptors participate in light-elicited aversion, another group of ChR2-eYFP mice was tested in the following manner. The chamber in which mice spent more time during baseline in the absence of light delivery was selected as the Paired chamber. A mixture of AMPA and NMDA antagonists (CNQX and AP5), or vehicle, was injected into LHb prior to being placed in the maze and light was delivered to LHb when mice entered the Paired chamber. Glutamate receptor antagonist injection in LHb resulted in no significant change from baseline time spent in any chamber. aCSF injection in LHb resulted in ChR2-eYFP mice spending more time in the Unpaired chamber and less time in Paired chamber, demonstrating that aversion elicited by light activation of ChR2 terminals from VTA VGluT2 neurons is dependent on LHb glutamate receptors. We conclude that a glutamatergic pathway from VTA to LHb plays a role in aversion and its conditioning.

Disclosures: **D.H. Root:** None. **C.A. Mejias-Aponte:** None. **M. Morales:** None.

Poster

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Program#/Poster: 654.13/UU17

Topic: F.03. Motivation and Emotion

Support: CNPq Grants

Title: Gabaergic activation in the basomedial amygdala reduces the tonic immobility response in guinea pigs: A innate fear behavior

Authors: ***B. B. DE PAULA**, J. R. MELO, R. L. SPINIELI, C. R. A. LEITE-PANISSI; Morphology, Physiol. and Pathology Basic, Univ. of São Paulo, Ribeirão Preto, Brazil

Abstracts: The defensive behavior of tonic immobility is associate with intense danger. In laboratory, this response is easily induced, and it may represent a model for study of the innate fear. Also, the amygdala has a critical role in the emocional mean of the sensory stimuli and modulation of the unconditioned and conditioned fear. In particular, the basomedial nucleus of the amygdala (BMA) participates in fear modulation, being responsable, together with lateral nucleus of the amygdala, by integration of the cognitive processing of the predator presence. In this context, GABAergic neurons exert a tonic control on neural substrates involved in expression of defensive reactions. The aim of this study was evaluated the involvement of GABAA and GABAB receptors of BMA in tonic imobillity response in guinea pig. Guinea pigs (n = 77, CEUA: 12.1.202.53.7) were anesthetized and submitted to surgery for guide canulla implate into the BMA. Afterward, guinea pigs were divided in groups for administration intra-BMA of Bicuculline (BIC; GABAA antagonist; 0.1µg/0.2µl and 0.5µg/0.2µl), Muscimol (MUS; GABAA agonist; 0.1µg/0.2µl and 0.5µg/0.2µl), MUS (0.5µg) preceded by BIC (0.1µg), Baclofen (BAC, GABAB agonist, 0.1µg/0.2µl and 0.5µg/0.2µl), Faclofen (FAC; agonist GABAA; 0.1µg/0.2µl and 0.5µg/0.2µl), FAC (0.5µg) preceded by BAC (0.1µg). Immediately after the treatment, the animals were submitted to TI response or the open field test (OF). Statistical analysis (One-Way ANOVA) was applied followed by Tukey test, with $P < 0.05$. The MUS and BAC reduced TI duration ($F_{6,4}=14.16$, $P < 0.001$ and $F_{5,29}=11.678$, $P < 0.001$, respectively) when compared with their respective controls ($P < 0.05$, Tukey). In contrast, BIC and FAC increased TI response ($F_{6,34}=5.827$, $P=0.002$ and $F_{5,29}=10.828$, $P<0.001$) compared to their respective controls. Further, BIC and FAC blocked the decrease in TI induced by MUS or BAC ($F_{4,24}=6.646$, $P=0.002$ and $F_{4,24}=5.445$, $P=0.006$). Furthermore, in the OF test, activation or blockade of GABAA and GABAB receptors into BMA did not alter the locomotion in guinea pigs, at the doses that were effective to produce changes in TI response. These results suggested that GABAA and GABAB receptors activation into BMA reduced TI responses, probably due to reduced innate/unconditioned fear. This reduce was not due to altered spontaneous motor activity, which may non-specifically affect TI behavior.

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Poster

654. Motivation and Emotions: Fear and Pain

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Topic: F.03. Motivation and Emotion

Support: FAPESP 2012/13804-8

Title: Evidence for the thalamic targets of the medial hypothalamic defensive system mediating emotional memory to social defeat

Authors: *M. J. RANGEL, JR, N. S. CANTERAS;
Univ. De São Paulo/Instituto De Ciências Biomédicas, São Paulo, Brazil

Abstracts: We have documented that the medial hypothalamic defensive system is involved in processing defensive behavior to social defeat, and that the dorsal premammillary nucleus (PMd) represents a key hypothalamic site for the expression of defensive behaviors to social threats. Anatomical findings suggest that the PMd is also in a position to modulate memory processing through a projecting branch to the ventral part of the anteromedial nucleus (AMv). In the present study, we investigated the role of this thalamic target in mediating emotional memory to social defeat. Using NMDA iontophoretic lesions, three experimental groups were analyzed: AMv lesioned animals; Reuniens (RE) lesioned animals and intact animals. During social attack by the aggressor (resident male), intruder animals from all experimental groups exhibited intense defensive responses remaining most of the time freezing and avoiding the aggressive co-specific. ANOVA analysis revealed that during exposure to the environment previously associated with the social defeat, the animals with bilateral AMv lesions (n=7) presented significantly less contextual fear responses, such as as risk assessment (29.3 ± 8.1; p=0.00009), and spent significantly more time in the resident cage (176.8 ± 41.1; p=0.00003), when compared to animals with RE lesions (n=4; risk assessment: 168.0 ± 45.2; time spending in resident cage: 85.2 ± 33.7) and intact animals (n=7; risk assessment: 177.8 ± 15.4; time spending in resident cage: 22.9 ± 10.3). Overall, the present results support the idea that the AMv, one of the main thalamic target of the medial hypothalamic system, is critically involved in the emotional memory processing related to social defeat.

Disclosures: M.J. Rangel: None. **N.S. Canteras:** None.

Poster

654. Motivation and Emotions: Fear and Pain

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Topic: F.03. Motivation and Emotion

Support: Fyssen foundation

LABEX BRAIN (ANR-10-LABX-43)

Title: CB1 receptors in the medial habenula control emotional behavior

Authors: *E. SORIA[1], A. MEHIDI[2], A. BUSQUETS-GARCIA[2], L. ROUX[2], L. ALONSO[2], I. LOUIT[2], T. WIESNER[2], D. VERRIER[2], A. CANNICH[2], F. GEORGES[3], G. MARSICANO[2]; [1]INSERM U862, Bordeaux, France; [2]INSERM, Bordeaux, France; [3]CNRS, Bordeaux, France

Abstracts: The habenula (Hb), divided in lateral (LHb) and medial (MHb) subregions, is involved in the regulation of emotion. Particularly, the projections from the MHb to the interpeduncular nucleus (IPN) play an important role in anxiety and fear responses. However, the molecular mechanisms participating in these processes are poorly understood. Many of the functions mediated by the MHb-to-IPN circuit, such as the control of anxiety, are also known to be under the control of the endocannabinoid system (ECS). The expression of CB1 receptors in this circuit suggests that ECS could mediate the functions of this brain region. However, no studies have addressed this issue so far. The presence of cannabinoid receptors (CB1) on habenular cells is the prerequisite for the definition of the impact of the ECS on the functions of this brain region. In this work we showed that CB1 receptors are functionally expressed in the MHb-to-IPN circuit, participating in the control of fear expression potentially by selectively modulating cholinergic transmission in the IPN.

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Poster

654. Motivation and Emotions: Fear and Pain

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Topic: F.03. Motivation and Emotion

Support: NHMRC grant

Title: Connections between the hippocampus, amygdala and prefrontal cortex: Circuits that drive fear learning and extinction

Authors: *P. SAH[1], R. MAREK[2];
[1]Queensland Brain Inst., St Lucia, Australia; [2]Queensland Brain Institite, The Univ. of Queensland, Brisbane, Australia

Abstracts: The hippocampus, amygdala, and medial prefrontal cortex (mPFC) form a tripartite neuronal circuit are key players in fear learning and extinction. Activity within the infralimbic medial prefrontal cortex (IL) has been shown to enhance fear extinction through synaptic connections to the amygdala. Moreover, the ventral hippocampus (vHPC) sends unidirectional projections to the mPFC. Lesioning of the vHPC alters IL activity and disrupt the encoding of fear extinction. Using retrograde tracers and optogenetics, combined with whole-cell recordings *in vitro* we have studied the neural circuits within the mPFC and their connections with the amygdala and vHPC. We describe the intrinsic connections with the mPFC and show that connections between the prelimbic medial prefrontal cortex (PL) and IL are unidirectional with L5/6 neurons in PL projecting to similar neurons in the IL. We show that vHPC projections to the mPFC largely target the IL. The PL receives moderate projections from the vHPC that show a rostro-caudal organisation. vHPC input is strongest onto fast spiking interneurons, which in turn produce large disynaptic inhibitory potentials in pyramidal neurons in both L2/3 and L5/6 pyramidal neurons, some of which send projection to the amygdala. This large inhibition shows adaptation to low frequency stimulation, and is capable of increasing the firing threshold of pyramidal neurons in the IL. These data show that vHPC-driven feed-forward inhibition controls the excitability of IL pyramidal neurons, and suggests a neural mechanism for the context dependency of extinction.

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Poster

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Support: H25-KAGAKU-IPPANN-003, Health and Labour Sciences Research Grants, Ministry of Health , Labour and Welfare, Japan

Title: Cross-fostering between Hatano high and low active avoidance rats altered emotional reactivity of male offspring

Authors: *Y. HORII[1,2], R. OHTA[3], K. TAKAHASHI[1], Y. SATO[1], K. SATO[1], S. NAKAJIMA[1], Y. SHIRAISHI[1], M. KAWAGUCHI[1];
[1]Meiji Univ., Kanagwa, Japan; [2]Natl. Inst. of Genet., Shizuoka, Japan; [3]Hatano Res. Inst., Kanagawa, Japan

Abstracts: It is important to develop a better understanding of how brain development and behavior are influenced by environmental stimuli such as maternal care, through these stimuli effects on epigenetic programming. For instance, rat offspring reared by high- licking and grooming (LG) dams are less fearful and show more modest hypothalamic-pituitary-adrenal (HPA) responses to stress than those reared by low-LG dams. Inbred strains of Hatano high- (HAA) and low- (LAA) active avoidance rats, which were originally selectively bred on the basis of respective high or low avoidance performance in an active avoidance task, show no strain differences in maternal LG behavior. However, in a preliminary experiment male HAA offspring reared by LAA dams showed reduced emotional reactivity in an elevated plus maze (EPM) test than HAA offspring reared by HAA dams. The aim of the present study is to confirm this earlier finding and to investigate detailed characteristics of maternal behavior. We prepared 4 experimental groups: HAA and LAA pups reared by unrelated HAA and LAA dams, respectively (in-fostering), and HAA and LAA pups reared by LAA and HAA dams (cross-fostering). First, we compared emotional reactivity of the male offspring using the EPM test and found both genetic and foster dam strain effects on emotional reactivity of male offspring. The HAA offspring showed higher anxiety-like behavior than the LAA offspring, regardless of the foster dam strain. Cross-fostering by HAA and LAA dams respectively increased or decreased emotional reactivity of the offspring. These results suggest that some maternal characteristics of each strain affected the development of emotional reactivity. Next we observed 18 types of maternal behavior, including LG, during the first 10 days postpartum. We found no differences in frequencies of LG or any of the other types of maternal behavior between the experimental groups. Thus these results suggest that some other maternal characteristics may influence the development of emotional reactivity of male offspring.

Disclosures: **Y. Horii:** None. **R. Ohta:** None. **K. Takahashi:** None. **Y. Sato:** None. **K. Sato:** None. **S. Nakajima:** None. **Y. Shiraishi:** None. **M. Kawaguchi:** None.

Poster

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Support: FAPESP (2009/17938-6)

Title: Chronic treatment with fluoxetine enhances antinociception and up-regulates 5-HT1A and 5-HT2C receptors within the periaqueductal gray in mice

Authors: *D. B. SOUZA[1], R. NUNES-DE-SOUZA[2], A. CANTO-DE-SOUZA[1];
[1]Univ. Federal De São Carlos, São Carlos, Brazil; [2]Pharmacol, FCF/Unesp/Araraquara, Araraquara, Brazil

Abstracts: AIM: It has been demonstrated that selective serotonin reuptake inhibitors (SSRI) attenuate pain response in human beings and animals. In addition, we have observed that the confinement of mice in the open arm of the elevated plus-maze (EPM) elicits antinociception (OAA). Besides to increase the levels of serotonin in the synaptic cleft, fluoxetine can also interact with 5-HT1A and 5-HT2C receptors. Here, we investigated the effects of chronic treatment with the SSRI fluoxetine on OAA, and on 5-HT1A and 5-HT2C receptors levels in the periaqueductal gray matter (PAG) of mice. METHODS AND RESULTS: Exp. 1: Male Swiss mice (n=7-9/group) were treated with fluoxetine (FLU: 0, 5, 10 and 20 mg/kg, s.c.) for 21 consecutive days. On 21th day, each mouse received an intraperitoneal injection of 0.6% acetic acid (0.1 ml/10g weight; nociceptive stimulus) and was then confined to either the open arm (OA) or enclosed arm (EA) of the EPM for 5 minutes, when the number of writhes was recorded. Two-way ANOVA (Factor 1: place of confinement x Factor 2: treatment) followed by Duncan's test confirmed that the OA confinement induced antinociception in comparison to EA-confined animals and revealed that FLU (20 mg/kg) enhanced this type of environmentally induced pain inhibition [Factor 1: $F(7,54) = 76.34$, $P<0.05$; Factor 2: $F(7,54) = 9.39$, $P<0.05$]. Exp. 2: Male Swiss mice (n=6-9/group) were treated with fluoxetine (FLU: 0, 5 or 20 mg/kg, s.c.) for 21 consecutive days. On 21th day, 40 minutes after the last FLU injection, the animals were sacrificed and their brains removed for analysis of the 5-HT1A and 5-HT2C receptors levels in the PAG through the Western Blotting assay. ANOVA followed by Duncan's test confirmed that FLU (5 and 20 mg/kg) increased 5-HT1A [$F(2,18) = 12.49$, $P<0.05$] and 5-HT2C receptor levels [$F(2,23) = 16.40$, $P<0.05$] compared to control group. CONCLUSION: Taken together, these results (i) confirm that the OA confinement induces antinociception, (ii) indicate that FLU (20 mg/kg) enhances OAA and (iii) suggest that chronic FLU increases 5-HT1A and 5-HT2C

receptor levels within the PAG, probably facilitating the OAA. FINANCIAL **SUPPORT:** UFSCar, CNPQ, FAPESP (2009/17938-6).

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Poster

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Topic: F.03. Motivation and Emotion

Title: The affective component of pain: The role of prostaglandins

Authors: *A. K. SINGH, M. FRITZ, A. M. KLAWONN, D. ENGBLOM;
Dept. of Clin. and Exptl. Med., Linköping Univ., Linköping, Sweden

Abstracts: Pain is characterized by two major components: the sensory (localization, intensity and quality) and the affective component (unpleasantness). The neural circuits and molecular mechanism underlying pain sensation have been extensively studied, but we are only now beginning to understand those responsible for the affective component. In the present study, we have investigated the role of prostaglandins in the affective component of pain. To study the affective component of pain we used a formalin-induced conditional place aversion (CPA) paradigm in mice. Normal mice showed a robust pain-induced aversion. The aversion was blocked by genetic or pharmacological inhibition of cyclooxygenase 2 (COX2) whereas inhibition of COX1 had no major effect. Deletion of COX2 in myeloid cells had no effect but COX2 deletion specific to the nervous system strongly attenuated the aversion. Mice lacking microsomal prostaglandin E synthase 1 (mPGES1) also showed an attenuated aversion indicating that prostaglandin E2 is the critical prostaglandin involved. Finally, we observed attenuation in the aversion of mice lacking EP2 prostaglandin E2 receptors while there was no change of CPA in mice lacking EP1 receptors. To determine if peripheral pain signaling is affected in the mutant mice, Fos-induction in the dorsal horn of the spinal cord will be measured. Overall, our results suggest that the discomfort and aversion induced by pain is controlled by neuronal COX2, mPGES1, and EP2 receptors.

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Poster

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Topic: F.03. Motivation and Emotion

Support: PADC/UNESP-FCFar

CNPQ

FAPESP process 2013/06764-2

Title: Role of TRPV1 channels of the dorsal periaqueductal gray in the modulation of phasic and tonic pain in mice

Authors: *D. C. MASCARENHAS[1,2], K. S. GOMES[1], R. L. NUNES DE SOUZA[1,2]; [1]Sch. of Pharmaceut. Sciences/ UNESP, Araraquara, Brazil; [2]Joint Grad. Program in Physiological Sci., Sao Carlos, Brazil

Abstracts: Previous findings have suggested that the TRPV1 channels located within the periaqueductal gray (PAG) play a role in the modulation of acute pain in rats. For instance, intra-PAG capsaicin (a TRPV1 agonist) increased the nociceptive responses assessed in the tail-flick and plantar tests. As far as we know there are no studies investigating the role of the TRPV1 channels of the PAG in the modulation of tonic pain. This study attempted to elucidate the role of TRPV1 channels within the dorsal PAG (dPAG) in the modulation of the phasic (assessed in the tail-flick test) and tonic (assessed in the formalin test) types of pain. Male Swiss mice (n=5-8) were surgically implanted with guide cannula targeted to the dPAG and five days later they received local injection of capsaicin (0, 0.01, 0.1 or 1 nmol/0.2 μL) or capsazepine (0, 10, 30 or 60 nmol/0.2 μL), TRPV1 agonist and antagonist, respectively, and were individually subjected to either the formalin or tail-flick tests. Results showed that both capsaicin (1 nmol; $F_{3,14}=10.31$) and capsazepine (60 nmol; $F_{3,16}=4.34$) attenuated the time spent licking the formalin-injected paw ($p<0.05$). Also, two-way ANOVA followed by Duncan test revealed that intra-dPAG capsaicin dose-dependently increased the latency of nociceptive response assessed in the tail-flick test [treatment ($F_{3,18}=42.09$, $p<0.05$); analgesia index ($F_{6,108}=31.34$, $p<0.05$); interaction ($F_{18,108}=13.96$, $p<0.05$)]. In contrast, intra-dPAG capsazepine decreased the tail-flick latency in mice [treatment ($F_{3,14}=9.45$, $p<0.05$); analgesia index ($F_{6,84}=7.16$, $p<0.05$); interaction ($F_{18,84}=2.5$, $p<0.05$)]. Our results suggest that activation (with capsaicin) of TRPV1 channels located within the dPAG leads to antinociception assessed in acute and tonic tests of nociception in mice. Curiously, the blockade of these channels with intra-dPAG capsazepine (60 nmol)

produces pro-nociceptive and antinociceptive effects in mice exposed to the tail-flick and formalin tests, respectively. Although further studies are needed to clarify these apparently inconsistent effects provoked by capsazepine, we cannot neglect that they may be due to the activation of distinct neural circuitry induced by different types of nociceptive stimuli [thermal stimulus (tail-flick test) × chemical stimulus (formalin test)].

Disclosures: **D.C. Mascarenhas:** None. **R.L. Nunes de Souza:** None. **K.S. Gomes:** None.

Poster

654. Motivation and Emotions: Fear and Pain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 654.21/UU25

Topic: F.03. Motivation and Emotion

Support: UFSCar

CNPq

FAPESP (2012/22238-6)

Title: Role of amygdala, insula and anterior cingulate cortex in the modulation of nociceptive response and pain empathy in mice

Authors: *A. CANTO-DE-SOUZA[1,2,3], V. PELARIN[1,3], D. BAPTISTA-DE-SOUZA[1,3]; [1]Psychobiology Group, Dept of Psychology, UFSCar, Sao Carlos, Brazil; [2]Grad. Program in Psychology, UFSCar/São Carlos, Brazil; [3]Joint Grad. Program in Physiological Sci., UFSCar/UNESP, Brazil

Abstracts: It has been suggested that limbic structures [e.g., amygdala (Amy), anterior cingulate cortex (ACC) and insula (Ins)] play a role in the modulation of pain and empathy in human beings. Previous studies have indicated that rodents also express pain empathy to a conspecific. Here, we investigated the role of Amy, ACC and Ins in the modulation of nociceptive response in mice living together with a cagemate with chronic pain. Male Swiss mice were housed in groups (Experiment 1) or in pairs (Experiment 2). The role of the brain areas in the modulation of the nociceptive empathy was assessed through the local injection of cobalt chloride ($CoCl_2$), which produces synaptic non-selective inactivation. In Experiment 1, mice received bilateral intracerebral injection of saline (0.1 µl) or $CoCl_2$ (1mM/0.1 µl) and, 10 min later, were subjected to the writhing test [intraperitoneal (i.p) injection of 0.6% acetic acid, nociceptive stimulus]

during a 5-min period, when the number of writhes was recorded. On the dyads (Experiment 2), animals lived together for 28 days since weaning. On 14th day, one animal of each pair was surgically subjected to a sciatic nerve constriction (SNC) or not (sham). On 24th day, the cagemate underwent a stereotaxic surgery to implant guide cannulae in the Amy, ACC or Ins. On 28th day, each mouse received intracerebral injection of saline or $CoCl_2$ and, 10 min later, was subjected to the writhing test, as described in Experiment 1. While inactivation of the Amy increased the number of writhes, inhibition of the ACC or Ins did not alter nociception, suggesting a distinct modulatory role of these structures on the sensorial compound of pain (Exp. 1). In Experiment 2, mice living together with a SNC-cagemate expressed higher frequency of abdominal writhing induced by 0.6% acetic acid i.p. in comparison to the control group (living with sham-operated mouse), suggesting that this experience activates the circuitry of neural representation of pain on the observer mouse (state of 'priming'). Inactivation of Ins and Amy produced opposite effects on nociception i.e. it decreased and increased, respectively, the abdominal contortions in those animals that lived together with a SNC cagemate mouse. ACC inactivation did not alter writhing behavior. Our results suggest that Amy and Ins differentially modulate pain empathy in mice.

Disclosures: A. Canto-de-Souza: None. **V. Pelarin:** None. **D. Baptista-de-Souza:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

Location: Halls A-C

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Program#/Poster: 655.01/UU26

Topic: F.03. Motivation and Emotion

Support: Fapesp Grant 2012/17626-2

CNPq

CAPES

Title: Role of neuronal nitric oxide synthase neurons located in the medial prefrontal cortex on restraint-induced long lasting anxiety in rats

Authors: *F. S. GUIMARAES, C. VILA-VERDE, A. Z. MARINHO, A. B. SONEGO; Pharmacol., Sch. Med. Ribeirao Preto-Usp, Ribeirao Preto, Brazil

Abstracts: Neurons expressing the neuronal isoform of the nitric oxide synthase (nNOS) enzyme are located in brain areas related to defensive responses such as the dorsolateral periaqueductal grey, dorsal premammilary nucleus, medial amygdala and medial prefrontal cortex (mPFC). Rats exposed to a live predator (a cat) show increased anxiety and expression of nNOS neurons in the mPFC one-week later. The present study aimed at investigating if restraint stress, another procedure known to induce long-lasting anxiogenic effects, would also be associated with increased nNOS expression in the mPFC. In addition, we also verified if inhibition of this enzyme in the mPFC would be anxiolytic in restrained animals. Male Wistar rats were forced restraint for 3-h. Twenty-four or 7 days later they were tested in the elevated plus maze. Immediately after, their brains were removed and nNOS expression in the mPFC was evaluated by immunohistochemistry. Independent groups of animals had bilateral cannulae implanted into the prelimbic (PL) mPFC. Five to seven days after surgery the animals were forced restraint (3-h) and tested in the EPM 24-h later. Ten min before the test they received bilateral microinjections of the selective nNOS inhibitor n-propyl-l-arginine (NPL, 0.04 nmol) or vehicle (saline, 0.2 μL). Restraint stress increased (28% to 49%) the number of neurons expressing nNOS in the prelimbic, but not in the infralimbic, mPFC both 24-h and 7 days after restraint. The number of nNOS neurons was negatively associated with the percentage of open arm entries in the EPM (r=-0.702 and -0.539). Restraint stress decreased open arm exploration 24-h later (percentage of open arm entries, control: 36.2 ± 4.6, restraint:19.8 ± 7.6). This effect was prevented by intra-PL microinjection of NPL (drug versus stress interaction, $p<0.05$). The results suggest that nNOS expression changes in the prelimbic mPFC could be related to the long-lasting anxiogenic effects of inescapable stressors such as forced restraint.

Disclosures: F.S. Guimaraes: None. **C. Vila-Verde:** None. **A.Z. Marinho:** None. **A.B. Sonego:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Program#/Poster: 655.02/UU27

Topic: F.03. Motivation and Emotion

Support: R15MH093918-01 A1

Title: Tempol protects anxiogenic drug induced anxiety-like behavior in rats

Authors: *G. D. PATKI, A. SALVI, H. LIU, F. ATROOZ, S. SALIM;
Univ. of Houston, Houston, TX

Abstracts: We have published that pharmacological induction of oxidative stress (OS) causes anxiety-like behavior in rats. We also have established that psychological stress induces OS and leads to anxiety-like behavior in rats. All evidence points towards a causal role of OS in anxiety-like behavior. In this study we have examined whether anxiety-like behavior induced directly via anxiogenic drugs can be prevented using agents (adenosine receptor agonist, caffeine, 50mg/kg or the partial inverse agonist of the GABAA receptor, FG-7142, 7.5mg/kg) that minimize OS. Osmotic pumps were either filled with antioxidant tempol or saline. The pumps were attached to the catheter leading to the brain cannula and inserted into the subcutaneous pocket in the back of the rat. Continuous i.c.v. infusion of saline or tempol in the third ventricle of the brain (4.3mmol/day) was maintained for 1 week by attachment of the infusion cannula to an osmotic pump. On the test days rats were injected i.p. with either saline vehicle or the anxiogenic drugs. Each of these drugs have been reported to cause increased vigilance and arousal behaviors in rat's home cage environments from 2-3 hr following drug administration compared with vehicle-injected controls. Two hours following drug injection all groups were subjected to behavioral assessments. Anxiety-like behavior tests (open-field, light-dark and elevated plus maze) suggested that tempol prevented anxiogenic drug-induced anxiety-like behavior in rats. Biochemical data suggests significant differences in the levels of stress (assessed via corticosterone assay) and oxidative stress (analyzed via 8-isoprostane and protein carbonylation) in rats.

Disclosures: G.D. Patki: None. **A. Salvi:** None. **H. Liu:** None. **F. Atrooz:** None. **S. Salim:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Program#/Poster: 655.03/UU28

Topic: F.03. Motivation and Emotion

Support: CONACYT scholarship 330090

PIFI scholarship 20120880

Title: Differential analgesic and anxiolytic effects of neuropeptide Y into periaqueductal gray of rat

Authors: *P. V. LEÓN[1,2], J. PACHECO-ROSADO[2], L. MENDOZA-RUIZ[2], A. MIRANDA-PÁEZ[2];
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Abstracts: Neuropeptide Y (NPY) which is highly conserved across animal species, is the most abundant neuropeptide in the central nervous system of mammals, is located mainly in the hypothalamus, septum, nucleus accumbens and periaqueductal gray (PGA). The PAG has gained importance due to their relation to analgesic mechanisms, immobility response, aggressive reactions, fear and fight or flight, all of which can be considered as integrated defensive behaviors. In the current study the differential effects of NPY in the dorsal and the ventrolateral columns of the PAG on analgesia, defensive behavior and anxiety were assessed. Adult male Wistar rats were used and subjected to stereotactic surgery to insert one guide cannula aimed at the dorsolateral or ventrolateral PAG columns for microinjection of NPY [Leu31, Pro34] dissolved in ISS (0.0, 0.23, and 0.47 nmol). The effect of NPY was assessed by testing tail flick (TF), immobility response by clamping the neck (IRCN), elevated plus maze (EPM) and defensive burying (DB). The NPY microinjected in VL-PAG or D-PAG exerts a significant analgesic effect because the tail flick latency was increased at all doses. The NPY injected in the VL-PAG significantly reduces the IRCN at both doses used, and into the D-PAG only with the dose of 0.47 nmol/1 µl compared between columns of PAG. NPY has a significant anxiolytic effect produced by the injection of 0.23 nmol/ 1 µl into the VL-PAG because it increases the dwell rate in open arms of EPM; oppositely, NPY applied into D-PAG has an anxiogenic effect at 0.47 nmol/1 µl, since it significantly reduces the dwell rate in open arms. It was confirmed in the DB test that NPY has an anxiolytic effect when injected into the VL-PAG at both doses; and within the D-PAG it has an anxiogenic effect only at a dose of 0.23 nmol / 1 µl; however, at 0.47 nmol/ 1 µl, its effect turns anxiolytic. NPY has a significant analgesic effect either ventral and dorsal PAG assessed through tail-flick test; NPY decreases IRCN probably due to an anxiolytic effect at this level and has a potent anxiolytic effect which is more apparent within the VL-PAG when assessed through aimed tests of EPM and DB.

Disclosures: P.V. León: None. **J. Pacheco-Rosado:** None. **L. Mendoza-Ruiz:** None. **A. Miranda-Páez:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 655.04/UU29

Topic: F.03. Motivation and Emotion

Support: NIMH

IOCDF

Title: Deep brain stimulation of the ventral striatum attenuates avoidance but not approach behaviors in rats

Authors: *T. J. BANASIKOWSKI, A. A. GRACE;
Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstracts: Approximately two-thirds of people with OCD exhibit compulsions that are performed to protect them from negative consequences, even though they cognitively know the fear is irrational. Such avoidance behaviors rely on environmental triggers that are linked to aberrant frontal-cortical-limbic-basal-ganglia circuit activity - implicated in impulse control, reward learning and approach behaviors. Recently, deep brain stimulation (DBS) of the ventral striatum (VS) was shown to reduce refractory OCD symptoms while increasing the effectiveness of "extinction with response prevention (EXT-RP)" therapy, a process modeled after patient exposure therapy where compulsion triggers are devalued of salience. Using a rodent model of OCD-like behavior we hypothesized that DBS of VS during EXT-RP will strengthen the reevaluation of no longer salient events, thus leading to subsequent attenuation of compulsive avoidance behavior in test. Using the platform-avoidance task originally conceived by Gregory Quirk and colleagues, we trained rats to lever-press for food and avoid a shock that was predicted by a 30 second tone. The shock could be avoided if a rat stepped on a safety platform located opposite from the sucrose-delivering lever. During EXT-RP training, the platform was present but obstructed and the tones were no longer punished with a shock (stimulus devaluation/reevaluation). On the test day, rats again had access to the platform to examine if tones continued to elicit avoidance behavior (now compulsive). Bilateral DBS stimulation of VS significantly enhanced devaluation of avoidance-triggering stimulus (tone) during EXT-RP compared to non-stimulated animals. The decrease in avoidance following EXT-RP was not associated with changes in approach behaviors as demonstrated by lack of significant difference in lever-press suppression ratios between DBS and non-stimulated animals. This effect was further confirmed by rats trained to lever-press for sucrose on a progressive ratio schedule in that they showed no significant change in lever-presses and break-points in sessions when DBS was given. Our findings suggest that therapeutic effects of DBS in ventral striatum are most likely due to a decrease in avoidance behaviors and not due to an increase in approach behaviors as animals failed to significantly alter their effort to obtain desirable rewards.

Disclosures: **T.J. Banasikowski:** None. **A.A. Grace:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 655.05/UU30

Topic: F.03. Motivation and Emotion

Support: NSERC Grant

Title: Anxiolytic properties of protium copal in rats

Authors: ***C. CAYER**[1,2,3], D. KOLMOGOROVA[3], Z. MERALI[1], J. T. ARNASON[2]; [1]Behavioral Neurosci., IMHR / Univ. of Ottawa, Ottawa, ON, Canada; [2]Biol., Ctr. for Advanced Res. in Envrn. Genomics / Univ. of Ottawa, Ottawa, ON, Canada; [3]Psychology, Univ. Of Ottawa, Ottawa, ON, Canada

Abstracts: Introduction: The main goal of this study was to determine whether copal, an aromatic resin derived from the Protium Copal tree, elicited anxiolytic-like behavior in rats. Despite the extensive Central and South American use of this resin, there are currently no known scientific behavioral studies done with this resin and the behavioural effects of this substance are largely speculative. Background: Burning of herbs and the herbal resins has always been common to most cultures around the world. Mayan people have traditionally used copal as incense and during sweat lodge ceremonies since pre-Columbian times. Many South American cultures continue practicing these traditions today. It is generally described that copal, when burned, is mentally uplifting and calming. Methods: Behavioral measurements in rats were assessed with the elevated plus maze (EPM), social interaction (SI) and conditioned emotion response (CER) paradigms. Rats were exposed to burning copal (100 mg) over 5 minutes in smoking chamber apparatus using a unique technique and then immediately tested for each behavioral paradigm. Quantification of triterpenes in the copal resin was compared to different types of sage by analysis using mass spectrometry. Results: Copal resin smoke exposure significantly reduced anxiety-like behavior in the SI and CER tests. Moreover of the anxiolytic time window of copal resin inhalation seems short and within 10 minutes and was more profound in the social interaction context. Phytochemical analysis revealed that copal has markedly more α- and β-amyrins compared to several types of sage. Conclusion: This study shows that copal incense from Protium copal does indeed elicit some anxiolytic-like effects in rats and possibly its high α and β-amyrin content may offer us a clue to an active ingredient.

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Poster

655. Motivation and Emotions: Rodent Anxiety Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 655.06/UU31

Topic: F.03. Motivation and Emotion

Title: The effects of acute vagus nerve stimulation on anxiety in rats

Authors: *L. J. NOBLE, C. K. MCINTYRE;
Univ. of Texas At Dallas, Dallas, TX

Abstracts: Anxiety disorders, such as posttraumatic stress disorder (PTSD) and obsessive compulsive disorder, are typically treated with cognitive behavioral therapy. In one form of cognitive behavior therapy, exposure therapy, patients are repeatedly exposed to the cues that elicit conditioned fear or maladaptive behavioral responses. Over time, conditioned responses are extinguished. Because successful extinction requires learning of new associations with conditioned cues, many studies have examined the effects of memory enhancing drugs as adjuncts to exposure therapy. Although this approach is highly effective in fear conditioned rats, results in humans with PTSD have been somewhat inconsistent. One possible explanation for

this discrepancy is, when the conditioned response is not sufficiently extinguished, memory-enhancing drugs could reinforce the association between the cue and the inappropriate fear response. Optimal anxiety disorder treatments should reduce the anxiety produced by the conditioned cues while enhancing consolidation of fear extinction. Unfortunately, most anxiety-reducing drugs impair memory consolidation and thus interfere with progress in exposure therapy. Vagus nerve stimulation (VNS) is an FDA-approved treatment for the prevention of seizures. Recent research indicates that VNS enhances memory consolidation in rats and humans, and training-induced cortical plasticity and rehabilitation in animal models of stroke and tinnitus. We recently found that VNS pairing with unreinforced exposure to conditioned cues enhanced extinction of conditioned fear in rats. This effect could be due to enhanced consolidation of fear extinction. Alternatively, or additionally, VNS may facilitate extinction learning by reducing anxiety during exposure to the conditioned cues. Reduced anxiety has been observed in humans and rats following chronic VNS. However, whether VNS has immediate anxiolytic effects remains unknown. The objective of this study was to examine the effect of acute VNS administration on anxiety in male Sprague-Dawley rats. VNS was administered 10 min before or during exploration on an elevated plus maze. Because rats are neophobic, VNS was administered 1 time/day for 3 days prior to testing in some groups. Results indicate that animals given VNS with habituation spent significantly more time in the open arms of the elevated plus maze and corticosterone levels were significantly reduced in this group compared to sham-stimulated controls. These findings suggest that, following habituation, VNS reduces anxiety in rats.

Disclosures: L.J. Noble: None. **C.K. McIntyre:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 655.07/UU32

Topic: F.03. Motivation and Emotion

Title: Lending a helping paw: GABAergic mechanisms in empathy and pro-social behaviors in rats

Authors: *B. CAMPBELL, J. E. MEYERS-MANOR, N. D. MATHEWS, E. P. WIERTELAK; Neurosci. Studies, Macalester Col., Saint Paul, MN

Abstracts: Non-human animals, here specifically rats, are capable of both pro-social behavior and responses implicating emotional contagion. The current studies set out to: 1) determine whether rats may demonstrate the ability to recognize a conspecific's distressed state from a non-distressed state, through patterns of behavior indicative of differential levels of accord with an empathetic response., and 2) Examine the role that GABAergic mechanisms may play in such responsivity. The activation of GABAergic mechanisms is highly correlated with relief from anxiety-related symptoms in humans; the question here was whether alterations in GABAergic activity might affect the performance of empathy-related activity in rats. Physiological symptoms of anxiety and distress originate in activation of brain areas associated with the limbic system. Administration of chlordiazepoxide, a prototypical GABAergic benzodiazepine agonist results in decreased levels of such responsivity. To examine whether activation of limbic system structures and GABAergic mechanisms are necessary for the emotional contagion involved in empathetically motivated behavior, in study 1, subjects from two groups of rats were placed in individual plexiglas restraining apparatuses; one habituated to the restrainer, one non-habituated (and therefore distressed). A free-roaming cagemate was then placed into the open-field containing the restrainer, which offered the option of opening the restrainer and freeing their cage-mate. Here, rats released the distressed cagemate at a greater rate on the first day of testing than those in the habituated, non-distressed group. In study 2, non-habituated distressed group received either 2 mg/kg of chlordiazepoxide or vehicle to evaluate the impact of benzodiazepines on pro-social behavior.

Disclosures: B. Campbell: None. **J.E. Meyers-Manor:** None. **N.D. Mathews:** None. **E.P. Wiertelak:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Topic: F.03. Motivation and Emotion

Support: NIH grant MH084906

Title: Effect of anxiety on spontaneous activity of the prefrontal cortex and its neuronal correlates of the extra-dimensional set-shifting task performance

Authors: *J. PARK[1], C. O. BONDI[1], A. DEL ARCO[2], J. WOOD[1], B. MOGHADDAM[1]; [1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Complutense Univ. of Madrid, Madrid, Spain

Abstracts: Anxiety is a debilitating disorder for approximately one-third of the population at some point in their lives, and it is a common symptom of many psychiatric disorders including PTSD, GAD, OCD, major depression, schizophrenia and addiction. Human and animal studies indicate that pathological manifestations of anxiety are associated with the PFC neuronal dysfunction. However, little is known about the prefrontal neuronal substrates of sustained anxiety. In this study, we first investigated the modulation of spontaneous PFC population activity in anxiety using *in vivo* electrophysiology from freely behaving rats in their home cage. The systemic administration of anxiogenic compound FG-7142 induced sustained suppression of PFC population activity, as the majority of PFC single units decreased their firing rates. We then examined how rats' cognitive task performance and its PFC neuronal correlates are modulated during the pharmacologically induced state of anxiety. Rats were trained and tested with an extra-dimensional set-shifting task that requires responses according to two discrimination rules, each involving a distinct perceptual dimension, spatial position and location of a light stimulus, to acquire food reward. The rats' task performance was bidirectionally modulated by the anxiogenic drug injection, as the response accuracy increased in trials guided by the location of a light stimulus, whereas the accuracy decreased in trials guided by the spatial position, regardless of the light stimulus. This suggests that anxiety may be associated with the response pattern biased to the sensory stimulus, but reduced cognitive control of behavior based on the task rule. The PFC single unit activities were recorded during the set-shifting task performance. Consistent with the home cage recording data, the baseline firing rates of single units tended to be lower after drug injection compared to vehicle injection. However, the PFC units tended to increase their firing rates with a greater magnitude in the peri-response period. In addition, their peri-response activity patterns differed in the two discrimination rules during performance after the drug injection, which may underlie the bidirectional modulation of response accuracy. Together, these suggest that anxiety is associated with disrupted spontaneous and task-related PFC neuronal activities that may lead to alterations in cognitive behavior.

Disclosures: J. Park: None. **C.O. Bondi:** None. **A. Del Arco:** None. **J. Wood:** None. **B. Moghaddam:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Program#/Poster: 655.09/UU34

Topic: F.03. Motivation and Emotion

Support: NIMH Grant MH088046

Title: Ketamine microinjected in the prelimbic (PL) region of the PFC blunts the behavioral and neurochemical effects of inescapable stress (IS), but the PL is not necessary for systemic ketamine to blunt the effects of IS

Authors: *K. H. KUBALA[1], J. AMAT[2], R. M. ALEXSEJEV[2], J. KIM[2], L. R. WATKINS[2], S. F. MAIER[2];
[1]Psychology, [2]Univ. of Colorado, Boulder, CO

Abstracts: Sub-anesthetic doses of ketamine have been shown to have potent and rapid antidepressant and anti-stress effects, and these effects likely involve the prefrontal cortex. We have reported (SFN, 2013) that ip ketamine given 2 hrs before inescapable tail shocks (IS) blunts the increase anxiety and the increase in basolateral amygdala (BLA) levels of 5-HT typically produced by IS. Here we tested whether the prelimbic (PL) region of the prefrontal cortex is involved in such protective effects of ketamine. In the first experiment, adult Sprague Dawley rats, were microinjected with a range of ketamine doses (1, 10, 100 & 1000 ng, in 0.5 microliters) into PL, 1 hr before IS. Twenty-four hrs later exploration of a juvenile in a novel environment was measured to assess anxiety. The 10 ng dose was the only dose that prevented the increased anxiety (decreased exploration) produced by IS. Consistent with this result, PL microinjection of 10 ng ketamine prevented the increase in BLA 5-HT usually produced by IS, and did so as robustly as systemic administration of ketamine. Given this result, we tested whether the PL region is necessary at the time of stress for the protective effect of systemic ketamine. Rats received an ip injection of ketamine and 90 min later muscimol was microinjected into PL. 30 min later subjects received IS. Contrary to expectation, inhibition of neuronal activity in the PL region did not prevent the IS protective effect of systemic ketamine. In conclusion, ketamine microinjected in the prelimbic region appears to be sufficient to block the anxiogenic effect of IS, but inactivation of this prefrontal cortex region at the time of stress does not appears to prevent the protective effect of systemic ketamine.

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Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Topic: F.03. Motivation and Emotion

Support: Deanship of Biomedical Sciences

Dept. Anatomy & Neurobiology

Title: Activation of group II metabotropic glutamate receptors exerts an anxiolytic-like effect in ovariectomized female rats

Authors: *C. PINEYRO RUIZ[1], L. RIVERA ROMAN[3], S. GONZALEZ[3], N. PEREZ-ACEVEDO[2];
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Abstracts: Anxiety disorders affect 40 million adults in the United States. Generalized anxiety disorder (GAD), one type of anxiety disorders, affects females twice more than males. It is possible that this predisposition might be due to differences in metabolite concentration such as estradiol. In hippocampal cells, estrogen receptors at the plasma membrane regulate metabotropic glutamate receptors (mGluRs). mGluRs have been also linked to anxiety modulation. 1S,2S,5R,6S)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740), a potent and selective group II mGluRs agonist, produces anxiolytic effects in male rodents. However, whether LY354740 produces the same effect in female rats, and whether estradiol might modulate anxiety through mGluRs interaction is still unknown. We hypothesized that the anxiolytic effect of LY354740 will be higher in ovariectomized female rats containing estradiol implants (OVX-EB) than animals containing empty implants (OVX). We evaluated GAD using the elevated plus-maze (EPM). We also evaluated risk assessment behaviors (RABs) within the EPM. RABs include flat back approach (FBA), stretch attend postures (SAP) and head dipping. We also analyzed freezing, grooming and sniffing. A Two-Way ANOVA was performed to evaluate the effect of LY354740, estradiol and the interaction between LY354740 + estradiol. We administered LY354740 (10 mg/kg) intraperitoneal 30 minutes prior to the EPM. Preliminary data shows that LY354740 significantly decreased closed arms entries in OVX-EB but not OVX female rats ($p = 0.039$). LY354740 significantly reduced FBA in OVX-EB and OVX female rats ($p = 0.001$) and SAP in OVX female rats only ($p = 0.006$). Our preliminary results suggest that in GAD, the anxiolytic-like effect of LY354740 is selective to OVX-EB female rats, suggesting an interaction between mGluRs and estrogen receptors. On the other hand, the anxiolytic-like effect of LY354740 in RABs seems to be independent upon estradiol treatment in female rats in the EPM, suggesting that the effect is due to group II mGluRs activation. Further experiments need to be done to evaluate these results.

Disclosures: C. Pineyro Ruiz: None. **L. Rivera Roman:** None. **S. Gonzalez:** None. **N. Perez-Acevedo:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Title: Novel acetylcholine detectors reveal that habenula cholinergic neurons regulate anxiety via nicotinic acetylcholine receptor signaling

Authors: *X. PANG[1], J. NGOLAB[1], L. LIU[1], R. ZHAO-SHEA[1], J. M. MCINTOSH[2], P. D. GARDNER[1], A. R. TAPPER[1];
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Abstracts: Anxiety disorders are associated with increased cigarette consumption and decreased smoking cessation, although the underlying neuroanatomical bases and molecular underpinnings of this association are unclear. Recently, the cholinergic neuron-rich habenulo-interpeduncular circuit has been implicated in modulation of anxiety. Interestingly, cholinergic neurons of the medial habenula (MHb) that project to the interpenduncular nucleus (IPN) robustly express nicotinic acetylcholine receptors (nAChRs), ligand-gated cation channels that are activated by the excitatory neurotransmitter, acetylcholine (ACh), as well as nicotine, the addictive component of tobacco smoke. To test the hypothesis that signaling through nAChRs expressed in either the MHb or IPN regulates anxiety-like behavior, we infused the nAChR receptor antagonist, mecamylamine into each region and measured anxiety-like behavior. Blockade of nAChRs in the MHb, but not the IPN, reduced anxiety-like behavior in both the elevated plus maze and marble burying test. To test the hypothesis that MHb nAChRs specifically in cholinergic neurons regulated anxiety, we expressed novel nAChR subunits that render nAChRs hypersensitive to ACh (ACh detectors) selectively in MHb cholinergic neurons of adult mice. Mice expressing ACh detector nAChR subunits exhibited increased baseline anxiety-like behavior compared to control animals. In addition, increased anxiety was alleviated by blockade of mutant receptors with a nAChR antagonist. During chronic nicotine exposure, nAChRs in MHb cholinergic neurons were functionally up-regulated. Selective blockade of these up-

regulated nAChRs in the MHb during withdrawal alleviated anxiety. Together, these data indicate that MHb cholinergic neurons regulate anxiety via nAChR signaling and point toward nAChR in the MHb as targets for novel anxiolytic and smoking cessation therapeutics.

Disclosures: X. Pang: None. **J. Ngolab:** None. **L. Liu:** None. **R. Zhao-shea:** None. **J.M. McIntosh:** None. **P.D. Gardner:** None. **A.R. Tapper:** None.

Poster

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Topic: F.03. Motivation and Emotion

Title: The dopamine D2 receptor is involved the anxiety-like behavior in light and dark choice test

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Abstracts: Anxiety disorders are one of the most frequent psychiatric illnesses and have a life-time prevalence of 15-20%. To overcome anxiety disorders, neuronal basis of anxiety should be clarified. Although it has been well documented that serotonin was one of the critical factor for anxiety disorders, a role of dopamine has not been evaluated. In the present study, we examined effects of quinpirole, a selective dopamine D2/D3 receptor agonist, on anxiety-like behaviors of mice. To evaluate anxiety-like behavior, we conducted open field test and light and dark preference test. Since the mice having increased anxiety show thigmotaxis in open field test, and a preference of dark room in light and dark preference test, we measured a percentage of time spent in the central area in open field test, and a percentage of time spent in the dark room in light and dark test. These typical anxiety-like behaviors as well as a reduction of locomotor activity were observed in both open field test and light and dark test after intraperitoneal injection of quinpirole to C57BL/6 mice in dose-dependent manners. A co-administration of haloperidol, a selective antagonist of dopamine D2/D3 receptor, with quinpirole markedly attenuated the anxiety-like behavior in light-and dark test, but not in open field test. Intraperitoneal injection of quinpirole to D2 receptor-deficient knock-out mouse still induced thigmotaxis in open field-test and a reduction of locomotor activity in open field test and light

and dark test, however no reduction of a percentage of time spent in the dark room in light and dark test was observed. From these results, we concluded that there are several distinct mechanisms for the expression of anxiety, and dopamine D2R participate at least in the expression of anxiety-like behavior in light and dark test.

Disclosures: **Y. Iida:** None. **T. Kojima:** None. **H. Nagayama:** None. **S. Yamamori:** None. **M. Itakura:** None. **T. Sasaoka:** None. **H. Miyaoka:** None. **M. Takahashi:** None.

Poster

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PIBIC-CNPQ

PADC/FCFAR-UNESP

CAPES PNPD 2748/2010

Title: Chemical stimulation and inhibition of the right (but not the left) medial prefrontal cortex changes the anxiety-like behavior in mice

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Abstracts: Several brain areas (e.g., amygdala, hypothalamus, periaqueductal gray) play a role in the modulation of defensive responses. Recently, the medial prefrontal cortex (mPFC) has also been suggested as an important structure that processes emotional reactions induced by aversive stimuli. **Objectives:** This study investigated the effect of chemical stimulation [with NOC-9, a nitric oxide (NO) donor] or inhibition [with cobalt chloride ($CoCl_2$)] of the mPFC on the behavior of mice exposed to the elevated plus-maze (EPM), a widely used animal test of anxiety. **Materials and Methods**: Male Swiss mice (n=9-12) received unilateral injection of vehicle or

NOC-9 [6-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-hexanamine; 37.5 nmol/0.2 µl] or $CoCl_2$ (0.1 mM/0.1 µl) into the left (L) or right (R) mPFC. Five minutes later, each mouse was exposed to the EPM to record the conventional measures of anxiety (percentage of open-arm entries and percentage of open-arm time: %OE and %OT) and locomotor activity (frequency of closed-arm entries: CE) for a period of 5 minutes. **Results**: Unilateral injection of NOC-9 into the L mPFC did not change anxiety indices [%OE (t=-0.44 p=0.66); %OT (t= -0.32; p=0.74]. However, when injected into the R mPFC NOC-9 (37.5 nmol) provoked an anxiogenic-like effect, reducing the open arm exploration [%OE (t= 2.5; p< 0.05); %OT (t= 2.4; p< 0.05)]. Neither intra-LmPFC nor intra-RmPFC injections of NOC-9 changed general activity [CE; LmPFC (t=1.12; p=0.24); RmPFC: (t= -1.8; p= 0.09)]. While intra-LmPFC injections of $CoCl_2$ did not change the behavior of mice exposed to the EPM [%OE (t=1.10; p=0.28); %OT (t=1.13; p=0.27)]; CE (t=0.80; p=0.43)], intra-RmPFC CoCl2 provoked an anxiolytic-like effect, increasing the open arm exploration [%OE (t=-3.7; p<0.05); %OT (t=-2.05; p<0.05)] without changing locomotor activity [CE (t=0.91; p= 0.37)]. **Conclusions:** While chemical stimulation or inhibition of the LmPFC does not change anxiety-like behavior, intra-RmPFC injections of NOC-9 and CoCl2 increase and attenuate, respectively, open arm avoidance in the EPM. **T**hese results are suggestive that the mPFC plays a functional lateralization in the modulation of anxiety-like behavior in mice exposed to the EPM.

Disclosures: R.L. Nunes-de-Souza: None. **N.S. Costa:** None. **T.T. Miguel:** None. **M.A. Vicente:** None.

Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Support: Fapesp

CNPq

PADC/FCF/UNESP

Title: Urocortin III injected into the amygdala does not change anxiety-like behavior in mice exposed to the elevated plus-maze

Authors: *A. C. CIPRIANO[1,2], K. GOMES[2], R. L. NUNES-DE-SOUZA[2,1];
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Abstracts: We have recently observed that injections of low doses of corticotrophin-releasing factor (CRF) into the amygdala produce anxiogenic-like effects in mice exposed to the elevated plus-maze (EPM). The hypotheses that CRF plays an anxiogenic-like role at CRF receptor type 1 (CRF1) in this limbic area was strengthened by a marked anxiolytic-like effect provoked by local injection of CP376395, a highly selective CRF1 receptor antagonist. However, the role of CRF2 receptors located within the amygdala in the modulation of anxiety remains to be determined. Here, we investigated the effects of intra-amygdala injections of urocortin III, a selective CRF2 agonist, on behavior of mice exposed to the EPM. Adult male Swiss mice (N = 10-14/group) received bilateral intra-amygdala microinjections of saline or urocortin III (4, 8 or 16 pmol/ 0.1µl), and 10 min later, were individually placed to the EPM to record the conventional indices of anxiety [percentage of open-arm entries (%OE) and percentage of open-arm time (%OT)] and locomotor activity (closed-arm entries: CE) for a period of 5 minutes. One-way ANOVA revealed that urocortin III did not significantly change any behavior of mice in the EPM [%OE ($F_{3,45} = 0.25$); %OT ($F_{3,45} = 0.48$); CE ($F_{3,45} = 0.26$); $p > 0.05$]. Taken together, these results suggest that the activation of CRF2 receptors within the amygdala does not change the behavior of mice exposed to EPM. However, present results do not rule out the hypotheses that the CRF2 receptors located within the amygdala of mice play a modulatory role on anxiety assessed in the EPM.

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Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Topic: F.03. Motivation and Emotion

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Title: Genetic mutations in GluN2A serine and tyrosine phosphorylation sites controlled by PKC decrease anxiety-related behaviors in mice

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Abstracts: Activity-dependent plasticity in neuronal synapses could be regulated by phosphorylation of specific protein subunits that are involved in synaptic transmission. NMDAR-activation dependent long-term potentiation is a well-studied phenomenon of synaptic plasticity. Heterotetrameric NMDA receptors are composed of two GluN1 subunits and two of four different GluN2 subunits (A-D). The subunit composition of the receptor renders different physiological properties (such as activation kinetics, gating, post-synaptic protein clustering). The long C-terminal domain (CTD) of the NMDAR contains a number of serines and tyrosine residues which could be phosphorylated by a number of kinases. Currents through GluN2A-containing NMDAR were found to be diminished when specific Ser and Tyr residues in the CTD were mutated to Ala and Phe respectively. Thus, it was established that the phosphorylation of the serines (S1291 and S1312) directly by Protein Kinase C (PKC) and tyrosines (Y1292 and Y1387) indirectly via PKC activation of Src Tyrosine Kinase positively modulate the receptor currents. To understand the behavioral and physiological consequences of phosphorylation at those sites, we employed a gene targeted replacement strategy and generated *Grin2adeltaPKC* mice. *Grin2adeltaPKC* mice have site-directed mutations in two Ser and two Tyr residues (S1291A, Y1292F, S1312A and Y1387F) in the CTD of GluN2A. These mice have similar expression levels of *Grin2a* mRNA compared to their littermate controls. These mice also have similar locomotor activity and spatial working memory in a plus maze. However, they show decreased alternation (lower than chance level alternation) in a T-maze continuous alternation task which might be related to the sensitivity of the behavioral paradigm. When these mice were subjected to anxiety-associated tasks, they exhibited reduced anxiety-related behaviors such as increased time spent in the center of an open field, increased time in light side of light/dark box, and increased entries and dwell times in the open arms of an elevated plus maze. Interestingly, LTP induced by theta burst stimulation of Schaffer collateral-CA1 synapses in hippocampal slices from *Grin2adeltaPKC* mice was not impaired and in fact may have been enhanced. Presynaptic mechanisms were not involved in the impairment since there were no significant differences in input-output curves and paired-pulse facilitation between the mutant and control mice. Thus, these results suggest that PKC phosphorylation of at least one of those sites regulates NMDAR-mediated signaling that modulates anxiolytic behaviors and possibly long-term potentiation in hippocampus.

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Poster

655. Motivation and Emotions: Rodent Anxiety Models

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Topic: F.03. Motivation and Emotion

Title: Cortical projections to the amygdala in mouse

Authors: *Q. WANG, L. NG, J. A. HARRIS, S. W. OH, A. BERNARD, A. M. HENRY, M. T. MORTRUD, B. OUELLETTE, J. J. HOHMANN, C. KOCH, H. ZENG;
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Abstracts: The mammalian amygdala is subdivided into several groups of nuclei. Its basolateral and centromedial nuclear groups play a pivotal role in fear-conditioned learning and emotional behavior. Recently, advantage has been taken from mouse amygdala to characterize the function of different cell types linked to these behaviors. Although cortical inputs to amygdala are important sensory sources, a comprehensive effort of brain-wide cortical inputs to mouse amygdala has not been done. Here we systematically analyzed the projections from various cortical regions to mouse amygdala using the data generated in the Allen Mouse Brain Connectivity Atlas, with viral tracer AAV as an anterograde tracer. We find that different cortical areas project bilaterally and/or ipsilaterally to different nuclei of the amygdala with different strengths. The primary auditory cortex has sparse ipsilateral projections to LA. The dorsal auditory cortex has bilateral projections to LA and ipsilateral projections to BLA. The ventral auditory cortex has ipsilateral projections to LA, BLA and medial amygdala nucleus (MEA). The primary and secondary motor cortices have bilateral projections to BLA and central amygdala nucleus (CEA). The primary somatosensory cortex has bilateral projections to LA and CEA. The supplemental somatosensory cortex has bilateral projections to BLA and CEA, and ipsilateral projections to LA. The ventrolateral orbital frontal area has ipsilateral projections to BLA and CEA. The medial orbital frontal area has bilateral projections to BLA and CEA, and ipsilateral projections to LA, basomedial amygdalar nucleus (BMA), MEA, anterior amygdala area (AAA) and cortical amygdala area (COA). The lateral orbital frontal area has bilateral projections to BLA, and ipsilateral projections to CEA, MEA, COA and AAA. The infralimbic area has bilateral projections to BLA and MEA, and ipsilateral projections to BMA, COA and AAA. The prelimbic area has bilateral projections to BLA and CEA, and ipsilateral projections to MEA, COA and AAA. The perirhinal cortex has bilateral projections to LA and BLA, and ipsilateral projections to BMA, MEA CEA, COA and piriform-amygdala area. The posterior part of the agranular insular area has bilateral projections to BLA, and ipsilateral projections to LA. The dorsal part of the agranular insular area has bilateral projections to LA, BLA and CEA, and ipsilateral projections to COA and AAA. These results show the convergence of cortical input in amygdala, and will help further explore how different cortical regions influence behaviors through the emotional gateway of the amygdala.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Chronic anxiety response - effects of diazepam, amphetamine and H4R antagonist

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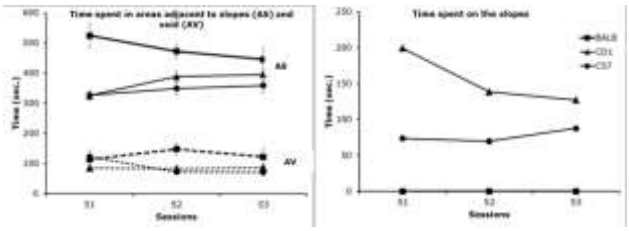
Abstracts: Most current tests of anxiety proved inappropriate for assessing chronic anxiety as they are limited to a single test session. In the present report we describe a novel behavioral test in which fear and anxiety response can be observed over a number of test sessions. This test consistently discriminates between anxious and non-anxious strains of mice and distinguishes between diazepam and amphetamine. When exposed to an unfamiliar elevated platform with downward steep slopes attached on two opposite sides, BALB/c, C57BL/6J, and CD-1 mice spent a large amount of time in the areas adjacent to the slopes than in any other parts of the platform. C57BL/6J, C57BL/6N and CD-1 crossed onto and explored the slopes while BALB/c mice remained the entire 12 min session on the platform. Repeated exposure (3 sessions/24hr intervals) to the test did not affect the difference between these strains of mice. In another configuration of the test, the two slopes were tilted upward. At the end of each slope there was a stand. Mice needed to climb the slope to reach the stand. In this configuration, all 4 strains of mice were able to climb onto the slopes but only C57BL/6J, C57BL/6N and CD-1 mice crossed onto the stand. Repeated exposure (3 sessions/24hr intervals) to the test did not affect the difference between these strains of mice. The above observations suggest that repeated exposure to the elevated platform induced fear and anxiety which was elevated in BALB/c mice and reduced in C57BL/6J, C57BL/6N and CD-1 mice. Diazepam facilitated crossing onto the slopes while amphetamine and H4R antagonist (JNJ7777120) had no effects. Repeated exposure (6 sessions/24hr intervals) to the test did not reduce anxiety in Balb/c mice.



Disclosures: R.M. Abuhamdah: None. **S. Abuhamdah:** None. **P.L. Chazot:** None. **A. Ennaceur:** None.

Poster

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COST Action CM1103

Title: Anxiety-like behavior in crayfish is controlled by serotonin

Authors: P. FOSSAT[1], J. BACQUE-CAZENAVE[1], P. DE DEURWAERDERE[2], J.-P. DELBECQUE[1], *D. CATTAERT[1];
[1]Univ. Bordeaux & CNRS - INCIA - UMR 5287, Talence, France; [2]Univ. Bordeaux & CNRS - IMN - UMR 5293, Bordeaux, France

Abstracts: Anxiety is a behavioral adaptation to stress and is a sustained apprehension about future uncertainties that prepares individuals to detect and address threats. Although this complex emotional state has been exclusively described and analyzed in humans and higher mammals, the question can be raised as to whether lower animals, including invertebrates, also express anxiety-like behaviors. Here, we show that crayfish that are stressed by repetitive exposure to electric fields subsequently adapt their behavior even when placed in a different context: in an aquatic dark/light plus-maze, the crayfish avoided aversive light arms. This avoidance was completely abolished by an injection of chlordiazepoxide, which is a benzodiazepine anxiolytic drug. Avoidance is associated with increases in brain serotonin (5HT) in stressed animals. In unstressed crayfish, 5HT injections induced avoidance, and chlordiazepoxide prevented this effect. The striking similarities between the behavioral

adaptations displayed by crayfish and rodents after exposure to stressful situations, the involvement of 5HT, and the suppressive effects of a benzodiazepine clearly indicate that crayfish can express a primitive form of anxiety and that the underlying mechanisms have undoubtedly been conserved during evolution. Analyses of this ancestral form of behavior in a simple model reveal a new route to understanding anxiety and may alter our conceptions of the emotional statuses of invertebrates.

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Poster

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Department of Defense CDMRP PTSD Program (ESB)

Title: Stress enables serotonergic tuning of fear memory consolidation

Authors: *M. V. BARATTA[1,2,3,4], J. YAO[2,4], M. D. WEBER[1], B. GISABELLA[2,4], P. E. MONAHAN[2,3,4], N. PETROSSIAN[2,4], E. S. BOYDEN[2,3,4,5], K. A. GOOSENS[2,4]; [1]Psychology & Neurosci., Univ. of Colorado Boulder, Boulder, CO; [2]McGovern Inst. for Brain Res., [3]MIT Media Lab., [4]Dept. of Brain and Cognitive Sci., [5]Dept. of Biol. Engin., MIT, Cambridge, MA

Abstracts: Serotonergic systems play a critical role in the regulation of emotion, and its dysregulation is associated with stress-related vulnerability to anxiety disorders such as post-traumatic stress disorder. Prior work from our laboratory and others suggest that the serotonergic dorsal raphe nucleus is critical for mediating the impact of stress on aversive processing. Here we investigated the role of serotonin 2C ($5\text{-}HT_{2C}$) receptors in the basolateral amygdala (BLA), a

target area in the fear network that receives serotonergic innervation from the dorsal raphe. As in prior studies, repeated immobilization stress prior to auditory fear conditioning enhanced long-term fear memory without impacting freezing levels during acquisition or during a short-term memory test. Intra-BLA administration of the highly selective 5-HT_{2C} receptor antagonist SB242084 (0.4 μg/0.4 μl) immediately following fear acquisition completely blocked the stress-induced enhancement of fear without impacting basal levels of fear in unstressed animals. Post-conditioning serotonin levels in the BLA did not differ between Stress and No Stress groups. In contrast, western blot analysis revealed that stress elevated the membrane expression of BLA 5-HT_{2C} receptors without altering whole cell levels of these receptors. Since edited forms of the 5-HT_{2C} receptor exhibit less internalization from the membrane surface, we examined expression of the editing enzyme for the 5-HT_{2C} receptor, adenosine deaminase acting on RNA 1 (ADAR1). Following fear training, we found that BLA ADAR1 protein levels were increased in subjects exposed to repeated stress. Taken together, these data show that stress bolsters the consequences of aversive reinforcement not by simply enhancing the neurobiological signals used to encode fear in unstressed animals, but rather by engaging a distinct serotonergic mechanism. Our results also point to 5-HT_{2C} receptors as a promising therapeutic G-protein target for restoration of normal, adaptive levels of threat processing in mood disorders.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Evaluating CF 50 kHz USVs as an indicator of anxious state

Authors: *J. O. TAYLOR, S. B. KEGLEY, A. LEMKE, C. M. URBANO, B. G. COOPER; TCU, Fort Worth, TX

Abstracts: Adult rat ultrasonic vocalizations (USVs) are a valuable tool for noninvasively assessing an animal's emotional state. USVs are produced in one of two frequency ranges, 22 kHz or 50 kHz. Within the 50 kHz range, one subtype of USV, constant frequency 50 kHz (CF 50 kHz) calls, is not viewed as a call signaling a particular emotional state. The current study tested the hypothesis that CF 50 kHz calls are an indicator of anxiety using behavioral and

pharmacological manipulations. Animals were injected (i.p.) with an anxiolytic (diazepam, 1 mg/kg), an anxiogenic (pentylenetetrazole, 10 mg/kg), or saline 30 min prior to behavioral testing. USVs were recorded while animals received a repeated sequence of mild, unsignaled footshocks to elicit anxiety; or animals were evaluated for anxiety using an elevated plus maze (EPM) without acute stressors. To determine whether previous aversive experience modulated anxiety, animals that received control injections in the unsignaled footshock paradigm were subsequently tested on the EPM . A low dose of diazepam decreased the rate of CF 50 kHz calling immediately following the initial unsignaled footshocks compared to saline- and PTZ-treated animals. Animals with previous footshock experience were more likely to produce CF 50 kHz calls on the EPM, and were significantly more anxious than experimentally naïve animals. The pattern of CF 50 kHz call production during both behavioral tests supports the novel interpretation that the production of CF 50 kHz calls is an indicator of anxiety in rats. These results suggest that anxiety state can be noninvasively measured by recording USVs and recording these USVs may aid the development of animal models of anxiety.

Disclosures: J.O. Taylor: None. **S.B. Kegley:** None. **A. Lemke:** None. **C.M. Urbano:** None. **B.G. Cooper:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.01/UU46

Topic: F.03. Motivation and Emotion

Support: The China Ministry of Science and Technology 973 Program 2012CB837700

Title: Reward signaling in the dorsal raphe nucleus

Authors: *Z. LIU, J. ZHOU, Y. LI, F. HU, Q. FENG, J.-E. ZHANG, D. WANG, J. ZENG, J. BAO, M. LUO;
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Abstracts: Reward processing is critical for animal's survival in a dynamic environment. The dorsal raphe nucleus (DRN) is a center of serotonin (5-hydroxytryptamine; 5-HT) neurons and has long been implicated in mood regulation. Although 5-HT has been theorized to encode punishment by opposing the dopamine system, the role of DRN in reward processing remains largely elusive. Here, combining optogenetics, *in vitro*/vivo physiology, immunohistochemistry

with a series of behavioral tests, we show that DRN neurons actually signal reward directly by releasing glutamate and serotonin. Glutamate contributes most of the reward motivation while 5-HT sustains this high motivation even in more challenging & complicated conditions. Furthermore, Ventral Tegmental Area (VTA) and Nucleus Accumbens (NAc) might be the downstream targets involved in the reward effects mediated by DRN. These results uncover the pivotal role of DRN in the brain reward system which has long been neglected before and have multiple implications on reward theory and 5-HT functions in mood & emotion regulation.

Disclosures: **Z. Liu:** None. **J. Zhou:** None. **Y. Li:** None. **F. Hu:** None. **Q. Feng:** None. **J. Zhang:** None. **D. Wang:** None. **J. Zeng:** None. **J. Bao:** None. **M. Luo:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.02/UU47

Topic: C.17. Drugs of Abuse and Addiction

Title: Sonic hedgehog signaling pathway is important in the maintenance and function of adult dopaminergic neurons

Authors: X. ZHOU[1], Y. JIN[1], E. FILICHIA[1], K. JIN[2], *B. J. HOFFER[3,1], Y. LUO[1]; [1]Neurolog. Surgery, Case Western Reserve Univ., Cleveland, OH; [2]Dept. of biomedical engineering, Univ. of Rochester, Rochester, NY; [3]Scientist Emeritus, NIDA/NIH, Lyndhurst, OH

Abstracts: During development, sonic hedgehog (shh) induce precursor cells to differentiate into dopaminergic (DA) neurons. However, whether shh signaling is also required in the maturation or maintenance of DA neurons is unknown due to the lethality of traditional shh knockout models. We utilized the cre-loxP system to achieve stage and cell type-specific deletion of shh or its receptor, smoothened (smo), in DA neurons during the late developmental stage (E15.5) by crossing DATcre (dopamine transporter) mice with loxP-shh or loxP-smo mice. Our results show that DATcreshh knockout (ko) mice demonstrate hyperactivity compared to wild type (wt) mice at 3 months and 5-6 months. TH positive neuron cell counts in substantia nigra (SN) showed that, at 7 months, there is no difference between wt and shh ko mice. However, in aged mice (20 months), there are significantly less TH-positive neurons in shh ko mice (110.625 ± 6.78 per section) as compared to wt mice (141.5 ± 8.17 per section, $p = 0.019$). In DATcresmo knockout mice, similar hyperactivity was found in 4-5 month old mice. TH positive neuron cell count

using unbiased stereology showed substantially fewer cells in 7 month old smo ko mice (6245.16 ± 71.44) than in wt mice (7361.72 ± 362.55, p = 0.039). In lesion studies of smo ko versus wt mice, we found no difference in survival of DA neurons in the classic unilateral striatal 6-OHDA lesion modeling Parkinson's disease. DATcreshh and DATcresmo mice also display differential response when exposed to psychostimulant and addictive drugs. In summary, our data demonstrate that shh signaling pathway plays critical role in maintaining the function of dopaminergic system in adulthood in mice.

Disclosures: **X. Zhou:** None. **Y. Jin:** None. **E. Filichia:** None. **K. Jin:** None. **B.J. Hoffer:** None. **Y. Luo:** None.

Poster

656. Reward II

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Program#/Poster: 656.03/UU48

Topic: F.03. Motivation and Emotion

Support: China Ministry of Science and Technology 973 Program (2010CB833902 & 2012CB837700)

Title: Encoding reward signals by dorsal raphe 5-ht neurons of freely behaving mice

Authors: ***Y. LI**[1,2], W. ZHONG[1,2], Z. LIU[1], J. ZHOU[1], D. WANG[1], J. ZENG[1], Q. FENG[1], J. BAO[1], C. JIA[1], M. LUO[1,3];
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Abstracts: The dorsal raphe nucleus (DRN) is the major source of serotonin (5-HT) in the forebrain, although a substantial number of neurons here are known to release other neurotransmitters such as glutamate, GABA, and dopamine. These neurons exhibit diverse correlations with different phases of reward-related behaviors, but it remains unclear how 5-HT neurons in the DRN encode reward signals in freely behaving animals. We carried out recordings of DRN 5-HT neurons from freely behaving Sert-Cre mice that underwent a simplified sucrose foraging behavioral task. 5-HT neurons were identified by selectively expressing ChR2 and then optical tagging with optetrodes. Our experiments reveal that 5-HT neurons exhibit low basal firing rate (~2Hz), become gradually activated in anticipation of sucrose delivery (~8 Hz), and fire burst of action potentials (>20 Hz) at the early phase of reward consumption. In contrast, a

substantial number of GABAergic neurons exhibit high basal firing rates and are inhibited during reward anticipation and consumption. Moreover, mouse poking behavior during reward anticipation phase is increased by optogenetic stimulation and reduced by optogenetic inhibition of DRN 5-HT neurons, respectively. These results strongly demonstrate that DRN 5-HT neurons encode reward-seeking signals with tonic and then bursting activation pattern, suggesting an important role of these neurons in reward processing during freely behaving states.

Disclosures: Y. Li: None. **W. Zhong:** None. **Z. Liu:** None. **D. Wang:** None. **J. Zeng:** None. **Q. Feng:** None. **J. Bao:** None. **J. Zhou:** None. **C. Jia:** None. **M. Luo:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.04/UU49

Topic: F.03. Motivation and Emotion

Title: Qualitative reward prediction errors and dopamine during reward reversal

Authors: *V. MARTINEZ[1], L. M. BURGENO[2], P. E. M. PHILLIPS[2];
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Abstracts: Phasic dopamine transmission in the nucleus accumbens core (NAC) has been implicated in the formulation of quantitative reward prediction errors. Using fast-scan cyclic voltammetry (FSCV) we examined phasic dopamine responses in the NAC during performance of a Dual Reward Discrimination (DRD) task. Rats were simultaneously deprived of food and water to elicit motivated task performance for food and water rewards. Sessions were comprised of 90 trials (30 food trials, 30 water trials and 30 choice trials) with all trial types evenly interleaved across each session. Operant performance requires that rats respond to cues (light flashes) that predict specific rewards (food or water) which are contingent on their spatial location (left-cues predict food and right-cues predict water). Rats were trained exclusively using this reward contingency until they reached performance criteria; at this time rats performed a 'Reversal' session that was accompanied by FSCV recordings. The first 30 trials of Reversal sessions were presented using standard reward contingencies (left=food; right=water); however during trials 31-90 reward contingencies were reversed so that the left responses now yielded water and right responses, food. This was done to elicit reward prediction errors that resulted from changes in reward identity, while leaving the quantity of rewards unchanged. Baseline

performance on choice trials indicated equal preference for food and water rewards; therefore, prediction errors elicited by reward reversal aren't necessarily ‘positive’ or ‘negative’, but rather reflect qualitative discrepancies in reward identity. Performance accuracy and number of omissions did not differ substantially following reversal. Peak dopamine concentrations were taken from distinct time periods associated with cue onset, lever extension and reward delivery. Preliminary voltammetric data indicated that food and water trials elicited phasic signals of equal magnitude when rats performed under standard reward contingencies. Interestingly, following reward reversal, phasic dopamine signals were augmented for both trial types relative to baseline. We interpret these findings to suggest that unforeseen contingency reversals alter the predictive values of cues based on their prior history and render subsequent rewards as qualitatively-unexpected.

Disclosures: V. Martinez: None. **L.M. Burgeno:** None. **P.E.M. Phillips:** None.

Poster

656. Reward II

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Program#/Poster: 656.05/UU50

Topic: F.03. Motivation and Emotion

Support: FAPESP

CNPq

Title: Brain structures involved in the reinforcement omission effects modulation

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Univ. de São Paulo, Ribeirão Preto, Brazil

Abstracts: Studies developed in our laboratory have showed that amygdala is involved in the modulation of the reinforcement omission effects (ROEs). For instance, rats with large amygdala lesions, trained to respond on a fixed-interval with a limited hold signaled schedule of reinforcement (FI LH signaled), failed to increase response rates in intervals following nonreinforcement. However, rats with lesions of the basolateral complex of amygdala (BLA), trained to respond on the same schedule of reinforcement, were more responsive to occasional reinforcement omission than rats of the sham-operated group. The view that amygdala lesions block ROEs is supported by evidence implicating the amygdala in responses correlated

motivational and attentional processes. However, these processes depend on the operation of separate amygdala areas, through their connections with other brain systems. We are investigating whether the ROEs may be modulated by different brain structures linked to the amygdala, like nucleus accumbens (NAC), medial pre-frontal cortex (mPC), orbitofrontal cortex (OFC), substantia nigra pars compacta (SNc) and tegmental ventral area (VTA). For example, recent experiments developed in our laboratory showed that nucleus NAC and mPC, but not OFC, also are involved in the modulation of ROEs. Thus, the present study aimed to verify if the neurotoxic lesions of the SNc interfere on ROEs modulation. Rats were trained on a FI 12 s LH 6 s signaled schedule in which correct responses were always followed by reinforcement (100% reinforcement schedule). After the training the rats were submitted to SNc lesions. In the test, the training was changed from 100% to 50% reinforcement schedule. The results showed that SNc lesions did not impair the ROEs. Rats of SNC lesioned group present ROEs as well as rats of the sham-operated group: the response in intervals following nonreinforcement was higher than in intervals following reinforcement. Furthermore, there were no differences among both groups. Together, the results obtained in our laboratory support the hypothesis that not only amygdala, but also NAC and mPC are related to ROEs modulation. However, the OFC and SNc do not seem to be involved in this process. Studies have showed that the dynamic interaction of BLA with the NAC, mPC and also VTA may be important to incentive motivational processes. Thus, it is possible that VTA could also be involved in the ROEs modulation.

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Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: Colorado College Natural Sciences Research and Development Grant

Colorado College Faculty/Student Collaborative Grants

Title: Hedonic reward and incentive salience in rats exposed postnatally to the polybrominated diphenyl ether commercial mixture DE-71

Authors: *L. L. DRISCOLL[1], E. BECKETT[2], J. BOESE[2], J. WATTS[2], J. SPERRY[2], R. KASEMODEL[2];
[1]Colorado Col., COLORADO SPGS, CO; [2]Neurosci., Colorado Col., Colorado Springs, CO

Abstracts: Polybrominated diphenyl eithers (PBDEs) are environmentally ubiquitous and bioaccumulative flame retardants used in the manufacturing of polymer products. Exposure to PBDEs in food, dust, and air can have endocrine (thyroid and sex hormone) and neurobehavioral (especially learning and attention) effects, particularly if exposure occurs in the perinatal period. Neurobehavioral assessments of PBDE exposure effects have not, to date, included measures of motivation or reward. The purpose of this study was to determine the effects of postnatal exposure to the PBDE commercial mixture DE-71 on sucrose water consumption, which is a measure of hedonic tone, and on a progressive ratio (PR) lever pressing task, which measures the amount of effort the subject is willing to exert to earn a reward. Male and female rat pups received daily oral doses of DE-71 (0, 30, or 60 mg/kg) in corn oil from postnatal days 6-12; half of the DE-71-exposed rats also received oral doses of 6 μg/kg/day levothyroxine sodium (LT4) to determine if thyroid hormone supplementation could "rescue" the impacts of DE-71 on behavior. All DE-71 exposed groups demonstrated a greater preference for sucrose water than did controls; this effect was present in both sexes. In contrast, PR lever press performance was relatively unaffected by DE-71, except that the 60 mg/kg exposed rats showed a sexual dimorphism in the number of lever presses completed (males more than females), whereas the other groups did not. LT4 supplementation had no effect on sucrose consumption or PR performance, suggesting that the alterations in hedonic tone due to DE-71 exposure are not secondary to toxin-induced hypothyroidism. Taken together, these results show that postnatal DE-71 exposure increases hedonic drive in both males and females, but it does not seem to impact motivated responding for rewards under a PR schedule.

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Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: Max Planck Society

Wellcome Trust

Title: Reward prediction error signals in major depressive disorder

Authors: *R. B. RUTLEDGE, M. MOUTOUSSIS, T. HERLT, L. HRYNKIEWICZ, J. LAM, O. OUSDAL, P. FONAGY, R. J. DOLAN;
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Abstracts: Dopamine neurons are believed to represent a reward prediction error (RPE) signal, the difference between experienced and predicted rewards. In healthy subjects, RPE signals have been measured from dopamine projection areas including the ventral striatum using functional neuroimaging. Subjects suffering from major depressive disorder have been found to have deficits in reward learning tasks and to lack normal RPE signals in the striatum. We tested whether RPE signals are represented in the striatum of depressed subjects in a task that does not require learning. We used an axiomatic model of the dopaminergic RPE hypothesis to formally test whether neural responses satisfied necessary and sufficient conditions for the class of RPE models. The axioms will be satisfied if activity: 1) increases with prize magnitude, 2) decreases with lottery expected value, and 3) is the same for outcomes from all lotteries with a single possible outcome. We tested medicated, unmedicated, and healthy subjects on a decision making task in which they chose between lotteries with different probabilities of monetary gains and losses and the outcomes of chosen lotteries were shown after a brief delay. Task earnings and reaction times did not differ between subject groups and all groups responded more quickly to choose the best than the worst option. Neural responses in the ventral striatum and in the ventral medial prefrontal cortex in depressed subjects satisfied all three conditions of the axiomatic model, demonstrating that there are intact neural RPE representations in subjects suffering from major depressive disorder.

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Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: NIH R21NS066115

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Title: Pattern analysis of low-frequency power predicts stopping behavior in human subthalamic nucleus

Authors: *J. M. PEARSON, P. T. HICKEY, S. P. LAD, M. L. PLATT, D. A. TURNER; Neurobio., Duke Univ. Med. Ctr., DURHAM, NC

Abstracts: Pharmacological treatment of Parkinson's Disease (PD) with dopaminergic drugs can lead to impulse control disorders such as compulsive gambling, hypersexuality, and punding. Recent evidence suggests that these disorders may also be evoked by deep brain stimulation (DBS). Studies in humans and animals indicate that the subthalamic nucleus (STN), the typical target for DBS treatment of PD, may be necessary for inhibiting prepotent motor responses, particularly those linked to reward, but little is known about other types of motivational information encoded by this area. We recorded neurons from the deeper part of STN (<3mm from ventral border, adjacent to substantia nigra) of patients undergoing DBS implantation for the treatment of PD while awake. Subjects performed an interactive task during the recordings, using a variant of the balloon analogue risk task (BART), a decision making paradigm used to assess impulsivity in humans. Here, instead of inhibiting a motor response, subjects were required to terminate the inflation of a balloon presented onscreen before it popped. Successfully terminating the inflation resulted in a reward proportional to the final balloon size, while a popped balloon resulted in no reward. Pop times were random, with balloon color signaling risk level. On control trials, balloons inflated to a predetermined size indicated onscreen by a gray circle before stopping; subjects simply initiated inflation. Balloons never popped on control trials, though some balloons (colored gray) resulted in 0 reward. Using multi-electrode arrays, we recorded 32 simultaneous channels of local field potentials (LFP) in a subset of patients. We found that low-frequency LFP power in the 0-10 Hz range rose in seconds in advance of stopping decisions, and that this pattern was absent on trials where subjects did not respond in time. A classifier analysis based on a regularized regression model successfully predicted stop decisions within 1 second, with area under the ROC curve measures of > 0.6 in multiple subjects. These results suggest that local activity in STN carries a mixture of motivational and motor preparatory behavior, and that machine learning methods might be useful in predicting impulsive behavior via monitoring of signals at the DBS electrode.

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Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: NIH Grant AA010761

Title: Different monetary incentive delay neural profiles in high and low risk social drinkers

Authors: M. DIBARTOLO[1], X. ZHU[1], J. SCHACHT[2], *B. FROELIGER[1], R. ANTON[2], J. JOSEPH[1];
[1]Neurosciences, [2]Psychiatry, Med. Univ. of South Carolina, Charleston, SC

Abstracts: The monetary incentive delay (MID) task is an effective probe for isolating neural circuitry in the human brain associated with incentivized motivation. Individuals at risk for substance abuse have been shown to exhibit differences in incentive elicited neural activation. This study examined whether or not young adults at high- or low- risk for alcohol dependence would show different neural activation patterns related to reward or loss-avoidance of high and low monetary values. It was hypothesized that high-risk social drinkers would exhibit attenuated reward sensitivity or hypersensitivity to loss. 30 young adults (ages 18-25, 15 male) were categorized as being at high- (N=12, 7 male) or low-risk (N= 18, 8 male) for future alcohol dependence based on the Alcohol Use Disorders Identification Test (AUDIT). Scores for the high-risk group ranged from 8-13, scores for the low-risk group ranged from 2-7, and no subjects had previous or current alcohol use disorder treatment. Subjects performed the MID task while undergoing functional magnetic resonance imaging. Five incentive levels were used (- $5, - $,5, 0, + $.5, + $5) in the MID task. We found that neurobehavioral MID profiles were different in high- and low-risk social drinkers. Neural activation in several frontal regions was consistently higher in low-risk drinkers than high-risk drinkers during the feedback stage, independent of incentive level. Of particular interest was the activation pattern in the left superior frontal gyrus: during the cue phase, the high-risk group showed greater activation during high loss trials than low risk group, but similar activation during high reward trials. During the feedback stage the opposite effect was observed: the low-risk group showed greater activation during high reward trials than the high risk group, but showed similar activation during the high loss trials. The left superior frontal cortex has been linked to working memory and complex executive functioning as well as to processes that are related to value determination and motivation. The present findings suggest that high-risk drinkers may engage in more elaborative, higher-level processing for potential negative outcomes whereas low-risk drinkers devote the same type of processing to positive outcomes.

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Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: T32 DA007268

P01 DA031656

Title: Neural encoding of incentive salience in the ventral pallidum of rats during Pavlovian conditioned approach

Authors: ***A. M. AHRENS**, T. E. ROBINSON, J. W. ALDRIDGE;
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Abstracts: Individual differences in the neural representation of incentive salience may be an important factor that predisposes certain individuals to addiction. Incentive motivation can be modeled in rats using a Pavlovian conditioned approach procedure, in which the presentation of a discrete cue (the conditioned stimulus, CS) is consistently followed by food reward (the unconditioned stimulus, US) in a response-independent manner. In this procedure there are clear individual difference in the form that conditioned behavior takes; with some rats approaching and interacting with the cue itself ("sign trackers", STs), some rats approaching the food cup ("goal trackers", GTs), and other rats alternating between the CS and the food cup ("intermediates", INs). The attraction to the CS seen in STs, but not GTs, is thought to reflect the attribution of incentive salience to the CS. The goal of the present study was to determine how these individual differences are represented in the neural firing patterns of the ventral pallidum, a mesolimbic structure that encodes both the predictive and incentive value of reward-paired cues. We used single-unit *in vivo* electrophysiology to record neural activity in the ventral pallidum of freely-moving ST, GT, and IN rats during the expression of Pavlovian conditioned approach behavior. We found that all rats showed clear increases in neural activity in response to both the onset of the CS and the delivery of the US. However, the STs showed heightened CS responses, both in terms of the percentage of responsive neurons and the magnitude of cue responses, compared to the GTs and INs. These results show that individual difference in approach behavior

are reflected in mesolimbic reward circuitry, and that by utilizing the ST/GT model it is possible to distinguish between the predictive and incentive value attributed to a reward-paired cue.

Disclosures: **A.M. Ahrens:** None. **T.E. Robinson:** None. **J.W. Aldridge:** None.

Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: NSERC Doctoral scholarship

CIHR Operating grant

Title: Interaction between inhibition of monoamine transporter function and environmental enrichment or social isolation on responding for conditioned reinforcement in mice

Authors: *C. BROWNE[1], P. J. FLETCHER[2], F. D. ZEEB[2];
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Abstracts: Stimuli associated with primary rewards can acquire motivational value through incentive motivational processes, as demonstrated by their ability to function as conditioned reinforcers. The expression of incentive motivation is regulated in large part by the activity of brain monoamine neurotransmitters. Environmental enrichment (EE) and isolation rearing can alter both monoamine function and motivational processes such as cue-induced reinstatement of reward seeking. This suggests that environmental factors may interact with the incentive value of reward-associated stimuli through altered monoamine function. Thus, the present experiments examined the effects of rearing condition on responding for a reward-associated stimulus serving as a conditioned reinforcer (CR) in environmentally enriched (EE), pair housed (PH), and single housed (SH) mice. We also determined how responding was affected by blocking the serotonin (SERT), norepinephrine (NET), and dopamine (DAT) transporters, with citalopram (CIT), atomoxetine (ATO) and GBR12909 (GBR), respectively. At post-natal day 21, male C57BL/6 mice were separated into EE, PH, and SH groups for the duration of the study (all groups n=12). PH and SH mice were housed in standard conditions, while EE mice were housed 6 to a large cage in an enriched environment. In adulthood, mice were tested on a conditioned reinforcement

procedure involving a Pavlovian conditioning phase followed by an operant conditioning phase. During the Pavlovian phase, water-restricted mice received 30 pairings of a conditioned stimulus (CS) prior to the delivery of saccharin over 14 sessions. No group differences were seen in learned approach to the reward location upon CS presentation. In the operant phase, mice were able to respond on a lever to obtain only the stimulus previously paired with saccharin (now a CR). Baseline levels of responding for the CR were similar between PH and EE mice, while SH mice showed significantly higher responding. Subsequently, the effect of the CIT (10, 20 mg/kg), ATO (0.3, 1, 3 mg/kg), and GBR (2.5, 5, 10 mg/kg) on responding was examined. For all groups, both doses of CIT decreased responding for the CR, whereas ATO decreased responding only at the highest dose tested. In contrast, GBR increased responding for the CR only in EE mice at the highest dose. These results suggest that mice in impoverished conditions (SH) may attribute greater incentive value to reward-associated stimuli, and that PH and EE mice do not differ greatly in responding for a CR. Further, blockade of SERT and NET produce decreases in CR responding in general, while EE mice show a susceptibility to the response enhancing effect of DAT blockade.

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Poster

656. Reward II

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Topic: F.03. Motivation and Emotion

Support: NIH P30 DK041301

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NIH K01 DK085133

Title: Resting state and functional connectivity of the nucleus accumbens with rewards regions in overweight and obese women

Authors: K. COVELESKIE[1], A. GUPTA[1], *L. A. KILPATRICK[1], E. D. MAYER[1], C. ASHE-MCNALLEY[1], J. STAINS[1], J. S. LABUS[1], E. A. MAYER[1,2];

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Abstracts: Background: Neuroimaging studies in obese subjects have identified abnormal activation of key regions of central reward circuits, including the nucleus accumbens (NAcc), in response to food related stimuli. We aimed to examine if subjects with elevated body mass index (BMI) show structural, and resting state (RS) functional connectivity alterations within regions of the reward network. Methods: 50 healthy, premenopausal women, 19 overweight and obese (high BMI=26-38 kg/m2) and 31 lean (BMI=19-25 kg/m2) were studied. Structural and RS functional scans were collected using a Siemens Allegra 3T magnetic resonance imaging (MRI) scanner. Group differences in grey matter volume (GMV) of the NAcc, oscillation dynamics of intrinsic brain activity, and functional connectivity of the NAcc to regions within the reward network were examined. Results: GMV of left NAcc was significantly greater in the high BMI group than in the lean group (p=0.031). Altered frequency distributions were observed in women with high BMI compared to lean in the left NAcc, right and left anterior cingulate cortex (ACC) and ventro-medial prefrontal cortex (vmPFC). Additionally, subjects with high BMI had greater connectivity of the left NAcc with bilateral ACC and right vmPFC in specific frequency bands. Conclusions: Overweight and obese women in the absence of food related stimuli show structural and functional alterations in reward related brain networks which may play a role in altered ingestive behaviors.

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Poster

656. Reward II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC

Title: Prenatal exposure to stress in combination with adolescent exposure to marijuana results in sexually dimorphic cognitive and physiological effects

Authors: *R. J. KEELEY, J. TROW, B. LOWRY, R. J. MCDONALD;
Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstracts: Adolescent marijuana exposure is associated with disruptions in cognitive function in some but not all animal models. In addition, human exposure to marijuana during adolescence can increase the risk of development of psychiatric illness. However, some populations are more at risk than others to the long-term consequences of adolescent marijuana use. One possible factor that could predispose an individual to the negative consequences of marijuana is early-life stress, particularly given the relationship between the endogenous cannabinoid system and the hypothalamic-pituitary-adrenal axis. Therefore, this research study examined the long-term consequences of exposure to the psychoactive component of marijuana, Δ9-tetrahydrocannabinol (THC), in rats prenatally exposed to stress. This prenatal stress paradigm has been shown to alter stress responses, anxiety behaviour as well as has epigenetic consequences for the offspring. Following adolescent exposure to THC, rats were aged to adulthood and exposed to a battery of behavioural tasks, including elevated plus maze, the Morris water task and discriminative fear-conditioning to context. Following a minimum 2 week acclimation period following the fear-conditioning task, blood was taken before and 30 minutes after a restraint stress. No differences were observed in anxiety-related behaviour in the elevated plus maze or spatial learning in the Morris water task. In the discriminative-freezing to context task, a sex-specific effect was observed such that only males showed any impairment in discriminative freezing or active avoidance with the administration of THC. Finally, basal corticosterone was unaltered with treatment conditions. However, sexually dimorphic effects of THC or just a vehicle injection were observed. Females showed altered corticosterone responses following restraint stress if they had been exposed to vehicle or THC in adolescence in comparison to a handled control group. Male corticosterone responses were less clear, such that THC may have returned corticosterone levels to that of controls in comparison to the vehicle group. This research project highlights the importance of considering at risk groups for the administration of THC and that these effect may be sexually dimorphic.

Disclosures: **R.J. Keeley:** None. **J. Trow:** None. **B. Lowry:** None. **R.J. McDonald:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.14/UU59

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant HD070888

Title: Effects of chronic oral methylphenidate exposure on behavior and brain metabolism in female rats

Authors: *L. S. ROBISON[1], M. VITALE[1], M. MICHAELOS[1], J. GANDHI[2], S. PAENG[3], J. LEE[4], E. MIAO[1], M. HADJIARGYROU, Ph.D.[6], D. KOMATSU[5], P. K. THANOS[1]; [1]Psychology, [2]Physiol. & Biophysics, [3]Biol., [4]Chem., [5]Orthopedics, Stony Brook Univ., Stony Brook, NY; [6]Life Sci., New York Inst. of Technol., Old Westbury, NY

Abstracts: Methylphenidate (MP; marketed as Ritalin) is a commonly prescribed stimulant used to treat Attention Deficit Hyperactivity Disorder (ADHD). MP has received attention for its possible abuse potential and side effects that may persist after cessation of treatment. Previously, we found that chronic MP treatment has significant effects on locomotor behavior, emotionality, and neurochemistry in male rats. In the current study, female Sprague-Dawley rats were split into 3 groups (n=24/group) at 4 weeks of age: control (water), low dose MP (LD), high dose MP (HD). A dual bottle 8-hour limited access drinking paradigm was utilized, which produces an MP pharmacokinetic profile similar to treated patients: 4 mg/kg MP (LD) or 30 mg/kg MP (HD) during hour 1, and 10 mg/kg (LD) or 60 mg/kg MP (HD) hours 2-8. Half of the rats in each group were treated for 13 weeks, while the other half were treated for 13 weeks then went through a one month abstinence period (given water for the 8h drinking period). Food and fluid intake and body weight were recorded daily for the duration of the experiment. Rats were tested for open field activity weekly throughout treatment and abstinence. Circadian activity testing was performed during weeks 1, 2, 4, 8, and 13 of treatment, as well 1, 2, and 5 weeks into the abstinence period. Novel object recognition (NOR) and social interaction tests were performed during the last weeks of treatment and abstinence. Brain metabolic effects of MP were assessed with [18F]FDG microPET scans at the end of treatment. HD MP increased food intake but decreased body weight, likely due to dramatic increases in energy expenditure. Open field tests revealed that MP dose-dependently increased general activity levels during treatment. These effects were greatest in later weeks of treatment, suggesting sensitization, and were corroborated by increased circadian activity levels in the dark cycle only. MP also increased rearing and center activity in the open field throughout treatment, suggesting increased exploratory behavior and reduced anxiety. MP had no significant effect on novel object recognition memory or social interaction. Preliminary PET findings reveal that MP resulted in alterations in neural activity in prefrontal, striatal, thalamic, and midbrain regions, as well as regions involved in memory & sensory processing. Collection of data during abstinence is ongoing.

Disclosures: L.S. Robison: None. **M. Vitale:** None. **M. Michaelos:** None. **J. Gandhi:** None. **S. Paeng:** None. **J. Lee:** None. **E. Miao:** None. **M. Hadjiargyrou:** None. **D. Komatsu:** None. **P.K. Thanos:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.15/UU60

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Intramural grant

Title: Persistent inhibitory circuit deficits emerge following chronic prenatal exposure to exogenous cannabinoids

Authors: *G. A. VARGISH, C. J. MCBAIN, K. A. PELKEY, X. YUAN, D. COLLINS;
Lab. of Cell. and Synaptic Neurophysiol., NIH/NICHD, Bethesda, MD

Abstracts: *In utero* exposure to exogenous cannabinoids (CBs) is correlated with cognitive defects in humans. However, the mechanism underlying these defects remains unknown. Disruption of cholecystokinin expressing interneuron (CCK INTs) development is a prime candidate as CCK INTs express cannabinoid receptor 1 (CB1R) embryonically and comprise much of the CB1R expression in the cortex and hippocampus. Further, endocannabinoid (eCB) signaling has been implicated in aspects of development such as proliferation, migration and circuit integration. As CB1R expressing CCK INTs proliferate and migrate beginning around embryonic day 10.5 (E10.5) in mice, maternal intake of CBs could interfere with CCK INT eCB signaling *in utero*. Thus, we examined the outcome of chronic prenatal exposure to CBs by comparing the offspring of pregnant mice treated from E10.5 to birth with CBs (Δ9-THC or WIN55,212-2 (WIN), a CB1R agonist) or a vehicle control (VH). Immunohistochemical characterization revealed a dramatic reduction in CCK INT density in mice prenatally exposed to WIN when compared to VH controls (55.5% decrease). This deficit was specific to CCK INTs as the density of cells expressing other INT markers was comparable between the two groups. Recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) in the dentate gyrus support anatomical findings as there is a reduction in basal sIPSC amplitude in mice prenatally treated with WIN (VH=63pA, n=64; WIN=54pA n=61). To assess the specific contribution of CCK INTs we applied ω-conotoxin (CTx), an N-type Ca2+ channel antagonist. As CCK INTs rely solely on N-type Ca2+ channels for transmitter release, CTx should specifically reduce CCK INT mediated IPSCs. CTx significantly reduced sIPSCs in VH but not WIN-treated animals (VH=28%, n=20; WIN=7%, n=17). sIPSCs in WIN-treated animals also showed a reduced sensitivity to acute WIN application compared to VH (VH=29%, n=11; WIN=12%, n=9). Further, recordings of disynaptically evoked feedback inhibition in CA1 of WIN treated animals exhibit a significantly reduced sensitivity to acute WIN application when compared to VH

(VH=54% n =20; WIN=30% n=17; p=0.01). Finally, recordings of connected CCK INT-pyramidal cell pairs in WIN treated mice show that the synaptic properties of residual CCK INTs remain intact, suggesting CCK INT loss accounts for the observed inhibitory deficits. Overall, our data reveal deficits in CCK INT mediated inhibitory drive following *in utero* exposure to CBs. These deficits could precipitate a significant change in excitation/inhibition balance, allowing us to understand, at a cellular level, the cognitive defects associated with prenatal CB exposure.

Disclosures: G.A. Vargish: None. **C.J. McBain:** None. **K.A. Pelkey:** None. **X. Yuan:** None. **D. Collins:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.16/UU61

Topic: C.17. Drugs of Abuse and Addiction

Support: Nipissing University, Internal Grant

Title: Chronic peripubertal WIN55212-2 administration is associated with changes in fear conditioning, amygdalar volume, and synaptic proteins

Authors: P. DELUCA, M. BOUDREAULT, J. ANDREWS, N. LANDRY, S. HILL, C. JESSO, M. J. SAARI, *A. C. WEEKS;
Nipissing Univ., North Bay, ON, ON, Canada

Abstracts: Although cannabis and its associated derivatives are the predominant illicit substances consumed by young people, experimental research regarding long-term adverse neurological effects of its chronic use is limited for this age group. This research is particularly important since the age when marijuana is first used in North America is steadily decreasing with the younger aged users being the fastest growing population. It has recently been demonstrated that, in animal models, chronic cannabinoid administration during the peripubertal period is uniquely capable of causing neurological damage. In this way the youngest age groups may be at the highest risk for long-term detrimental effects of cannabis consumption. Research has also indicated that increased levels of anxiety may be associated with cannabis use. For this reason, the amygdala, which has long been implicated in anxiety and fear responses, was the focus of the neurological examination in the current study. This experiment involved chronic administration

of the cannabinoid agonist WIN55212-2 mesylate to peripubertal male Wistar rats. Following chronic injections, experimental and control rats went through a standard auditory fear conditioning procedure to determine whether the drug treatment altered fear-learning. Neurological effects were examined by measuring co-localized synaptic protein density in the lateral and central amygdaloid nuclei (LA and CE respectively) using a confocal microscopic approach. Co-localizations were determined by applying primary antibodies to the synaptic proteins SV2 and PSD-95. Specific fluorophore-conjugated secondary antibodies were then applied and synapses were defined by the unique colour produced when both proteins co-localized. The behavioural results confirmed normal fear conditioning in both groups but indicated that the drug condition rats were more likely to show a fear response to the tone only (no shock). Preliminary synaptic results did not show any significant differences but there was a significant change in neural volume where the LA in the drug group was significantly larger than in the vehicle controls. Taken together, these results suggest that chronic cannabinoid use during adolescence results in a heightened level of emotional response and changes in amygdalar volume.

Disclosures: P. DeLuca: None. **A.C. Weeks:** None. **M. Boudreault:** None. **J. Andrews:** None. **N. Landry:** None. **S. Hill:** None. **C. Jesso:** None. **M.J. Saari:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.17/UU62

Topic: F.03. Motivation and Emotion

Support: Swedish Research Council and the Sahlgrenska University Hospital (JW and HO)

Marianne and Marcus Wallenberg Foundation (HO)

Research Council of Norway (SL)

Title: Expectation of specific therapeutic effects induces generalized placebo improvement of both pain and pleasure

Authors: *D.-M. ELLINGSEN[1], S. LEKNES[2], C. TRISCOLI[3], H. OLAUSSON[4], J. WESSBERG[1];
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Psychology, University of Oslo, Norway; [3]Univ. of Gothenburg, Inst. of Neurosci. and Physiol., Goteborg, Sweden; [4]Dept. of Clin. and Exptl. Med., Linköping Univ., Linköping, Sweden

Abstracts: Placebo improvement of negative symptoms, like pain, is often thought to arise from an "unconscious decision" that the pain is less important, because of an impending treatment that is believed to be efficient. We have recently shown that placebo improvement of positive (pleasant touch) and negative (pain) hedonic feelings rely on activation of a similar modulatory circuit. Here, we investigated whether suggestion of treatment benefit on either touch pleasantness or pain unpleasantness can bring about placebo improvement of both pleasure (hyperhedonia) and pain (analgesia). Forty-seven healthy volunteers participated in a crossover design, where they self-administered a nasal spray suggested to either (1) improve the pleasantness of touch (HYP group) or (2) reduce pain unpleasantness (ANA group). To strengthen the participants' expectations of treatment benefit, they were shown one out of two brief video documentaries that summarized scientific findings supporting either treatment-induced improvement of pain (ANA group) or touch pleasantness (HYP group). The video documentary was presented immediately before nasal spray treatment. Next, they rated pleasantness/unpleasantness and sensory intensity of gentle stroking touch and moderate heat pain. After placebo treatment, relative to a control condition without treatment, both the HYP and the ANA groups reported increased touch pleasantness and reduced pain unpleasantness. There was a similar placebo-induced improvement of sensory intensity, whereby pain intensity was reduced and touch intensity was increased, in both the HYP and the ANA groups. The results are consistent with a view of placebo responses as a generalized mechanism of reward prediction, by which a placebo-induced "motivational state" affects both positive and negative hedonic feelings.

Disclosures: D. Ellingsen: None. **S. Leknes:** None. **C. Triscoli:** None. **H. Olausson:** None. **J. Wessberg:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.18/UU63

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIMH grant R03MH086078

UCR Collaborative Seed Grant

Title: Animal model of adolescent cannabis abuse exhibits permanent deficit in the endocannabinoid system signaling

Authors: *A. CORCHES, J. W. LOVELACE, A. HIROTO, E. KORZUS;
Univ. of California, Riverside, Riverside, CA

Abstracts: Whereas current data link adolescence cannabis abuse to increased risk for dependence on other drugs, depression, anxiety disorders and psychosis, the mechanism underlying these adverse effects remains controversial. Here we show that mouse model of adolescent cannabis abuse shows deficits in an endocannabinoid (eCB)-mediated signaling and plasticity at adult central glutamatergic synapses in prefrontal cortex. Blockade of the monoacylglycerol lipase, the primary enzyme responsible for degrading the endocannabinoid 2-arachidonoylglycerol, with the specific inhibitor JZL184 amelioratesthese deficits. The observed deficit in cortical eCB-dependent signaling may represent a neural maladaptation underlying network instability and abnormal cognitive functioning.

Disclosures: **A. Corches:** None. **J.W. Lovelace:** None. **A. Hiroto:** None. **E. Korzus:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.19/UU64

Topic: F.03. Motivation and Emotion

Support: DA006886

DA032270

Title: A longitudinal analysis of changes in accumbens and dorsolateral striatal drug processing across protracted cocaine self-administration

Authors: *D. J. BARKER[1], K. COFFEY[2], N. GAYLIARD[2], J. KULIK[2], M. WEST[2];
[1]Rutgers Univ., Ellicott City, MD; [2]Rutgers Univ., New Brunswick, NJ

Abstracts: One of the most important hallmarks of substance dependence is addicts' perseverative drug seeking behavior. Indeed, once drug abuse has become chronic, drug-seeking behaviors often persist despite an overwhelming array of negative consequences associated with continued use. For this reason, it is important to understand changes in neural activity that

develop as drug abuse transitions from acute to chronic use and critical to examine drug-related processing in the dependent subject. Theories of addiction have suggested that long-term changes in striatal activity may be responsible for the transition from goal-directed drug seeking behaviors to automatic or habitual responding. Goal-directed behaviors are those whose responding is outcome driven, while habitual responding develops over repeated stimulus-response associations such that responding becomes stimulus elicited (i.e., insensitive to changes in the outcome). Evidence supporting these theories includes the data demonstrating that neural activity gradually shifts from the ventromedial striatum (i.e. nucleus accumbens) to the dorsolateral striatum across chronic drug self-administration. Nevertheless, a thorough longitudinal study of changes in striatal firing has yet to be conducted. Thus, the goal of the present study was to record neurons in the ventral striatum (accumbens core and shell) and dorsolateral striatum across 24 sessions of long-access cocaine self-administration and to examine changes in the processing of drug-seeking behaviors across this period.

Disclosures: D.J. Barker: None. **K. Coffey:** None. **N. Gayliard:** None. **J. Kulik:** None. **M. West:** None.

Poster

656. Reward II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 656.20/UU65

Topic: G.04. Physiological Methods

Support: EWU FGRCW 2012-13

Title: Characterizing the chronic dopamine microsensor

Authors: E. P. MARR, B. W. YAZEL, T. R. VANDERHOLM, S. L. DAVIS, P. W. RINNE, B. R. SORENSEN, *D. P. DABERKOW;
Biol., Eastern Washington Univ., Cheney, WA

Abstracts: Dopamine (DA) is a neurotransmitter involved in reward learning and drug addiction. Therefore, monitoring DA *in vivo* is critical in investigating DA activity related to these behaviors. Fast-scan cyclic voltammetry (FSCV) at the carbon fiber microelectrode has long been an indispensable tool in monitoring real-time DA signaling. Previously, glass-housed DA electrodes have limited this technique to short-term DA monitoring (hours) *in vivo*. Development of polyimide fused silica-encased microelectrodes has advanced the technique for

long-term DA monitoring (months) *in vivo*. In this study, we electrically stimulated and recorded DA signals at silica-encased microelectrodes long-term (months) in effort to characterize the recovery time and electrode stability after implantation. Silica-encased recording, bipolar stimulating, and silver chloride reference electrodes were surgically implanted and affixed in the dorsomedial striatum (+1.0 AP, +2.0 ML, -5.0 DV) of male Sprague-Dawley rats (~400 g). Electrically evoked (60Hz, 60p, 300 μA) DA signals were obtained during surgery and biweekly for four months post-surgery. Recorded DA signals were subsequently modeled by kinetic analysis to resolve parameters describing DA release ($[DA]_p$; the concentration of DA release per stimulus pulse) and uptake (V_{max}; maximal rate of DA uptake). Preliminary data suggest that peak amplitude of evoked DA signals (DA_{max}) has an initial decrease after surgery and then stabilizes several weeks later. The same trend holds for parameters describing DA release and uptake determined by kinetic analysis. The results of this study indicate that once recovered, electrically evoked DA signals recorded at the silica-encased microelectrode, and resulting kinetic parameters describing DA release and uptake, are stable long-term (months).

Disclosures: **E.P. Marr:** None. **D.P. Daberkow:** None. **B.W. Yazel:** None. **T.R. Vanderholm:** None. **S.L. Davis:** None. **P.W. Rinne:** None. **B.R. Sorensen:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.01/UU66

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: INSERM

Région Basse-Normandie

Title: Combinations of housekeeping genes for normalization of qPCR data in cerebral age-related transcriptomic studies

Authors: *G. BRUCKERT, M. HEBERT, D. VIVIEN, F. DOCAGNE, B. ROUSSEL; INSERM U919, Caen, France

Abstracts: Numbers of studies use quantitative PCR (qPCR) for investigating gene expression because of its precision and accuracy to treat many samples. Internal and external controls are needed to set up qPCR experiments including normalization processes with housekeeping genes.

Nowadays more than one housekeeping gene is recommended that do not vary with experimental paradigms. This point is particularly important in age-related cerebral studies in which standard "housekeeping" genes are usually highly variable. Because of this limitation, such studies usually use only one housekeeping gene to normalise their specific gene expression or use the direct Ct values. Altogether, this implies a major approximation of the results which could lead to possible interpretation mistakes. In the present study, we tried to develop a more rigorous approach for analysing qPCR data during aging and thus in different mouse brain areas. To address this question, we used the geNorm algorithm (QBase+ software) for the identification of a set of relevant housekeeping genes which should allow us to normalized aged-related qPCR data. As controls, we first investigated the expression of 8 genes largely used as housekeeping genes (pPIB, HMBS, RPL13A, actin, HPRT, pPOX, SDHA, Polr2a) in studies performed from hippocampus, striatum, cerebellum and cortex of C57Bl/6J mice aged from 8 weeks to 22 months old. In each of these structures, although none of these putative housekeeping genes displayed a stable expression from 8 weeks to 22 month old, we proposed a combination of at least two of them allowing an acceptable normalization. Our data revealed a unique combination of housekeeping genes that are specific of each of the brain structures analysed, that could be useful for normalization procedure of qPCR data in cerebral age-related transcriptomic studies.

Disclosures: G. Bruckert: None. **M. Hebert:** None. **D. Vivien:** None. **F. Docagne:** None. **B. Roussel:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.02/UU67

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Brain Disorder Award from the McKnight Foundation

David Weil Fund to the Semel Institute at University of California, Los Angeles (UCLA)

NASARD Young Investigator award

Title: Genetically-Directed sparse and stochastic labeling of striatal direct-pathway neurons in mice: Application to study neurodevelopment and neurodegeneration

Authors: *X. LU, X. W. YANG;
Dept Psychiatry & Biohav Sci., Ctr. For Neurobehavioral Genetics, Semel Inst., LOS ANGELES, CA

Abstracts: To tackle the complexity of brain, tools for sparse and random labeling of individual neurons are needed to study neuronal development, connectivity, plasticity, physiology and pathology. Current reagents are limited by their availability for use in multiple cell types and developmental stages, the time consuming process of generating and combining the necessary mouse alleles (usually bi-, or tripartite system), and the inability to perform genetic-based tagging of endogenous proteins in single defined neuronal cell types. We have developed a simple and versatile method (i.e. MORF) to label single neurons. We generated a dopamine D1 receptor (Drd1a) bacterial artificial chromosome transgenic mice (D1-bacMORF) to demonstrate such method. The D1-bacMORF mice reveal fine dendritic structures of the sparse and stochastic labeled neuronal cell types normally expressing dopamine D1 receptor, including the striatal direct pathway medium spiny neurons (D1-MSNs), hippocampal and cortex pyramidal neurons. By crossing with D1-BAC-TdTomato mice, we showed D1-bacMORF mice selectively labels about 1% of the D1-MSNs. As a demonstration of the functional utility of such method, we used D1-bacMORF mice to reveal the increasing elaborated dendritic morphology of D1-MSNs during postnatal ages between P0 and P90. Furthermore, we crossed D1-bacMORF mice with a Huntington's disease knock-in mice and was able to show age-dependent, progressive dendritic pathology in D1-MSNs of HD mice but not wildtype control mice. Together, our study established a novel and powerful transgenic method to confer genetically-directed sparse and stochastic expression of a marker protein in specific neuronal cell types, and the resulting "Golgi-like" morphological details of the molecularly-defined neurons in intact mouse brains may help to greatly facilitate the study of development, function and dysfunction of the mammalian central nervous system.

Disclosures: X. Lu: None. **X.W. Yang:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.03/UU68

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: ERC Synergy Grant 2012

Title: A transgenic mouse model expressing a luficerase-based sensor to study proteostasis *in vivo*

Authors: *E. SCHULZ-TRIEGLAFF, R. KLEIN, I. DUDANOVA;
Molecules - Signaling - Develop., Max Planck Inst. of Neurobio., Munich, Germany

Abstracts: The decline in protein homeostasis (proteostasis) mechanisms is believed to be a major cause of age-related neurodegenerative disorders, such as Alzheimer's, Parkinson's or Huntington's disease. It has been shown that modulating the proteostasis machinery protects from or enhances pathological changes in cellular and animal models of neurodegenerative diseases. However, proteostasis has not been monitored during the course of disease of mammalian models *in vivo*. To study the chronological sequence of proteostasis impairment, protein aggregation and neuropathological and functional changes in the brain, we are using protein sensors, which consist of firefly luciferase (Fluc) fused to GFP. Fluc is a metastable protein that requires chaperone assistance for proper folding and full enzymatic activity. A decrease in protein folding capacity of a cell can be detected by decreased enzymatic activity of Fluc and changes in GFP distribution in the cytoplasm, as the sensor forms inclusions when not folded correctly. Point mutations have been introduced into this protein, leading to sensor proteins with increased sensitivity to disturbances of proteostasis (Gupta R, et al. 2011. Nature methods 8: 879-84). We first tested the sensor variants` sensitivity and effectiveness in murine cortical cultures by applying different stress paradigms to transfected primary neurons. Either application of a proteasome inhibitor or co-expression of aggregating proteins leads to the formation of GFP+ inclusions in transfected neurons. Subsequently, we have generated transgenic mice expressing the sensor under a pan-neuronal promoter, to drive expression in the nervous system. Promising founder lines show expression in several brain regions, including cortical layer V pyramidal neurons, thalamus, hypothalamus, ventral tegmental area and cerebellar granule cell layer. We are currently characterizing the properties of the sensor in neuronal cultures of these transgenic animals. We plan to cross these sensor mice to different disease models to assess proteostasis in *ex vivo* fixed tissue and in cultures of dissociated neurons. In addition, luciferase activity could be visualized *in vivo* in anesthetized mice in a noninvasive manner, providing the opportunity for studies of disease progression in the same animal. Our sensor mice represent a tool to monitor proteostasis status in the living animal, an important basis for developing new therapies based on improved proteostasis.

Disclosures: E. Schulz-Trieglaff: None. **R. Klein:** None. **I. Dudanova:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.04/UU69

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: R00 DA024719

Title: Isolation of DNA from genetically-defined populations of neurons

Authors: *P. S. LAMBETH, B. A. NEWMYER, D. M. WARTHEN, E. PEREZ-REYES, M. M. SCOTT;
Dept. of Pharmacol., Univ. of Virginia, Charlottesville, VA

Abstracts: A clear description of how epigenetic and transcriptional changes occur in defined populations of neurons is crucial to understanding how stimuli modify neuronal function. Currently, no method exists for both flexibly and efficiently tagging nuclei for either genetic or epigenetic analysis. To address this shortcoming, we have developed a broadly applicable method of tagging neuronal cells in a Cre-dependent manner. Sun2 is a protein anchored in the inner nuclear membrane and has redundancy with Sun1, therefore it is an ideal target as it has been shown that manipulations to one of the pair do not affect function. By fusing the myc epitope tag to the C-terminus of Sun2 and inserting it into a DIO (double floxed inverted) adeno-associated viral vector, we have developed a way to limit nuclear tag expression to genetically defined cell types that express Cre recombinase. In the development of this technique, we report on our ability to isolate whole nuclei and to separate genetically distinct populations of cells from heterogeneous cortical tissue. We anticipate combining this approach with other methods that permit the isolation of mRNA from defined cell types through the Cre recombinase dependent expression of HA-tagged or GFP-tagged ribosomal proteins. Consequently, this approach will allow for an analysis of how genetic or epigenetic changes correlate with transcriptional changes in defined populations of neurons. We envision our approach being suitable for a wide variety of downstream applications, including ChIP-seq and multiple forms of DNA methylation analysis.

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Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.05/UU70

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: start-up funds from Georgia Regents Univ.

Title: Generation of a bacterial artificial chromosome transgenic rat line for specifically expressing Cre recombinase and channelrhodopsin in dopamine neurons

Authors: *Y. LU, S. FREY, P. WANG, J. U. FREY;
Lab. of Functional Plasticity, Learning and Memory (BBDI), Georgia Regents Univ., Augusta, GA

Abstracts: Dopamine is known for its importance in many cognitive functions and neurodevelopmental processes. Understanding of mechanisms regulating and regulated by dopaminergic neurons lags however, partially because of shortage of genetic tools to selectively and precisely manipulate these neurons. Comparing with mice, rats offer several advantages both in behavioral tests and electrophysiology. The dopamine transporter (DAT) is only expressed in a subpopulation of dopaminergic neurons to regulate the extracellular dopamine levels. Comparing with the more widely expressed tyrosine hydroxylase (TH) gene, DAT expression is more selective for dopaminergic neurons. Here we generated a transgenic rat line expressing a codon-improved Cre recombinase (iCre), and bicistronically a red shifted chimeric channelrhodopsin (C1V1) using the self-cleaving 2A peptide derived from porcine teschovirus-1 (p2A). These two genes were placed under the control of a large genomic regulatory sequence of DAT gene in a bacterial artificial chromosome (BAC). This DAT-C1V1-p2A-iCre BAC transgenic rat line could present a unique tool allowing genetic manipulations using the Cre/LoxP system and simultaneously an optogenetic control of the DA neuron activity.

Disclosures: **Y. Lu:** A. Employment/Salary (full or part-time):; Georgia Regents University, Laboratory of Functional Plasticity, Learning and Memory (BBDI). **S. Frey:** A. Employment/Salary (full or part-time):; Georgia Regents University, Laboratory of Functional Plasticity, Learning and Memory (BBDI). **P. Wang:** A. Employment/Salary (full or part-time):; Georgia Regents University, Laboratory of Functional Plasticity, Learning and Memory (BBDI). **J.U. Frey:** A. Employment/Salary (full or part-time):; Georgia Regents University, Laboratory of Functional Plasticity, Learning and Memory (BBDI).

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.06/UU71

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF (GRFP)

Title: Cell-type specific control of gene function in *Drosophila* melanogaster

Authors: *Y. E. FISHER[1], D. M. GOHL[2], T. R. CLANDININ[1];
[1]Stanford Univ., Stanford, CA; [2]Univ. of Minnesota Genomics Ctr., Univ. of Minnesota, Minneapolis-St Paul, MN

Abstracts: The ability of a neuron to perform a specific computational role within a circuit is tightly shaped by its gene expression profile. Therefore, a common experimental goal is to disrupt a gene of interest in a cell-type specific manner. Due to its immense genetic toolkit, *Drosophila* is a powerful model system for the study of circuit function. However, current conditional gene disruption techniques often only incompletely reduce gene activity or require time-consuming genome engineering. We have developed FLP-STOP, a conditional gene disruption tool for *Drosophila* that uses a targeted insertional mutagenesis strategy that takes advantage of a large collection of existing transposable elements targeting most genes. In this approach, a transgenic animal is created in which a recombinase-dependent STOP-cassette is placed within an intron of the gene of interest. By design, this cassette will only disrupt gene function when it is in one of two possible orientations. The cassette is flanked by recombinase recognition sites (creating a FLEx switch) such that it becomes inverted in the presence of FLP recombinase (Schnutgen et al. 2003). We demonstrate that intronic insertions of the STOP-cassette in the neutral or "non-disrupting" orientation retain normal gene function while the "disrupting" orientation is sufficient to severely impair gene function and can even produce null alleles. Cassette inversion is highly efficient and can be achieved when FLP recombinase is expressed using a heatshock promotor or under GAL4/UAS control, demonstrating compatibility with existing tools for targeting and manipulating neuronal populations. We present progress toward a tool kit of transgenic tools for manipulating genes that target GABAergic, glutamatergic and cholinergic transmission as well as various voltage-gated ion channels. This tool facilitates experiments that investigate the interaction between genes and circuit function.
Schnütgen, F., Doerflinger, N., Calléja, C., Wendling, O., Chambon, P., & Ghyselinck, N. B. (2003). A directional strategy for monitoring Cre-mediated recombination at the cellular level in the mouse. Nature Biotechnology, 21(5), 562-5. doi:10.1038/nbt811

Disclosures: Y.E. Fisher: None. **D.M. Gohl:** None. **T.R. Clandinin:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.07/UU72

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Howard Hughes Medical Institute

NIH

JPB Foundation

Title: Profiling of midbrain dopamine neuron projections to the striatum using Retro-TRAP

Authors: ***A. R. NECTOW**[1], M. I. EKSTRAND[1], K. N. LATCHA[1], J. M. FRIEDMAN[2]; [1]Dept. of Mol. Genet., Rockefeller Univ., New York, NY; [2]Dept. of Mol. Genet., Rockefeller University/HHMI, New York, NY

Abstracts: Translational profiling methodologies have allowed for the systematic characterization of neural circuitry in health and disease states. We have recently developed a methodology, Retro-TRAP, for profiling neurons based on their connectivity utilizing an anti-GFP nanobody fused to ribosomal protein Rpl10a (NBL10). This approach can be further extended to be cell-type-specific by restricting NBL10 expression to Cre-expressing populations of neurons through viral-mediated gene transfer (AAV-FLEX-NBL10), allowing for molecular interrogation of genetically-defined neurons based on connectivity. Building on these methods, we added an N-terminal 3xFLAG tag to our construct (AAV-IV-NBL10), allowing for normalization to the whole Cre-expressing population to identify projection-specific marker genes within a molecularly defined population. We used these approaches to comprehensively profile and compare mesolimbic and nigrostriatal projections, with a particular focus on dopamine neurons. Through comparative analysis of high-throughput RNA sequencing (RNA-seq) data generated from each of these populations, we've identified a number of projection-specific marker genes differentiating these two populations, some of which were confirmed via immunohistochemistry, FISH, and/or the Allen Brain Atlas. Importantly, the current approach is generalizable to neural circuitry throughout the central and peripheral nervous system, allowing for comprehensive molecular profiling of defined cell-types based on their connectivity.

Disclosures: **A.R. Nectow:** None. **M.I. Ekstrand:** None. **K.N. Latcha:** None. **J.M. Friedman:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.08/UU73

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Natural Sciences and Engineering Research Council of Canada

Alfred P. Sloan Foundation

The Scottish Rite Charitable Foundation of Canada

NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

Title: Quantification of protein levels in single cells *in vivo*

Authors: C.-A. LO[1], I. KAYS[1], F. EMRAN[2], T.-J. LIN[2], V. CVETKOVSKA[1], *B. E. CHEN[1]; [1]Ctr. for Res. in Neurosci., McGill Univ., Montreal, QC, Canada; [2]Ctr. for Res. in Neurosci., Res. Inst. of the McGill Univ. Hlth. Ctr., Montréal, QC, Canada

Abstracts: Accurate measurement of the amount of specific protein a cell produces is important for investigating basic molecular processes of the cell. The current methods for determining protein amounts have poor cellular resolution and are inherently destructive to cells, limiting the accuracy and relevance of the measurements. We have developed a technique that allows for quantitation of protein levels in single living cells. This Protein Quantitation Ratioing (PQR) technique uses a genetic tag that produces a stoichiometric ratio of a fluorescent protein reporter and the protein of interest during protein translation. The fluorescence intensity (i.e., brightness of the cell) is directly proportional to the number of molecules produced of the protein of interest, and thus is used to determine the relative protein amount within the cell. Using quantitative imaging and electrophysiology, we demonstrate that PQR can produce stoichiometric separations and linear relationships between different genes. Using the circadian system, we demonstrate cyclical changes in fluorescence in small lateral ventral neurons in the *Drosophila* brain. We use genome editing techniques to insert Protein Quantitation Reporters into endogenous genomic loci in three different genomes for quantitation of endogenous protein levels. Fluorescence quantification of endogenous RPL13A protein levels in a neuron can be used to normalize across experimental and optical conditions, such as spherical aberrations, optical distortions, calcium imaging, and imaging depths during *in vivo* imaging. The Protein Quantitation Ratioing technique allows for measurements of endogenous or exogenous protein

amounts in single living cells *in vivo*, to relate cellular phenotypes as a function of protein concentrations.

Disclosures: **C. Lo:** None. **I. Kays:** None. **F. Emran:** None. **T. Lin:** None. **V. Cvetkovska:** None. **B.E. Chen:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.09/UU74

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Efficient transfection of native sensory neurons

Authors: *J. SVENSSON DALÉN[1], A. GARZA CARBAJAL[2], A. KARLSSON[1], M. KARLSSON[1], T. HUCHO[2], P. KARILA[1];
[1]Cellectricon AB, Mölndal, Sweden; [2]Experimentelle Anästhesiologie und Schmerzforschung Klinik für Anästhesiologie und Operative Intensi, Uniklinik Köln, Köln, Germany

Abstracts: The introduction of plasmid DNA into cells with the aim to manipulate the protein expression is a widely used method in cell biology research. Several transfection methods are available, although all methods do not suit all cellular systems. Specifically, many primary cell types, such as neurons, have proven to be difficult to transfect with high efficiency and viability. We have developed a protocol for in-situ electroporation of primary dorsal root ganglion (DRG) neurons from adult rats using the Cellaxess Elektra Discovery Platform. The Elektra platform utilizes a capillary electroporation concept that can be applied for gene transfer to primary cultures in 384-well plates. The focused electrical field delivered by the platform minimizes electrochemical toxicity and joule heating, compared to conventional cuvette-based electroporation methods. The platform has the capability to transfect neuronal cells, e.g. rat, mouse & IPS-derived human neurons, with excellent viability and retained morphology, and the method is well-suited for high content readouts. Importantly, as the transfection procedure is performed directly in 384-well HCA compatible plates, the protocol is ideally suited for a screening workflow where additional manipulations, such as microscopy-based readouts or other follow-up assays, are easily accomplished without additional cell processing. With the Elektra platform, we were able to develop stable protocols for transfection of rat DRG neurons with a high degree of viability and a retained and healthy morphology. Using a CopGFP-plasmid a transfection efficiency of 40-60% was achieved and cultures could be transfected at different

time points after plating in a straightforward manner. In our lab, we use primary DRG neurons in culture as a cell model for chronic pain. These neurons retain their sensory functionality after transfection and remain responsive to thermal, mechanical and functional stimuli, and when supplemented with nerve growth factor (NGF), they can be used to mimic peripheral sensitization. Through the use of the transfection protocol described here, we now have the capability to knock in, and/or knock out, gene targets of interest in these cells to further refine and enhance the relevance of our disease models.

Disclosures: **J. Svensson Dalén:** A. Employment/Salary (full or part-time):; Cellectricon AB. **A. Garza Carbajal:** None. **A. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB. **T. Hucho:** None. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.10/UU75

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Brain Sciences Project of the Center for Novel Science Initiatives, National Institutes of Natural Sciences (BS251004 to K.I.)

Title: Intravascular administration of an AAV vector to neonatal macaques results in widespread gene transduction into neurons throughout the primate brain

Authors: ***K. INOUE**[1], K. KIMURA[1,2], R. YASUKOCHI[1], N. SUGAWARA[1], Y. OKUDA[1], M. FUJIWARA[1], M. TAKADA[1];
[1]Systems Neurosci. Sec., Primate Res. Inst., Kyoto Univ., Inuyama, Japan; [2]Dept. Neurol. and Stroke Med., Sch. of Medicine, Yokohama City Univ., Yokohama, Japan

Abstracts: Recombinant adeno-associated virus (rAAV) vectors constitute a powerful tool for delivering target genes into the brain. Previous studies reported that serotype 9 of AAV (AAV9) was able to cross the blood-brain barrier, and that systemic application of a self-complementary AAV9 vector to neonatal mice, rats, and cats yielded efficient gene transduction into neurons. However, gene transduction into neurons of neonatal non-human primates has not yet been performed successfully. Here we show that intravascular administration of a single-stranded AAV9 vector to neonatal macaques resulted in widespread gene transduction into neurons

throughout the brain. We injected 1.0 x 10^{14} viral genome/kg of the AAV vector expressing green fluorescent protein (GFP) under CMV promoter control through the saphenous vein. Robust GFP expression was found in both neurons and glia over the cerebral cortex, subcortical regions, and cerebellum. Neuronal tropism and transduction efficiency varied depending on the brain structure and cell type. Purkinje cells in the cerebellar cortex exhibited the highest transduction efficiency. The present data indicate that systemic delivery of the AAV9 vector in neonatal macaques is a potential methodology to transduce target genes efficiently throughout the primate brain and is useful in creating genetically manipulated primate models of neuropsychiatric disorders and developing their gene therapeutic approaches.

Disclosures: K. Inoue: None. **K. Kimura:** None. **R. Yasukochi:** None. **N. Sugawara:** None. **Y. Okuda:** None. **M. Fujiwara:** None. **M. Takada:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

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Program#/Poster: 657.11/UU76

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Whitehall Foundation (C.J.P)

NIH (R01DC013070, C.J.P)

NIH (R01NS079584, M.N.W.)

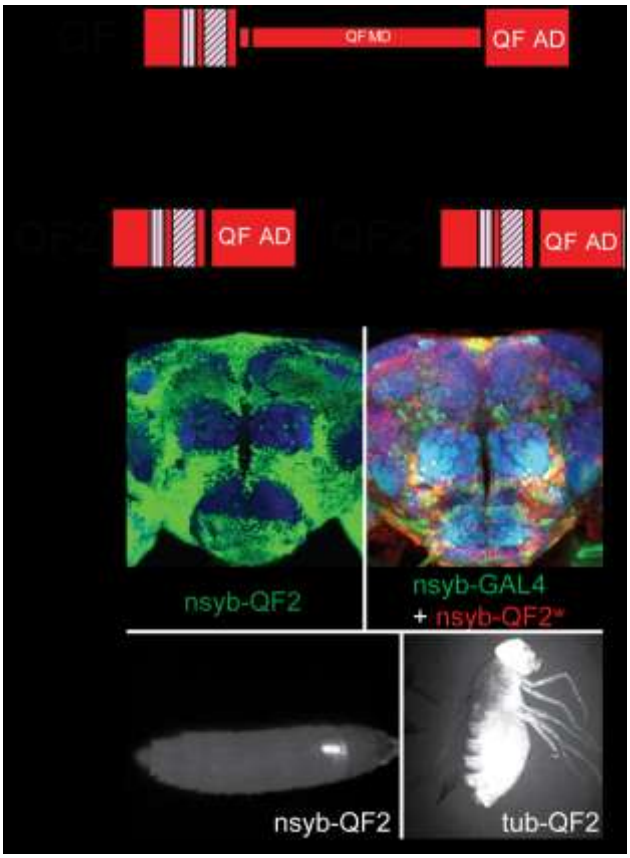
HHMI (L.L.)

Title: Improved and expanded Q-system toolkit for transgene expression

Authors: *O. RIABININA[1], D. LUGINBUHL[3], E. MARR[1], S. LIU[2], M. N. WU[2], L. LUO[3], C. J. POTTER[1];
[1]Dep of Neuroscience, SOM, [2]Dep of Neurology, SOM, Johns Hopkins Univ., Baltimore, MD; [3]Howard Hughes Med. Inst., Stanford Univ., Stanford, CA

Abstracts: The Q-system is a binary transcription factor system for expression of transgenes *in vivo*. The system was originally developed for use in *Drosophila* (Potter et al, 2010), and has since been adapted for *C. elegans* (Wei et al, 2012) and D. rerio (Subedi et al, 2014). In its original form, the transcriptional activator of the Q-system QF appeared to be toxic when

expressed pan-neuronally or ubiquitously in *Drosophila*. To solve the toxicity problem, we created a number of chimeric transactivators by combining functional subunits (DNA binding domain, middle domain and activation domain) of QF, GAL4 and the third *Drosophila* expression system, LexA. We discovered that the middle domain of QF is chiefly responsible for its toxicity. By removing the middle domain we developed the second-generation version of QF, named QF2. By introducing a mutation in the activation domain, we also generated QF2w, a weaker variant useful in cases of very broad QF2 expression. Both QF2 and QF2w are non-toxic, can drive strong expression and are QS-suppressible. QF2 and QF2w function equally well in the range of temperatures (18-29C), typically used in *Drosophila* experiments. For use in the nervous system, we generated transgenic *Drosophila* that express QF2 and QF2w pan-neuronally under the control of a strong synaptobrevin promoter. To extend the use of the Q-system beyond nervous system, we also generated fly lines where QF2/QF2w are expressed ubiquitously by actin and tubulin promoters. These lines can be used to express high levels of reporter genes in nervous system, imaginal discs and muscles of larva, and various tissues in adult flies. In the course of our study, we also developed two chimeric transactivators, GAL4-QF and LexA-QF, that bind the UAS and LexAop, respectively, and are QS-suppressible. Pairwise combinations of all new transactivators and GAL4 functions effectively during pan-neuronal expression, thus allowing greater flexibility in intersectional studies that make use of the Q, GAL4/UAS and LexA systems.



Disclosures: **O. Riabinina:** None. **D. Luginbuhl:** None. **E. Marr:** None. **S. Liu:** None. **M.N. Wu:** None. **L. Luo:** None. **C.J. Potter:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.12/UU77

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant NS045926

NIH Grant NS086604

NIH Grant MH099587

Title: Cas9 nickase increase efficiency in producing transgenic human embryonic stem cell lines

Authors: ***J. CAO**, Y. CHEN, M. XIONG, Z. DU, S.-C. ZHANG;
Waisman Center, Univ. of Wisconsin Madison, Madison, WI

Abstracts: The ability to precisely modify mammalian genome has revolutionized biomedical researches over the past decades. Nevertheless, generation of genetically modified human stem cells with traditional transgenic tools has been challenging. The RNA-directed sequence-specific Cas9 endonuclease enables homology-directed repair (HDR) in the presence of a template, making it possible to produce genetically modified human stem cell lines. The newly developed Cas9 nickase system further improves the specificity of sgRNA-guided targeting on a DNA locus. However, it is not known whether the nickase system affects the HDR efficiency, especially in human pluripotent stem cells (PSCs). By using the Cas9 system to target Ago2 and Sox2 in human embryonic stem cells (ESCs), we found that the HDR efficiency is enhanced from 1.3% in the regular Cas9 system to 55.8% in the nickase system at Ago2 locus. Similarly, the HDR efficiency is improved from 21.8% to 67.8% by the nickase system at Sox2 locus. Importantly, we also observed an increased homozygous HDR ratio by Cas9 nickase (is from 0% to 20% for Ago2 and from 2.3% to 16.1% for Sox2). Interestingly, when we tested the genomic DNA cleavage efficiency by regular Cas9 or nickase, we found that nickase has a lower cleavage efficiency than regular Cas9. Our results thus indicate that Cas9 nickase increasesthe HDR efficiency and homozygous HDR ratio in human ESCs, suggesting that Cas9 nickase system is

ideal for producing transgenic human ESC lines, including knockout lines that require targeting both alleles.

Disclosures: **J. Cao:** None. **Y. Chen:** None. **M. Xiong:** None. **Z. Du:** None. **S. Zhang:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.13/UU78

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: ERC

Title: Fluorescence-activated sorting of fixed nuclei (FAST-FIN): A general method to study nuclei from specific cell populations that preserves post-translational modifications

Authors: L. MARION-POLL[1], E. MONTALBAN[1], A. MUNIER[2], D. HERVÉ[1], *J.-A. GIRAULT[1];
[1]UMR-S 839, Inserm UPMC Inst. du Fer A Moulin, Paris, France; [2]Cell Imaging and Flow Cytometry facility, UPMC, Paris, France

Abstracts: A major difficulty in the study of transcriptional changes and epigenetic modifications comes from the cellular heterogeneity of brain tissue. A promising approach is to directly purify identified nuclei. Using mouse striatum we have developed a rapid and efficient method to isolate cell type-specific nuclei from fixed adult brain (fluorescence-activated sorting of fixed nuclei, FAST-FIN). The protocol we describe can be performed within one or two days. Animals are quickly perfused with a formaldehyde fixative that stops enzymatic reactions and maintains the tissue in the state it was at the time of death. Tissue is subsequently dissociated with a Dounce homogenizer and nuclei prepared by centrifugation in an iodixanol density gradient. The purified fixed nuclei can then be immunostained with specific antibodies and either analyzed or sorted by flow cytometry. Simple criteria allow identification of neurons. Immunolabeling and transgenic mice that express fluorescent proteins can be used to identify specific cell populations, and the nuclei from these populations can be efficiently isolated, even rare cell types such as parvalbumin-expressing interneurons. This method allows the preservation and study of dynamic and labile post-translational protein modifications. FAST-FIN should be applicable to other tissues and species.

Disclosures: **L. Marion-Poll:** None. **E. Montalban:** None. **A. Munier:** None. **D. Hervé:** None. **J. Girault:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

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Program#/Poster: 657.14/UU79

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Brain-specific delivery of rabies virus glycoprotein modified nanoparticles as a function of route of administration

Authors: ***A. PRAKAPENKA**[1,2,3,5,6], R. L. MCCALL[6], R. W. SIRIANNI[6,4], H. A. BIMONTE-NELSON[5,3,6];
[2]Sch. of Life Sci., [3]Dept. of Psychology, [4]Dept. of Biomed. Engin., [1]Arizona State Univ., Tempe, AZ; [5]Arizona Alzheimer's Consortium, Phoenix, AZ; [6]Brain Tumor Res. Center, Barrow Neurolog. Inst., Phoenix, AZ

Abstracts: The efficacy of hydrophobic drugs in the central nervous system (CNS) is limited by poor solubility, rapid clearance, and inefficient delivery to target tissue. Nanoparticles composed of poly (lactic co-glycolic) acid (PLGA) release drugs over a time period of days to weeks, sustaining the presence of drug in tissue to improve efficacy. By modifying the surface of the nanoparticle, it may be possible to achieve tissue-specific targeting. For instance, rabies virus glycoprotein (RVG) is a 26-amino acid peptide from the coat protein of the rabies virus that plays a crucial role in its penetration of the CNS. This study examined the biodistribution of both plain PLGA and RVG modified PLGA nanoparticles using encapsulated fluorescent hydrophobic dye. The nanoparticle formulations were administered to mice by intravenous, intraperitoneal, and subcutaneous routes. Tissue was extracted at 30 minutes, 2 hours, 6 hours, and 24 hours after injection and homogenized to measure fluorescent signal. Results showed that total dye concentration in the brain was highest following intravenous administration, with a 40% concentration increase at 2 hours using RVG modified PLGA relative to plain PLGA. An increase in brain concentration with RVG modified PLGA was also seen with intraperitoneal (at 2 hours) and subcutaneous (at 6 hours) administration relative to plain PLGA. These results suggest that the brain-targeting effect of RVG modification is maintained across administration techniques, but that the kinetics of delivery are a function of the route of administration. That is, intravenous administration of nanoparticles produced the highest payload delivery to the brain.

These data will be helpful to identify drug delivery strategies that are capable of producing the highest accumulation of encapsulated payload in the brain while reducing peripheral exposure. In particular, we are developing surface-modified nanoparticles to study brain-specific effects of estrogen on cognitive performance.

Disclosures: A. Prakapenka: None. **R.L. McCall:** None. **R.W. Sirianni:** None. **H.A. Bimonte-Nelson:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

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Program#/Poster: 657.15/UU80

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01 DK098994 02

Hilda and Preston Davis Postdoctoral Fellowship in Eating Disorder Research

Title: Two-key recombinase system for neural ensemble labeling and manipulation of ventral striatal D1R- and D2R-MSN neural ensembles encoding primary appetitive and aversive stimuli

Authors: *D. M. OPLAND, C. W. BOND, D. S. ABRAMOV, R. J. DILEONE;
Mol. Psychiatry, Yale Univ., New Haven, CT

Abstracts: The ventral striatum is known to take part in encoding information relative to both primary appetitive and aversive stimuli, a component of its role in mesocorticolimbic reward processing. Previous research has elucidated the role of D1R-containing medium spiny neurons (MSNs) in encoding appetitive stimuli and potentiating behavior ("Go" neurons). Conversely, D2R-MSNs encode aversive stimuli and attenuate behavior ("No-Go" neurons). However, recent evidence suggests that there might be encoding of aversion within D1R-MSN populations and encoding of reward within D2R-MSNs. These populations represent an understudied subset of striatal neurons that potentially contribute significantly to reward-mediated behaviors. Limits of molecular genetic labeling techniques prevent sufficiently identifying and manipulating functionally relevant subsets of active neural ensembles. Recent studies have used c-Fos or Arc IEG promoter driven Cre recombinase mediated expression of fluorophore labels in order to label activated neural ensembles participating in discrete behaviors. We have developed a similar IEG-mediated neuronal ensemble labeling technique that uses two different recombinase

enzymes (a 2-key system) which allows for both labeling (flourophore-tagging) and manipulation (optogenetically with hChR2) of active neural ensembles restricted by a population-specific marker (in this case a Cre-driver transgenic mouse line). This construct uses Arc as a marker for neural activity and tamoxifen as a means of restricting the labeling window. Using this new molecular tool we are able to specifically label in D1R-Cre and D2R-Cre mouse lines the relative response of each type of MSN to either primary appetitive or aversive stimuli as well as how each contribute to the modulation of behavior. This strategy allows labeling, and ultimately manipulation, of specific subsets of active neurons within brain regions.

Disclosures: D.M. Opland: None. **C.W. Bond:** None. **D.S. Abramov:** None. **R.J. DiLeone:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.16/UU81

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: McGovern Institute for Brain Research

Picower Institute for Learning and Memory

MIT Department of Brain & Cognitive Sciences

Simons Center for the Social Brain

Title: Towards nontoxic monosynaptic transduction

Authors: *I. R. WICKERSHAM;
McGovern Inst., MIT, CAMBRIDGE, MA

Abstracts: Monosynaptically restricted transsynaptic tracing using rabies viral vectors has become a widely used technique, allowing identification of synaptically connected neurons and expression of transgenes within them. However, this technology has so far been used almost exclusively for anatomical experiments because of its rapid cytotoxicity. Solving the toxicity problem would be a major advance, allowing the monitoring and manipulation of identified synaptically connected neuronal networks in the context of long-term behavioral experiments.

Here I present an approach to identifying and expressing transgenes within monosynaptically connected neurons while leaving them alive and physiologically normal indefinitely.

Disclosures: I.R. Wickersham: None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.17/UU82

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Genome BC

CIHR

NSERC

NRC-IRAP

Title: ApoE-mediated lipid nanoparticle delivery of RNA for loss-of-function and gain-of-function studies in primary neurons *in vitro* and *in vivo*

Authors: *E. RAMSAY, C. WALSH, A. ANSARI, A. THOMAS, K. OU, A. WILD, T. LEAVER, R. J. TAYLOR, D. ZWAENEPOEL;
Precision Nanosystems Inc., Vancouver, BC, Canada

Abstracts: Objective To demonstrate Apolipoprotein E (ApoE)-mediated Lipid Nanoparticle (LNP) delivery of RNA modulates gene expression in primary neurons *in vitro* and *in vivo* providing a rapid and simple mechanism to manipulate gene regulation. Methods RNA-LNP were prepared using a proprietary microfluidic-based manufacturing platform (SUB9KITS™). For uptake studies, fluorescent RNA-LNP (100 ng - 1000 ng RNA/mL) were incubated (24 h) with primary hippocampal neurons grown +/- astrocyte feeder layer. A mixed culture of primary cortical neurons (PCN) were treated with siRNA-LNP targeted against phosphotensin homolog 1 (PTEN). Gene knockdown was determined by mRNA (qPCR) and protein (western blot (WB)). Neuron viability was assessed using the lactate dehydrogenase (LDH) assay. A single dose of siRNA-LNP (500 nL at 5 mg/mL siRNA/10mins) was directly injected into the somatosensory cortex. Acute cortical slices (5 days post injection) were assessed for siRNA-LNP uptake and PTEN gene knockdwon *in vivo*. Gene expression was assessed in PCN using GFP mRNA-LNP

(500 ng/mL mRNA). GFP expression was measured using flow cytometry 72 h post-transfection. Results RNA-LNP were avidly taken-up (>98%) by primary hippocampal neurons grown on an astrocyte feeder layer. Neurons cultured without astrocytes had minimal uptake. Addition of ApoE4 recovered RNA-LNP uptake. ApoE delivers essential lipids, e.g. cholesterol to neurons via LDLR. PCN incubated with PTEN siRNA-LNP (100 ng/mL siRNA) for 72 h showed > 90% knockdown (qPCR, WB). Gene knockdown was sustained for 21 days after a single treatment. PTEN siRNA-LNP (100 ng/mL) mediated > 80% knockdown in PCN (DIV 6, 9, 13 and 16) at 72 h (qPCR). PCN incubated with 500 ng/mL GFP mRNA-LNP for 72h showed > 95% GFP positive neurons (Flow cytometry). The tested RNA-LNP were well tolerated (no difference in LDH signal compared to untreated control). A single intracortex of siPTEN-LNP (500nL at 5mg/mL siRNA/10mins) mediated > 85% knockdown in cortical slices 5 days post-injection (Western Blot). Conclusion RNA-LNP (SUB9KITS™) demonstrated ApoE-enhanced rapid uptake by neurons, which mediated effective, sustained and widespread modulation of a target gene expression both *in vitro* and *in vivo*, with no detectable toxicity at concentrations tested. This technology offers a a rapid and simple mechanism to manipulate gene regulation.

Disclosures: **E. Ramsay:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **C. Walsh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **A. Ansari:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **A. Thomas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **K. Ou:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **A. Wild:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **T. Leaver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **R.J. Taylor:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc. **D. Zwaenepoel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Precision NanoSystems Inc..

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.18/UU83

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: A Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Science, Sports and Culture of Japan. CREST, JST

Title: Evaluation of a novel reporter rat line which conditionally expresses red fluorescent protein (tdTomato)

Authors: *H. IGARASHI[1], K. KOIZUMI[2], R. KANEKO[3], K. IKEDA[4], H. ONIMARU[5], Y. YANAGAWA[3], S.-I. MURAMATSU[6], T. ISHIZUKA[2], H. YAWO[1,2];
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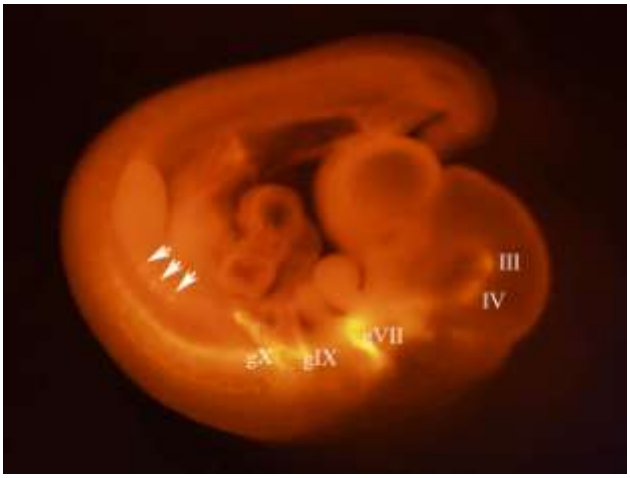
Abstracts: The Cre/loxP recombination system is one of the conditional chromosomal mimics to investigate systemic function of targeted genes (Rabbitts et al., 2001), and has been adopted to examine a function of specific gene. For *in vivo* experiments, rat offers potential advantages of larger body size and progressed ability to accomplish more complex behavioral task as compared to mouse. Here we evaluated a conditional reporter rat line which has red fluorescent protein (tdTomato) gene in the downstream of loxP-flanked STOP cassette. Firstly we injected AAV-Cre into striatum, hippocampus and cerebellum of the reporter rats to test the conditional expression of tdTomato. Each Cre-immuno-positive cells sparsely located in the injection part and merged with tdTomato. Secondly, site-specific expression was evaluated by *in utero* electroporation of AcGFP-NCre plasmid. Cortical layer 2/3 neurons were visualized by tdTomato fluorescence in the newborn pup. Thirdly, we evaluated this reporter system using *phox2b*-Cre driver rat, which specifically expresses Cre in cells of several hindbrain regions involved in the autonomic nervous system including neurons that are responsible for respiratory rhythm generation. When the double transgenic rats with *phox2b*-Cre and *floxed* tdTomato were examined at embryonic day (E)12.5, tdTomato was expressed in the neurons of parafacial respiratory group (pFRG), epibranchial ganglia, the forming oculomotor/trochlear nuclei and autonomic ganglia. The neuronal projection fibers were clearly visualized by tdTomato signal. It is suggested that the tdTomato was strongly expressed in neurons including Cre with high specificity. Our reporter rat would facilitate the neurophysiological studies and the connectomics of identified neurons which express Cre under a certain promoter. All animal procedures were

conducted in accordance with the guiding principles of Physiological Society of Japan and NIH.



Disclosures: **H. Igarashi:** None. **K. Koizumi:** None. **R. Kaneko:** None. **K. Ikeda:** None. **H. Onimaru:** None. **Y. Yanagawa:** None. **S. Muramatsu:** None. **T. Ishizuka:** None. **H. Yawo:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.19/UU84

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Restricted expression of transgene to astrocytes in the central nervous system following systemic injection with a novel self-complementary AAV9 vector

Authors: *J. DASHKOFF[1,2], E. HUDRY[2], S. TAKEDA[2], Z. FAN[2], N. TRUONG[2], C. A. MAGUIRE[3], B. T. HYMAN[2];
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Abstracts: Alzheimer's disease (AD) is a devastating neurodegenerative disease for which there is currently no cure. We previously demonstrated that expression of the *epsilon2* allele of human apolipoprotein E (*APOE*) via intraventricular injection of adeno-associated virus serotype 4 (AAV4) can reduce pathological processes in transgenic mouse models of AD slowing down the

progression of amyloid deposition and alleviating A-associated neurotoxicity *in vivo*. As an alternative preclinical approach to further enhance the safety and efficacy of this strategy, we engineered a novel self-complementary AAV9, scAAV9-GFA', designed to drive expression of a transgene specifically in astrocytes after peripheral intravascular infusion. Intravenous delivery of scAAV9-GFA' encoding green fluorescent protein led to robust and long-lasting transduction of astrocytes throughout the entire cerebral tissue, in the absence of neuronal transduction or more than trivial levels of peripheral expression of the transgene. scAAV9-GFA' led to transduction of ~10% of cortical astrocytes, two orders of magnitude higher levels than the comparable traditional ssAAV9 serotype. Both GFAPactivated and resting astrocytes expressed the reporter gene. These data suggest the potential of scAAV9-GFA' as a system to drive expression of therapeutic genes specifically in astrocytes of the CNS.

Disclosures: J. Dashkoff: None. **E. Hudry:** None. **S. Takeda:** None. **Z. Fan:** None. **N. Truong:** None. **C.A. Maguire:** None. **B.T. Hyman:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.20/UU85

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: ERC

Swedish Research Council

Wennegren Foundation

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Magn Bergwall Foundation

EU

Title: Understanding the genetic basis for functional heterogeneity in telencephalic interneurons

Authors: A. B. MUNOZ-MANCHADO, A. ZEISEL, S. LINNARSSON, *J. HJERLING LEFFLER, Dr;
Karolinska Institutet, STOCKHOLM, Sweden

Abstracts: The overarching goal is to discover and characterize the distinct transcriptional states that generate most of the distinct interneuron cell types in the mammalian neocortex. Despite a century of efforts, we have only a broad understanding of the cell types that make up the mammalian forebrain. For example, GABAergic interneurons are often classified by their expression of neurochemicals such as parvalbumin, somatostatin, calretinin and NPY, yet these classes overlap in complex patterns and still are not enough to describe the known functional diversity. During development the GABAergic interneurons are ventrally derived from the ganglionic eminences in the embryonic brain, which can be anatomically divided into lateral, medial and caudal parts (LGE, MGE and CGE respectively). The two latter regions give rise to non-overlapping cortical interneuron populations and the developmental origin remains the only unequivocal way to group cells. Nonetheless, there are distinct functions described for more than five different classes of interneurons and we are asking whether functional heterogeneity is reflected in reproducible gene-expression patterns. Furthermore, we know very little about the transcriptional states and chromatin organization that create and maintain distinct cell types. This lack of knowledge stymies efforts at large-scale brain connectivity mapping, since we do not fully understand the properties of the connected nodes and reduces reproducibility of experiments between labs. Recent progress in single cell RNA sequencing allow us to describe each cell individually in terms of RNA expression and thus address many of the questions mentioned above. We focus our effort in studying the cellular make up of interneurons from the somatosensory cortex and the striatum in p21-28 mice using the method described by the Linnarsson group (Islam et al., Nat Methods, 2012). We are validating our findings with immunohistochemistry and/or *in situ* hybridization. The cortical interneurons constitute a minority of all cortical cells, and to address this heterogeneous population in more detail, we use FACS isolation of, genetically labelled interneurons, based on developmental origin and/or the expression of markers. In order to infer functionality we will validate our findings using single-cell RT-PCR on electrophysiologically recorded neurons.

Disclosures: A.B. Munoz-Manchado: None. **J. Hjerling Leffler:** None. **A. Zeisel:** None. **S. Linnarsson:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.21/UU86

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Intramural Research Program

Title: Characterization of a transgenic rat expressing Cre recombinase from the dopamine transporter promoter

Authors: *Y.-J. ZHANG[1,2], C. T. RICHIE[1], L. R. WHITAKER[1], A. F. HOFFMAN[1], C. E. SPIVAK[1], C. R. LUPICA[1], H. A. BALDWIN[1], J. J. HINKLE[1], G. YEH[1], C. MEJIAS-APONTE[1], M. MORALES[1], S. M. UNDERHILL[3], J. C. SMITH[4], J. M. PICKEL[3], B. T. HOPE[1], B. K. HARVEY[1];
[1]NIDA IRP, Baltimore, MD; [2]NIAAA, Rockville, MD; [3]NIMH IRP, Bethesda, MD; [4]NINDS IRP, Bethesda, MD

Abstracts: The dopamine transporter (DAT) protein is a target of the widely abused psychostimulants cocaine and amphetamine, and loss of DAT expressing cells is often a marker of neurodegeneration associated with Parkinson's disease and methamphetamine abuse. Given the relevance of DAT-expressing neurons to rat models of addiction and neurodegeneration, we have generated and characterized three lines of transgenic Long Evans rats expressing Cre recombinase under the DAT promoter (DAT::iCre). The tissue-specific expression of Cre can be used in combination with Cre-dependent transgenes to obtain selective transgenesis in DAT(+) neurons. A bacterial artificial chromosome (BAC) containing the rat DAT gene was recombineered to replace the start codon of DAT with Cre recombinase. Pronuclear injections of DAT::iCre BAC into fertilized rat eggs ultimately resulted in three independent, phenotypically positive DAT::iCre lines. All three lines were immunoreactive for Cre recombinase in the midbrain and Cre-immunoreactivity colocalized with tyrosine hydroxylase-immunoreactivity (TH-IR), a marker for dopaminergic neurons. Injection of adeno-associated viral (AAV) vectors expressing Cre-dependent mCherry reporter protein resulted in expression restricted to TH-IR cells in the midbrain. *In situ* hybridization for DAT mRNA also colocalized with the Cre-driven mCherry. When DAT::iCre rats were crossed to DIO-mCherry (Cre-dependent) reporter rats, mCherry expression was primarily restricted to TH-IR cells in midbrain with few mCherry-labelled cells sparsely distributed in cortical regions including hippocampus. We also examined the effects of the genomic integration of the DAT::iCre transgene on endogenous DAT function and dopaminergic neuron phenotype. The presence of DAT::iCre did not alter endogenous levels of TH protein or TH-immunoreactivity in the striatum and midbrain compared to wild-type (WT) rats. Both basal levels of DAT and amphetamine-induced internalization of DAT in the midbrain were similar for WT and DAT::iCre rats. Fast-scan cyclic voltammetry was performed in brain slices from WT and DAT::iCre rats and no significant difference was observed in evoked dopamine release,dopamine uptake or concaine sensitivity in the dorsal striatum. *In vitro* intracellular recordings revealed no significant difference in firing and membrane properties of midbrain dopamine neurons from WT and DAT::iCre rats. The development of transgenic rat lines for targeting transgene expression to midbrain DAT-expressing neurons provides a promising approach to selectively asses the function of these neurons in animals models of drug abuse and neurodegeneration.

Disclosures: **Y. Zhang:** None. **C.T. Richie:** None. **L.R. Whitaker:** None. **A.F. Hoffman:** None. **C.E. Spivak:** None. **C.R. lupica:** None. **H.A. Baldwin:** None. **J.J. Hinkle:** None. **G. Yeh:** None. **C. Mejias-Aponte:** None. **M. Morales:** None. **S.M. Underhill:** None. **J.C. Smith:** None. **J.M. Pickel:** None. **B.T. Hope:** None. **B.K. Harvey:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.22/UU87

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Identification of astrocytes derived from neural progenitor cells by high-throughput screening using a live cell mRNA detection technology

Authors: *D. WELDON, V[1], Y. WILLIAMS[1], A. KO[1], V. KOONG[1], W. JASTROMB[2]; [1]EMD Millipore, Temecula, CA; [2]Nikon, Melville, NY

Abstracts: Neural progenitor cells (NPCs) contain the capability to differentiate into neuronal and glial cell lineages, and have been used by researchers to develop ways to repair the central nervous system following damage. One example is the derivation of astrocytes from NPCs, which is effective in generating functional terminally differentiated neuronal cells that can then be subjected to molecular and biological assays. Currently, astrocytes are mainly identified using intracellular antibody staining of proteins such as GFAP, which prevents those characterized cells from use in further experiments, due to the need to permeabilize the cells for successful staining. Here, we successfully demonstrate the detection of intracellular GFAP mRNA during the NPC-to-astrocyte differentiation process using a novel probe-based live-cell RNA detection technology, which can specifically measure RNA expression in whole cells without altering cell viability, function, or integrity. This allows researchers to not only monitor the regulation of specific mRNA during the course of the differentiation protocol, but also enables interrogated cells to be used in downstream cell-based functional assays. Here, we employed a high-throughput screening (HTS) method to screen our NPC-derived astrocytes expressing GFAP mRNA, which retained their biological function as evident in a subsequent migration assay. This enabled the quick and accurate identification of highest GFAP mRNA expressing cells, thus maximizing the likelihood of success in our downstream assay. The ability to monitor gene expression during NPC differentiation to astrocytes in a live cell setting not only removes the need to set up extra duplicate wells for downstream studies, but also enhances the relevance of

the data generated, due to the fact that the same cell is being tracked throughout the entire experimental workflow. Using live-cell RNA detection probes in HTS experiments not only enables researchers to vastly increase the number of cells that can be accurately interrogated at a time, but also allows them to retain their best candidate live samples for use in downstream functional assays, something that is not currently possible using traditional antibody-based methods.

Disclosures: **D. Weldon:** A. Employment/Salary (full or part-time):; EMD Millipore. **Y. Williams:** A. Employment/Salary (full or part-time):; EMD Millipore. **A. Ko:** A. Employment/Salary (full or part-time):; EMD Millipore. **V. Koong:** A. Employment/Salary (full or part-time):; EMD Millipore. **W. Jastromb:** A. Employment/Salary (full or part-time):; Nikon.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.23/UU88

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: MEXT 23700489

MEXT 24220008

MEXT 26830004

JSPS 235569

JSPS 256060

CREST

JST

Title: Dissecting multiple neuronal pathways in mammalian brain using Avian sarcoma and leukosis virus envelope-receptor pairs

Authors: *M. MATSUYAMA[1,2], Y. OHASHI[1], T. TSUBOTA[1], M. YAGUCHI[1], K. MAMADA[1], S. KATO[3], K. KOBAYASHI[3], Y. MIYASHITA[1];
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Japan; [3]Dept. of Mol. Genetics, Inst. of Biomed. Sciences, Fukushima Med. Univ., Fukushima, Japan

Abstracts: Methods for manipulating specific neural pathways are a powerful way to disentangle complex neural systems; however, conventional genetic tools (e.g., Cre/loxP and Tet system) cannot control gene expression in more than two neural pathways simultaneously. To overcome this limitation, we developed a "Gate-Tag vector system" using modified avian sarcoma and leukosis virus (ASLV) receptors and envelopes; this system simultaneously can be used to deliver three different genes into three separate cell targets. The vector system comprises two elements: a Gate vector expressing a specific receptor "Gate" on the target-cell surface and a Tag vector that enters the target-cell through the Gate. The specificity of the Gate-Tag vector system was confirmed by demonstrating the selective expression of fluorescent proteins only for specific receptor/envelope pairs both *in vitro* (>97% in HEK293T cells) and *in vivo* (>98% in rat brain). Moreover, when combined with highly efficient retrograde gene transfer (HiRet) (Hirano et al., 2013), this vector system achieved three distinct pathway-specific gene transfers of three different fluorescent genes into rat thalamocortical neurons. This system can be easily adapted to manipulate the function of selected neuronal pathways by expressing other genes such as those encoding neurotoxins and opsins. Thus, the Gate-Tag vector system is an independent, simultaneous, and specific genetic tool that can be used to dissect multiple neural pathways *in vivo*.

Disclosures: **M. Matsuyama:** None. **Y. Ohashi:** None. **T. Tsubota:** None. **M. Yaguchi:** None. **K. Mamada:** None. **S. Kato:** None. **K. Kobayashi:** None. **Y. Miyashita:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.24/UU89

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: R01 DA030807-01

R21 DA035577-01

Title: RiboTag: A novel method for measuring gene expression within discrete populations of primary neuronal cultures

Authors: *A. J. LESIAK, J. F. NEUMAIER;
Psychiatry and Behavioral Sci., Univ. of Washingotn, Seattle, WA

Abstracts: Measurement of dynamic changes in gene expression within cellular sub-types from a diverse cellular milieu has been a significant challenge in neuroscience; however recent techniques have been developed to resolve this issue. The HA-tagged RPL22 ribosomal protein (RiboTag) construct was originally developed for translational profiling in animal models and allows for the isolation of ribosome-bound mRNA transcripts specifically within the cells in which the RiboTag construct is expressed; however it may also be a useful tool for numerous *in vitro* applications. Primary neuronal cultures are a commonly used tool for the study of a variety of neurophysiological processes, but experimental analysis of gene expression within these cultures is often confounded by low transfection efficiency and co-culturing with non-neuronal cell types. To determine if RiboTag expression can address this problem, we prepared primary cultures and co-transfected RiboTag plasmids and various experimental plasmids using lipofection. 24-48 hours later, sample lysates were collected, and a small portion of this lysate was reserved as an in-well representation of mRNA within the entire sample (input sample) prior to isolation of RiboTag complexes using magnetic bead immunoprecipitation (pull-down sample). RiboTag pull-down yielded a significant enrichment of co-transfected genes relative to the input control. When a neuron-specific promoter was used to drive RiboTag expression selectively in neurons, mRNA recovered by RiboTag pull-down was enriched for neuronal markers and absent of glial markers, while the input samples contained both neuronal and glial transcripts. Co-transfection of RiboTag with sh-RNA constructs was also used to quickly and easily confirm sh-RNA knockdown of endogenous genes despite low transfection rates. Lastly, we demonstrate how RiboTag can be used to measure the transcriptional and translational effects of pharmacological and cell signaling events using DREADD-receptors, which are selectively activated by clozapine-N-oxide (CNO), an otherwise inert ligand. Treatment of neurons with forskolin induced an equivalent increase in cFos expression in both the pull-down sample and input sample relative to unstimulated controls; however, in Gs-coupled DREADD expressing neurons cFos expression was induced specifically in the pull-down sample but not in the input sample following CNO-treatment. In summary, RiboTag represents a convenient but powerful tool to isolate mRNA within a specific sub-set of cultured cells, and provides a tool for analyzing the translational profile accompanying a variety of neurophysiological events.

Disclosures: **A.J. Lesiak:** None. **J.F. Neumaier:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.25/UU90

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: The creation and characterization of Cre Rat Drivers

Authors: Z. LIU, *X. CUI;
Res. & Develop., SAGE Labs, Saint Louis, MO

Abstracts: The Cre-loxP system has been widely applied in the mouse, providing accurate spatiotemporal control of gene expression. Yet its adaptation in the rat, the preferred model system for neuroscience research, has been hindered by technical challenges until the recent but rapid development of nuclease-mediated site-specific genome editing technologies, such as zinc finger nucleases and CRISPR/Cas9, allowing efficient site-specific insertion of Cre expression cassette and loxP sites (Brown et al., 2013). Cre rat drivers will undoubtedly be of greatly impact, especially on the fast rising field of optogenetics. However, few Cre rats have been generated to date, preventing the rat from reaching their full potential as research models. We have reported previously the creation and preliminary characterization of two Cre rats: Th-Cre and Dat-Cre. Here we present further characterization of these two lines, including demonstration of the neuron-specific expression of Cre in different lines by using a combination of immunohistochemistry and mRNA fluorescence *in situ* hybridization (FISH) as well as the confirmation of Cre-dependent excision of the floxed alleles in cells harvested with laser-assisted microdissection and discuss breeding strategies. Additionally, we report our progress on the effort to expand the Cre-loxP rat tool box by creating six more Cre driver rats and three reporter lines. In the Cre rats, 2A-Cre will be under the control of Tph2, Sst, Slc32a1, Calb2, VIP and HTR3A promoters from each respective endogenous locus. A conditional fluorescent reporter and two conditional opsin (excitatory channelrhodopsin2 or inhibitory Halorhodopsin) expression cassettes will each be inserted to the readily targetable rat Rosa26 locus to obtain Cre-dependent expression. We hope these rat lines will not only provide the research community useful tools but encourage researchers to join force to make more Cre rats. Reference Brown, A., Fisher, D., Kouranova, J., McCoy, A., Forbes, K., Wu, Y., Ji, D., Henry, R., Chambers, A., Shu, W., Weinstein, E. J., Cui, X. (2013) Whole rat conditional knockout via genome editing. Nature Meth. 10, 638-640.

Disclosures: Z. Liu: None. **X. Cui:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.26/UU91

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Core Research for Evolutional Science and Technology of Japan Science and Technology Agency

the Uehara Memorial Foundation

Title: Improvement of gene transduction efficiency for neuron-specific retrograde gene transfer lentiviral vector with a novel fusion envelope glycoprotein

Authors: *K. KOBAYASHI[1], S. KATO[1], K. KOBAYASHI[2];
[1]Dept Mol Genet., Fukushima Med. Univ., Fukushima 960-1295, Japan; [2]Sec Viral Vector Dev, Natl. Inst. Physiol Sci., Okazaki 444-8585, Japan

Abstracts: Viral vectors for retrograde gene transfer mediate the delivery of transgenes into neuronal cell bodies innervating the region around the injection site of the vectors. Pseudotyping of a human immunodeficiency virus type 1 (HIV-1)-based lentiviral vector with different types of fusion envelope glycoproteins composed of rabies virus glycoprotein (RVG) and vesicular stomatitis virus glycoprotein (VSVG) domains produces a high efficiency of retrograde gene transfer. The vector for neuron-specific retrograde gene transfer (NeuRet) is a pseudotype of HIV-1 vector with fusion glycoprotein type C (FuG-C), which consists of the N-terminal region of the extracellular domain of RVG and a short C-terminal part of the extracellular domain (membrane proximal region) and the transmembrane/cytoplasmic domains of VSVG. The NeuRet vector has a high efficiency of retrograde gene delivery and transduces only neuronal cells around the injection site. In the present study, we tested gene transfer of HIV-1 lentiviral vectors pseudotyped with various types of fusion glycoproteins, in which the junction of RVG/VSVG segments diverged in the C-terminal portion of their extracellular domain. We found a novel type of fusion glycoprotein, termed type E (FuG-E), that displayed improved efficiency of retrograde gene transfer compared with the NeuRet vector having FuG-C. This FuG-E-pseudotyped vector transduced selectively neuronal cells around the injection site, showing the property of the NeuRet vector. Our NeuRet vector system with FuG-E will provide a powerful tool for gene therapeutic trials of neurological and neurodegenerative diseases and for the study of the mechanisms of neural networks underlying a variety of brain functions.

Disclosures: K. Kobayashi: None. **S. Kato:** None. **K. Kobayashi:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.27/UU92

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Swedish Research Council

Title: A novel method to uniquely label transcripts for massively parallel functional dissection in the mammalian brain

Authors: *T. BJORKLUND[1], M. DAVIDSSON[1], O. SCHWICH[1], P. ALDRIN-KIRK[1], P. DIAZ-FERNANDEZ[1], M. TORROBA CALVO[1], L. QUINTINO[2], C. LUNDBERG[2]; [1]Mol. Neuromodulation, Wallenberg Neurosci., Lund, Sweden; [2]CNS Gene Therapy, Wallenberg Neurosci. Ctr., Lund Univ., Lund, Sweden

Abstracts: Sequencing by synthesis technologies have enabled cost-effective deep transcriptome sequencing and has also triggered the development of novel molecular technologies to prepare and amplify mRNA from a single cell. We have taken these technologies and utilized them to develop a novel platform for massively parallel functional dissection *in vivo*. Through the implementation of linear insertion of unique identifiers (a.k.a barcodes) in viral vector plasmids, we can identify and quantify large numbers (≈3E5 unique clones) if functionally different vector clones in the same animal. This has been combined with a novel technology to randomly fragment a longer genetic fragment and insert each subfragment into either a lentiviral or AAV backbone. We here show that the barcodes in libraries produced through this technique are entirely orthogonal and that thus multiple libraries can be studied in the same animal (up to four studied in parallel to date). This platform can potentially be utilized to dissect a plethora of biological functions such as promoter and enhancer regions, 3' and 5' UTRs and more. Here we will present a proof-of-principle study where both lentiviral and AAV vectors are barcoded and utilized to study mRNA processing and trans-splicing in the mouse and rat brain.

Disclosures: **T. Bjorklund:** None. **M. Davidsson:** None. **P. Aldrin-Kirk:** None. **O. Schwich:** None. **P. Diaz-Fernandez:** None. **M. Torroba Calvo:** None. **L. Quintino:** None. **C. Lundberg:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

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Program#/Poster: 657.28/VV1

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: KAKENHI 26860157

KAKENHI 26118507

KAKENHI 26640041

KAKENHI 26293046

KAKENHI 23115103

Title: Trans-synaptic retrograde transportation of Cre recombinase

Authors: K. SHIMIZU[1], A. INUTSUKA[2], A. INUI[2], S. OHNISHI[1], *A. YAMANAKA[2]; [1]Dept. of Med., Nagoya Univ., Nagoya, Japan; [2]Res. Inst. of Envrn. Medicine, Nagoya Univ., Nagoya, Japan

Abstracts: Past studies have identified specific neuronal populations in the brain according to their genetic property such as expressing neurotransmitters. However, these genetically identified neuronal populations sometimes consist of subpopulations which have distinctive projections and functions. Although it is easy to trace forward axons from specific neuronal populations, it is difficult to trace back the upstream neurons and manipulate their activity selectively. In this study, we developed Cre recombinase fused with tetanus toxin fragment C (Cre-TTC) which can be expressed by adeno-associated virus (AAV) vectors. TTC is used for neuronal tracing, taking its advantage of retrograde trans-synaptic transport. To examine their retrograde transport, we selected dopamine neurons in the ventral tegmental area (VTA) and the substatia nigra pars compacta (SNc). It is known that dopamine neurons in the VTA project to the nucleus accumbens (NAc), while dopamine neurons in the SNc project to the caudate putamen (CPu). When we injected Cre-TTC vector in the CPu and FLEX-GFP vector in the VTA-SNc region, we detected GFP only in the SNc not in the VTA. On the other hand, when we injected Cre-TTC vector in the NAc and FLEX-GFP vector in the VTA-SNc region, we observed GFP only in the VTA not in the SNc. These findings show that Cre-TTC is trans-synaptically and retrogradely transported from projected neurons and induce Cre-dependent gene expression in upstream

projecting neurons. Our new tool provides projection pathway selective tracing and manipulation.

Disclosures: **K. Shimizu:** None. **A. Inutsuka:** None. **A. Inui:** None. **A. Yamanaka:** None. **S. Ohnishi:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.29/VV2

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Bio-Rad

Title: Rapid and ultra-sensitive single-cell transcript profiling with Droplet Digital PCR - application to neuronal differentiation

Authors: ***C. LITTERST**, S. WANG, T. LEGLER, N. KLITGORD, E. HEFNER, Y. JOUVENOT, G. KARLIN-NEUMANN;
DBC Bio-Rad, Pleasanton, CA

Abstracts: Single-cell transcript profiling is undoubtedly the ideal approach for gene expression analysis in neuronal tissues composed of various cell types. However, the robust detection of transcripts in isolated single cells or cytoplasm samples is technically challenging. Droplet Digital PCR (ddPCR) developed at Bio-Rad Digital Biology Center directly counts individual molecules with superior precision and reproducibility. The ddPCR-based single-cell gene expression protocol measures transcripts at molecular levels with minimal sample processing for defined targets. Furthermore, ddPCR is performed in 96-well plates and is well-suited to high throughput studies of focused sets of genes in large numbers of single cells. In this work, we demonstrate the single-cell gene expression analysis of *in vitro* differentiated neuronal cells. We present a simple and robust workflow for profiling multiplexed, transcript targets in flow-sorted, neuronal single-cells. We characterize a panel of validated assays targeting stem cell, proliferation and differentiation marker genes including nanog, p21, and Synapsin, respectively. We compare expression levels of these genes in non-differentiated versus differentiated single cells and bulk RNA preparation from the same cell populations prior to sorting. We demonstrate that ddPCR provides absolute counts of transcripts from >100,000 copies to <10 copies per cell. Our findings are discussed with current data in the literature.

Disclosures: **C. Litterst:** A. Employment/Salary (full or part-time):; Bio-Rad. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Bio-Rad. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bio-Rad. **S. Wang:** None. **T. Legler:** None. **N. Klitgord:** None. **E. Hefner:** None. **Y. Jouvenot:** None. **G. Karlin-Neumann:** None.

Poster

657. Techniques for Profiling and Manipulating Defined Neuronal Populations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 657.30/VV3

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Howard Hughes Medical Institute

Title: Co-opting fluorescent proteins for cell-specific gene manipulation

Authors: ***C. TANG**[1,2], T. SZIKRA[3], Y. KOZOROVITSKIY[1,2], M. TEXEIRA[3], B. SABATINI[1,2], B. ROSKA[3], C. CEPKO[1,2];
[1]Harvard Med. Sch., Boston, MA; [2]Howard Hughes and Med. Inst., Chevy Chase, MD; [3]Neural Circuit Laboratories, Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland

Abstracts: Detailed understanding of neural circuits and dynamics underlying animal behavior would be greatly facilitated by the ability to manipulate gene expression and neural activity in any desired cell type. However, it remains a challenge to achieve cell-specific gene manipulation in many model systems, especially the mouse nervous system. Fluorescent proteins are commonly incorporated into transgenic organisms for labeling specific cell populations, but the unmodified forms cannot control biological activities. Using GFP-binding proteins derived from Camelid antibodies, we co-opted GFP as a scaffold for inducing formation of biologically active complexes, developing hybrid transcription factors and split-Cre recombinases that control gene expression only in the presence of GFP or its derivatives. The modular design of the hybrid transcription factors allows for variation in key properties such as DNA specificity, transcriptional potency, and drug dependency. Production of GFP controlled cell-specific gene expression and facilitated functional perturbations in the mouse retina. Further, retrofitting existing transgenic GFP mouse and zebrafish lines for GFP-dependent transcription enabled applications such as optogenetic probing of neural circuits. This work establishes GFP as a multifunctional scaffold and opens the door to selective manipulation of diverse GFP-labeled

cells across transgenic lines. This approach may also be extended to exploit other intracellular products as cell-specific scaffolds in multicellular organisms.

Disclosures: **C. Tang:** None. **T. Szikra:** None. **Y. Kozorovitskiy:** None. **M. Texeira:** None. **B. Sabatini:** None. **B. Roska:** None. **C. Cepko:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 658.01/VV4

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Research Incubator Award from the Duke Institute for Brain Sciences.

Title: *In vivo* bioluminescence-driven optogenetics for neuronal activation and inhibition

Authors: *K. A. CLISSOLD, K. BERGLUND, M. E. KLEIN, V. PREVOSTO, M. KOVAL, Z. M. ABZUG, M. A. SOMMER, H. H. YIN, U. HOCHGESCHWENDER;
Psychology and Neurosci., Duke Univ., Durham, NC

Abstracts: Chemical genetic approaches for neuronal manipulation are advantageous for their minimal invasiveness and their ability to influence entire neuronal populations in large brain structures, while optogenetic approaches are advantageous for their ever-expanding diversity and temporal precision. To combine and complement the advantages of each technique, we recently developed a hybrid technology that allows control of a neuron by both chemical genetic and optogenetic means: luminescent opsins. These “luminopsins” are an engineered chimeric fusion of a light-generating enzyme (luciferase) to a light-activated opsin. Luminopsins enable tonic activation or silencing of neurons by bioluminescence upon application of a BBB-permeable substrate, coelenterazine (CTZ), while maintaining the ability to control the neurons rapidly with conventional light sources such as lasers. Here we investigated this approach for its efficacy *in vivo*. To activate a population of neurons, we developed an AAV vector containing a human Synapsin promoter-driven Gaussia luciferase fused to Volvox channelrhodopsin 1 (VChR1). To inhibit neurons, VChR1 was replaced by a light-driven proton pump, Maculans opsin (Mac). Virus was unilaterally injected into the substantia nigra pars reticulata (SNr) of the mouse. After incubation of 4-6 weeks, CTZ was applied directly to the SNr via micro-injections or injected peripherally in tail veins. Effects were compared to laser activation through an optic fiber implanted in the SNr. Upon activation or inhibition of SNr neurons by bioluminescence, the mice

showed ipsilateral or contralateral turning behavior, respectively. These results are consistent with previous research on the motor effects of unilateral activation or inhibition of the SNr. Using the same virus, we also evaluated the efficacy of luminopsins in non-human primates, Macaca mulatta, whose brain size has been the major obstacle for conventional optogenetics. These results demonstrate the first *in vivo* applicability of bioluminescence-driven excitation and silencing of neurons utilizing Gaussia luciferase.

Disclosures: K.A. Clissold: None. **K. Berglund:** None. **M.E. Klein:** None. **V. Prevosto:** None. **M. Koval:** None. **Z.M. Abzug:** None. **M.A. Sommer:** None. **H.H. Yin:** None. **U. Hochgeschwender:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

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Program#/Poster: 658.02/VV5

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 2R56NS045130

Michael J Fox Foundation

Brain Research Foundation

Title: Conventional invasive and next-generation non-invasive optogenetic modulation of the thalamic reticular nucleus

Authors: *B. HIGASHIKUBO[1,2], S. CRANDALL[1], B. CONNORS[1], U. HOCHGESCHWENDER[3], C. MOORE[1];
[1]Brown Univ., Providence, RI; [2]MIT, Cambridge, MA; [3]Duke Univ., Durham, NC

Abstracts: The thalamus relays sensory information from the periphery to the cortex, and thalamocortical dynamics are central to normal perception and to a number of pathological states. The thalamic reticular nucleus (TRN) provides inhibitory input to thalamic relay nuclei and is thus poised to gate the transmission of sensory information and to modulate activity in the thalamocortical circuit. The involvement of the TRN in diverse processes like sleep and epilepsy makes it an attractive target for modulation in experimental and clinical contexts. We have developed techniques for manipulating TRN activity using conventional optogenetics as well as a novel, non-invasive method using bioluminescence to drive optogenetic elements. In order to

characterize the response of TRN neurons to standard optogenetic drive, we combined optical stimulation and extracellular recording in a transgenic mouse expressing the light-sensitive ion channel ChR2 in GABAergic neurons. In a population of ~100 well isolated TRN units, we parametrically mapped the influence of stimulus duration on spike and burst probability. In addition, we characterized the influence of stimulus variation on the generation of persistent oscillatory activity in the thalamocortical circuit. Subsequent work utilized a non-invasive method of stimulating optogenetic elements using bioluminescent light production. In this preparation, a "luminopsin" protein consisting of Gaussia luciferase tethered to Volvox channelrhodopsin-1 was virally expressed in the TRN of wild-type mice. This luminopsin enables optogenetic activation with application of the luciferase substrate, coelenterazine (CTZ). *In vitro* whole cell recordings in brain slices showed consistent small depolarization in expressing cells when CTZ was applied to the bath. *In vivo* multielectrode recordings showed an increase in TRN spike rate after systemic CTZ delivery. Simultaneously recorded putative relay neurons decreased overall firing rate, while still firing bursts. This work is proof of concept for the use of luminospins to modulate neural activity, and could provide a means to optogenetically influence thalamocortical circuits without external light delivery.

Disclosures: B. Higashikubo: None. **S. Crandall:** None. **B. Connors:** None. **U. Hochgeschwender:** None. **C. Moore:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

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Program#/Poster: 658.03/VV6

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: 25640024

25290014

Title: Development of Tango system for the monitoring of 5-HT2CR activity

Authors: *Y. WATANABE, A. TSUJIMURA, M. AOKI, K. TAGUCHI, M. TANAKA; Basic Geriatrics, Kyoto Pref. Univ. Med., Kyoto, Japan

Abstracts: 5-HT2C receptor (5-HT2CR) is a member of the seven transmembrane-spanning G-protein coupled receptors (GPCRs) and couples to Gq/11 that subsequently activates

phospholipase C. It is widely distributed in the central nervous system and is involved in the regulation of anxiety, mood, and feeding. 5-HT2CR is known to undergo RNA-editing at five sites of second intracellular loop by adenosine deaminases acting on RNA (ADARs). 5-HT2CR RNA-editing leads to decrease in 5-HT potency, agonist binding affinity, constitutive activities, and G protein coupling activity. To date, the measurement of 5-HT2CR activity has been performed by G-protein dependent functional assays, such as inositol-1,4,5-trisphosphate (IP3) production assay and calcium flux assay. Although these assays are excellent methods, they are not appropriate for high-throughput drug screening (HTS) and application to *in vivo* live-imaging. Now, we are developing a novel method of 5-HT2CR monitoring to solve these problems. Tango system is a powerful tool for GPCRs assays based on ligand binding to a specific GPCR that triggers desensitization, a process mediated by the recruitment of arrestin to the activated receptor. For validation of 5-HT2CR Tango system, we constructed the following plasmids: pEF1_5HT2CR-TEVc-LexA-2A-arrestin-TEVp and pLexOP-EGFP. A cleavage site (TEVc) fused to the C-terminus of 5-HT2CR is cleaved by the TEV protease-tagged arrestin (arrestin-TEVp), resulting in the release of LexA transcriptional factor. Released LexA is translocated to the nucleus and induces EGFP-expression driven by the LexA promoter. These two plasmids were transfected into 293 cells, and EGFP expression was monitored with a fluorometer. We verified that 5-HT induced the expression of reporter gene in a dose-dependent manner and that its expression was inhibited by the 5-HT2CR antagonist SB242084. Moreover, this system was able to detect the difference of 5-HT2CR activities among RNA-editing isoforms (VGV and INI). These results suggest that our system will be useful for live-imaging of 5-HT2CR activity and HTS for drug discovery.

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Poster

658. Techniques to Image or Modulate Neural Activity

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Program#/Poster: 658.04/VV7

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: DARPA N66001-11-C-4016

Title: Optogenetic-induced glutamate release in the rodent hippocampus and frontal cortex

Authors: *E. MILLER[1], F. POMERLEAU[1], P. HUETTL[1], J. E. QUINTERO[1], S. R. BATTEN[1], J. S. BECKMANN[2], Y. AI[1], M. LUNDBLAD[3], J. JAKOBSSON[3], R. E. HAMPSON[4], S. A. DEADWYLER[4], G. A. GERHARDT[1];
[1]Anat. and Neurobio., [2]Psychology, Univ. of Kentucky, Lexington, KY; [3]Exptl. Med. Sci., Lund Univ., Lund, Sweden; [4]Physiol. & Pharmacol., Wake Forest Univ., Winston-Salem, NC

Abstracts: Specific, targeted control of neural systems to establish causality between neuronal activity and behavior has remained difficult to achieve until the recent advancements in optogenetics, which introduces light sensitive proteins (opsins) into neurons that regulate transmembrane ion conductance. Electrophysiological studies have shown that optical excitation or inhibition of neuronal activity is correlated with behavior, but to date, very few studies have examined neurotransmitter release combined with optical stimulation. We have combined our expertise of direct electrochemical measurements of neurotransmitter release (glutamate) *in vivo* with optogenetics in order to examine glutamate dynamics in the CNS. We infused (1 µl/each) AAV5-Syn-ChR2-EYFP into the right dentate gyrus (DG) of the hippocampus and the left infralimbic (IL) region of the frontal cortex. Histological analysis using yellow fluorescence revealed that, 5 weeks post-infusion, EYFP was present throughout the left frontal cortex and right hippocampus. There was also some evidence of bilateral distribution. We attached an optical fiber (200 µm o.d. ~200 µm from the recording sites) to our ceramic-based microelectrode array (MEA) configured to directly record tonic and phasic glutamate transients and lowered the assembly in DG or IL. We used constant light activation (DC: 488 nm, 1 to 10 mW) or pulses (train (TR): 10 ms; 40 Hz) to directly observe light-dependent glutamate release. Glutamate dynamics were in the same range as we have previously reported using other forms of stimulation (high potassium, drug induced or behavior). We observed glutamate release in the range of 1 to 70 µM and uptake rates of 0.1 to 10 µM/sec. These results show the feasibility of directly measuring glutamate release *in vivo* while controlling glutamatergic systems using optogenetics.

Disclosures: E. Miller: None. **F. Pomerleau:** None. **P. Huettl:** None. **J.E. Quintero:** F. Consulting Fees (e.g., advisory boards); Consultant for Quanteon LLC. **S.R. Batten:** None. **J.S. Beckmann:** None. **Y. Ai:** None. **M. Lundblad:** None. **J. Jakobsson:** None. **R.E. Hampson:** None. **S.A. Deadwyler:** None. **G.A. Gerhardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of Quanteon LLC.

Poster

658. Techniques to Image or Modulate Neural Activity

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH/NIMH Grant 1R01MH085500-01

NIH/NIMH Grant 1R01DA028298-01

Title: High level expression of genetic tools with improved cell type specificity

Authors: *L. MADISEN[1], A. GARNER[1], A. CHUONG[2], N. KLAPOETKE[2], L. LI[1], A. CHENG[1], B. TASIC[1], H. GU[1], M. MILLS[1], T. NGUYEN[1], T. KNÖPFEL[3], E. BOYDEN[2], R. C. REID[1], H. ZENG[1];
[1]Allen Inst. for Brain Sci., SEATTLE, WA; [2]MIT, Cambridge, MA; [3]Imperial Col. London, London, United Kingdom

Abstracts: The ongoing development of increasingly sensitive optical molecules allows for probing and perturbing neuronal states with unprecedented control. Despite their evident potential, the practical utility of such tools relies on their high-level expression in restricted cell populations. We previously generated a Rosa26-targeted expression platform that directs Cre-dependent expression of functionally high levels of fluorescent reporters, biosensors and actuators in transgenic mice. Although this system enables study and manipulation of Cre-driver defined cell populations, sporadic or transient expression of the Cre-driver during development and/or expression of the driver in multiple cell types in the adult often preclude limiting transgene expression to singular cell types. In addition, for optimal application of some genetic tools, even higher levels of expression than reached in our Rosa26 reporters would be advantageous. Accordingly, we continue to work on strategies to generate genetic tools with increased specificity and level of expression. As intersectional approaches for gene regulation offer the potential for increased specificity, we've generated Rosa26-targeted reporters that require co-expression of multiple site-specific recombinases, as well as a Cre-dependent reporter targeted to the neuronally-limited Snap25 locus. Analyses of informative double and triple transgenic mice demonstrate that both approaches achieve more precise expression of transgenes than previously obtainable. To achieve higher levels of reporter expression, we've targeted alternative genomic loci with constructs of various designs, including a Cre and tetO regulated reporter based in an alternative genomic locus known as TIGRE. Notably, we've found that our new TIGRE reporter directs robust expression of fluorescent markers, voltage and calcium sensors, and novel optogenetic tools to levels higher than those obtained using our comparable Rosa26-based reporters. Functional characterizations of TIGRE lines carrying different types of optical tools under Cre and tetO control demonstrate their utility for high performance studies of neuronal activity, both in tissue slices and *in vivo*. Our data establish this new reporter strategy as

a useful foundation for robust and highly-regulated transgene expression, enabling more effective labeling, activity monitoring, and activity control.

Disclosures: **L. Madisen:** None. **A. Garner:** None. **A. Chuong:** None. **N. Klapoetke:** None. **L. Li:** None. **A. Cheng:** None. **B. Tasic:** None. **H. Gu:** None. **M. Mills:** None. **T. Nguyen:** None. **T. Knöpfel:** None. **E. Boyden:** None. **R.C. Reid:** None. **H. Zeng:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

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Program#/Poster: 658.06/VV9

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Recent developments for neurotransmitter analysis

Authors: M. EYSBERG, *L. M. VAN HEERWAARDEN, H.-J. BROUWER, N. REINHOUD; Antec, Zoeterwoude, Netherlands

Abstracts: In the field of neurotransmitter analysis, there is a continuing demand for faster and more sensitive analyses. -Concentrations of some neurotransmitters from particular brain regions are very low. -There is a demand for measurements in ever smaller sample volumes. -In each sample the levels of many components are of interest. The recent development of a new electrochemical detector (DECADE Elite) and a new electrochemical flow cell (Sencell) in combination high efficiency separation columns makes it possible to improve the methods for neurotransmitter analysis. We present an overview these methods for analyzing neurotransmitters in microdialysate samples with the ALEXYS Neurotransmitter Analyzer. The DECADE Elite with an increased data rate and high temperature fully supports fast UHPLC separations. The Sencell from Antec has an Adjustable Spacer Technology (ATS) that enhances the sensitivity and improves detection limits. Optimized applications have been developed for the following neurotransmitters in microdialysate samples: -Monoamines -Acidic metabolites -Acetylcholine -Amino acids glutamate and GABA The ALEXYS Neurotransmitter Analyzer is a versatile and flexible UHPLC system dedicated to analyzing a wide range of neurotransmitters in very small samples and at low concentrations.

Disclosures: **M. Eysberg:** None. **L.M. Van Heerwaarden:** None. **H. Brouwer:** None. **N. Reinhoud:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: The Ottawa Hospital Foundation

NSERC

CIHR

CRC

Title: Optogenetics manipulation of cortical and subcortical neurons in the macaque monkey. Assessing expression of excitatory and inhibitory opsins

Authors: *B. W. CORRIGAN[1], R. A. GULLI[2], J. C. MARTINEZ[2], A. SACHS[3];
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Abstracts: Electrical stimulation has been the gold standard for control of neural circuits in clinical practice such as deep brain stimulation (DBS) in the treatment of Parkinson's Disease and depression. Optogenetics is an alternative method of manipulation of neural circuits, with the added benefits of targeting genetically and spatially defined cell groups, and avoiding the use of immunoreactive metal electrodes in favour of inert optic fibres. Using this method, it is possible to either excite or inhibit different light-sensitive opsins and proton pumps with millisecond time resolution. Research using this technique for treatment of clinically relevant rodent models is progressing rapidly, but much more research is needed in primate models before therapeutics can be developed for humans. We carried out injections in three different *Macaque* monkeys, targeting both cortical and subcortical areas and compared results of optical stimulation/electrophysiological recording, and transfection. In one female *Macaca fascicularis*, we conducted visually guided injections under anaesthesia of the excitatory opsin ChR2 (*Lenti-hThy-1-ChR2(H134R)-eYFP*) into the prefrontal cortex. During *in vivo* recordings, we measured increases in firing rates of up to 70 times above baseline. We also transfected the prefrontal cortex of a male *Macaca mulatta* with the inhibitory opsin ArchT (*AAV2-CaMKIIa-ArchT-eYFP*). These injections were done through a recording chamber in the awake, behaving monkey using a microinjectrode, allowing for electrophysiological identification of target region. During subsequent recordings, target neurons were effectively silenced during optical stimulation. We

carried out transfection of deep brain structures using a frameless stereotax system to target the MR-identified regions of a female *Macaca fascicularis,* including the basal ganglia: the striatum (*Lenti-hThy-1-ChR2(H134R)-eYFP*) and the medial septum(*Lenti-hThy-1-eNpHR3.0-eYFP*). Despite the similarity of the delivered viral envelopes, immunohistological analyses of these regions showed different transfection patterns, suggesting that different brain structures can express different patterns when the same viral vector is used. We are currently using this frameless stereotaxic system to transfect neurons of the subthalamic nucleus (STN) of a female *Macaca fascicularis* using *AAV2-CaMKIIa-ArchT-eYFP* and *AAV2-CaMKIIa-hChR2(H134R)-EYFP*. We will also test the effects of optical excitation and inhibition of the STN on behaviour by measuring saccade accuracy and latency. This is an important structure in the pathology of Parkinson's disease and an important target for DBS.

Disclosures: B.W. Corrigan: None. **R.A. Gulli:** None. **J.C. Martinez:** None. **A. Sachs:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

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Support: Wellcome Trust Grant 094385/Z/10/Z to KT

Royal Society International Joint Project to KT and DO

Title: Development of a new fast-response GCaMP3 family for monitoring calcium flux *in vivo*

Authors: *N. HELASSA[1], E. ESPOSITO[2], I. CONTE[1,3], T. CARTER[1,3], J. BRADLEY[2], D. OGDEN[2], K. TOROK[1];
[1]St George's Univ. of London, London, United Kingdom; [2]Lab. de Physiologie Cérébrale, CNRS and Univ. Paris Descartes, Paris, France; [3]MRC-National Inst. for Med. Res., London, United Kingdom

Abstracts: Green Fluorescent-Calmodulin Proteins (GCaMPs) have been the reporters of choice for visualizing neuronal network activity *in vivo* as they can monitor Ca^{2+} flux that accompanies membrane depolarisation and action potentials. GCaMPs are based on a circularly permutated EGFP molecule (cpEGFP) flanked at the N and C termini by the smooth muscle myosin light chain kinase derived RS20 peptide and calmodulin (CaM), respectively. Upon Ca^{2+} binding, the

formation of a tight complex between RS20 and CaM, stabilises the deprotonated form of cpEGFP inducing a fluorescence enhancement. However, the slow Ca^{2+}-response kinetics of the current GCaMPs does not make them suitable for measuring high frequency Ca^{2+} transients e.g. action potentials. To accelerate the Ca^{2+}-response kinetics of GCaMP3, we decreased the binding affinity of the Ca^{2+}.CaM.RS20 complex by point mutations in the EF-hands of CaM[1] (mutants EF-1 to EF-4) and in the RS20 target peptide sequence[2] (mutant RS-1). Newly engineered GCaMP3 proteins were characterised in terms of dynamic range, two-photon cross-section, Ca^{2+} affinity and association and dissociation kinetics. Ca^{2+} responses and dynamic range *in vivo* were tested in endothelial cells (HUVEC) stimulated by ionomycin. Dissociation constants (K_d) for Ca^{2+} obtained from the equilibrium Ca^{2+} binding experiments were in the μM range (0.5-5.6 μM) with Hill coefficients from 2 to 5. The fastest mutant GCaMP3 RS-1 EF-3 showed half times ($t_{1/2}$) for Ca^{2+} decay decreasing with temperature ($t_{1/2}$ of 10.1 ms at 20°C to $t_{1/2}$ of 2.5 ms at 37°C), which is 52-fold faster than GCaMP3. Fluorescence changes on Ca^{2+} association were highly cooperative and characterized by a rate limiting conformational change. The association rates of GCaMP3 wild-type were not temperature-dependent ($t_{1/2}$ for Ca^{2+} rise of 36.0 ± 1.0 within the 20-37°C range). However, GCaMP3 RS-1 EF-3 showed $t_{1/2}$ for Ca^{2+} rise from 4.5 ms at 20°C to 0.9 ms at 37°C, which is 38-fold faster than GCaMP3. Two-photon cross-sections and fluorescence responses to Ca^{2+} influx of mutated GCaMPs in endothelial cells were comparable to those of GCaMP3 wild-type. The systematic mutations employed accelerated the Ca^{2+} response kinetics of GCaMPs to be suitable for monitoring fast, high frequency Ca^{2+} transient, and the principles established can be applied to develop new GCaMPs with fast-response and low affinity properties. [1]Jama A et al. JBC, 2011, 286:12308-12316 [2]Török K and Trentham DR. Biochemistry, 1994, 33:12807-12820

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Poster

658. Techniques to Image or Modulate Neural Activity

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Program#/Poster: 658.09/VV12

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH

HHMI

Title: Elucidating input-output relations of VTA dopamine neurons

Authors: K. BEIER[1], K. MIYAMICHI[2], L. SCHWARZ[2], R. C. MALENKA[3], *L. LUO[2]; [1]Stanford University, Stanford Univ. Sch. of Med., STANFORD, CA; [2]Stanford Univ., STANFORD, CA; [3]Stanford Univ. Sch. of Med., STANFORD, CA

Abstracts: The ventral tegmental area (VTA) has been suggested to contain functionally heterogeneous dopamine (DA) neurons encoding signals that include reward, novelty, salience, motivation, and aversion. While initial studies suggested that DA neurons themselves were a homogenous population that exhibited phasic excitation in response to rewards and cues that signaled rewards, subsequent research showed that subpopulations of VTA DA neurons that project to different target areas exhibit different anatomical, molecular and electrophysiological properties. A thorough investigation of these subpopulations has been hampered by a paucity of tools capable of granting genetic access to anatomically-defined neural populations intermingled in heterogeneous brain structures. This limitation has made it challenging to definitively identify functional roles for distinct VTA DA neuron populations in signaling reward and aversion *in vivo*. Particularly useful would be the ability to concurrently map both input and output connectivity of each subpopulation. Classic neuroanatomical techniques have provided invaluable information about the connectivity of the brain, but are limited by an inability to identify direct synaptic connections or distinguish cell types. Recent rabies virus tracing experiments have identified direct synaptic input onto VTA DA neurons originating from over fifty distinct anatomical structures, suggesting that DA neurons receive input from surprisingly broad and diverse populations. In addition, more recent studies suggest that unique VTA neuron subtypes, defined by projection site, receive distinct inputs. However, the global connectivity of unique neuron types in the VTA has not been examined. Here, we employed a novel technique we developed called TRIO (trace the relationships between input and output) to more thoroughly study the connectivity of VTA DA neurons. TRIO combines rabies virus mediated retrograde transsynaptic tracing with a helper virus that is transduced via the axon terminals, such that we can examine input to VTA DA neurons that project to a specific brain region. We will report our systematic examination of global similarities and differences in input connectivity between VTA DA neuron subpopulations defined by their output sites. This approach has the potential to yield fundamental insights into the architecture of the midbrain dopamine system.

Disclosures: K. Beier: None. **K. Miyamichi:** None. **L. Schwarz:** None. **R.C. Malenka:** None. **L. Luo:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 658.10/VV13

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Wellcome Trust 095667, 095668

Title: Imaging cortical sensory dynamics in a transgenic mouse expressing a voltage indicator

Authors: *D. SHIMAOKA[1], L. ROSSI[1], T. SATO[1], A. BENUCCI[1], T. KNÖPFEL[2], L. MADISEN[3], H. ZENG[3], M. CARANDINI[1];
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Abstracts: Genetically-encoded voltage indicators (GEVIs) enable chronic imaging of selected neuronal populations across large portions of cortex, with fine temporal resolution. GEVIs, however, have hitherto required viral delivery or *in utero* electroporation, which are invasive and result in local expression. The Allen Institute recently developed a transgenic mouse (Ai78) that promises to overcome these limitations, as it expresses a FRET-based GEVI, VSFP-Butterfly 1.2. To test these new transgenic mice we imaged their cortical responses using wide-field optical imaging. Using a dual recombinase intersectional approach, we crossed Ai78 mice with Camk2a-tTA and Rasgrf2-Cre lines to express VSFP-Butterfly 1.2 in excitatory neurons of layer 2/3. For comparison, we also imaged the cortical responses of C57BL/6 mice electroporated *in utero* at E15.5 with the same VSFP construct. We implanted the adult mice with a head plate and a coverslip over thinned skull, and then repeatedly imaged the underlying cortex while the mice were head-fixed and awake. We exposed a large region of dorsal part of cortex (8x6 mm) to image somatosensory, auditory, and visual cortex. Voltage signal was estimated by taking the ratio between signals from the acceptor (594 nm) and the donor (543 nm) fluorophores on a single-trial basis. The transgenic mice showed similar voltage response amplitude (S/N = 8.5 ± 1.8 (S.E.), n = 3) compared to the electroporated mice (S/N = 5.5 ± 0.5, n = 3), and the signal could be obtained in a broader region of cortex including visual, auditory, somatosensory and motor areas. By presenting visual flickering bars we mapped the retinotopic organization in visual areas V1, LM, and AL. Trains of tones revealed tonotopic organization in at least 2 auditory areas. Trains of air puffs directed to the whiskers revealed activation in both barrel cortex and motor cortex, consistent with studies based on classical voltage-sensitive dyes. Response latency was examined by analysing the phase of oscillatory responses to the visual flickering bars or trains of air puffs or trains of tones. Visual latencies were in the order of 50 ms, with earliest responses seen in V1 and later responses in higher visual areas. Much shorter latencies were seen in somatosensory cortex (~5 ms) and in auditory cortex (~10 ms). We conclude that Ai78 mice provide a promising transgenic tool for GEVI imaging. This tool allows for noninvasive, genetically targeted monitoring of neuronal voltage activity at mesoscopic spatial scales and millisecond temporal scales.

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Poster

658. Techniques to Image or Modulate Neural Activity

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: CONACyT grant: 105 807

Title: High temporal resolution method for glutamate quantification in hippocampal slices

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Abstracts: Neurotransmitters quantification in specific regions of the central nervous system provides important information for linking specific regions with chemical and electrophysiological events. The available methodologies do not have the needed temporal resolution. Therefore, it is essential to design a method for accurately measurement of these compounds in brain tissue with high specificity and temporal resolution. We developed a method for accurate measurement of glutamate (Glu) in brain slices microregions that can be correlated to simultaneous electrical recordings. We obtained hippocampal slices of 400 micron thick of brains Wistar male rats. The slice was placed and stabilized in a dual superfusion chamber and DL-TBOA (DL-Theobenziloxyaspartate) 10 mM was administered, Glu concentrations and the electrical activity were simultaneously evaluated. This method consists in continuous collection of samples every 200 milliseconds from the hippocampal slice with a tubing probe (200 μm diameter). Sample collection was simultaneously mixed with an enzymatic reactor prepared to measure Glu concentration. The mixture was introduced into a fused silica tubing that was inside of an incubation chamber at 37 °C to carry out the reaction. A fluorescence cell was placed at the end of the tubing. The resorufin obtained as a product of reaction was excited with a laser beam (532 ηm). Fluorescent signal was taken at 590 ηm. Signal intensity was correlated with Glu concentration. Data show that DL-TBOA administration produces and increased of 25.00% in glutamate concentrations in the hippocampal slice compared to the basal concentration. With respect to the electrical recordings an increase of 217% in the amplitude after DL-TBOA administration appeared. The frequency remained without any evident change. The Glu increase

was directly correlated with the change in electrical activity produced by DL-TBOA administration. It can be concluded that using this technique, it is possible to quantify glutamate concentrations in a near real time resolution way that can be correlated with changes in the electrical activity.

Disclosures: C.L. Lopez Valenzuela: None. **A. Morales-Villagrán:** None. **K. Pardo-Peña:** None.

Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

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Program#/Poster: 658.12/VV15

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Intramural Research Program, NINDS-NIH

Title: Development of transgenic marmosets using nonsurgical embryo collection for imaging neural activity *in vivo*

Authors: J. E. PARK[1], S.-H. CHOI[1], X. F. ZHANG[1], T. P. SANTISAKULTARM[2], E. SASAKI[4,5], J. PICKEL[3], *A. C. SILVA[2];
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Abstracts: An attractive way to study brain function is to use optical imaging techniques such as Confocal and Two-Photon Microscopy, which allow simultaneous sampling of hundreds of fluorescently tagged neurons. Recently, genetically encoded calcium indicators of the GCaMP family have been synthetized. When expressed in excitable cells of the brain, these molecules fluoresce upon calcium binding, becoming a visible marker of neural activity. While these molecules can be delivered to discrete areas of the brain via local injection of a viral vector, it would be extremely valuable to create a transgenic animal in which these molecules were expressed ubiquitously in neurons all over the brain. The common marmoset (Callithrix jacchus) is an important nonhuman primate animal model in neurophysiological research because its brain organization resembles that of humans. Lately, the successful generation of transgenic marmosets with germline transmission of the transgene has boosted the attractiveness of this species as a valuable animal model for biomedical research. In this work, we aimed to develop

transgenic marmosets expressing GCaMP for imaging neural activity *in vivo*. High-titer lentiviral vectors expressing GCaMP5G and GCaMP6S under control of the CMV promoter were designed. Previously, we determined that these vectors, when injected stereotaxically in the brain, produced high levels of expression and mostly expressed in neurons. For non-surgical embryo collection and transfer, female marmosets greater than two years old were used. Ovarian cycles were controlled by a prostaglandin F2 alpha analogue and monitored by measurements of serum progesterone. We recovered up to 81.6% (31/38) of the ovulated embryos and repeated the flushing up to 10 times in the same animals. Repeated uterine flushing did not affect the integrity of the reproductive tract as well as quality or quantity of the embryos produced. Lentiviruses encoding EGFP, GCaMP5G or GCaMP6S were injected into the perivitelline space. Injected embryos were transferred to 2, 4 and 6 surrogate mothers, respectively. Four of them (one EGFP, two GCaMP5G, and one GCaMP6S) were diagnosed to be pregnant by ultrasonography around 21 days after embryo transfer. The EGFP recipient had a miscarriage around day 40 of pregnancy. The other three recipients are doing well and we are waiting for the pups to be born in GCaMP5G and GCaMP6S group. These results are important steps in developing the transgenic marmosets that robustly express calcium sensors for functional optical imaging of neural activity.

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Poster

658. Techniques to Image or Modulate Neural Activity

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Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 658.13/VV16

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant GM103503

Title: A novel upright low-dose brain imager: A new tool in neuroscience and its potential applications

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Abstracts: Positron Emission Tomography (PET) is traditionally used to image patients in the supine or prone position, and only few research devices allow for upright, brain dedicated imaging. Here, we present a novel device that allows for functional brain imaging in a freely-moving upright individual. This imager is extremely compact and fits tightly around the head, allowing for both the usage of lower radiation dose and the potential for longitudinal imaging. **Methods:** Four (4) individuals already scheduled for F^{18}-FDG PET/computed tomography (CT) imaging as part of their standard diagnostic package volunteered to also be imaged with the Helmet_PET wearable imager. In order to simulate the lower injected dose that this device can tolerate, the Helmet_PET imaging was performed 4 hours post F^{18}-FDG injection (lower dose by radioactive decay). Brain images were first obtained while patients were seated comfortably, and then when they rotated their head continuously by +/- 45^0. **Results:** The obtained images showed reasonable agreement with standard PET images with respect to the FDG distribution pattern in pronounced brain regions. In our ROI analysis, it was shown that there was only a 9.9% (Head of Caudate/Putament), 8.55% (Thalamus), and 4.74% (Medial Occipital) difference between the clinical images and the images obtained by Helmet_PET in the indicated ROI's (averages among patients). In addition, the metabolic activation recorded by Helmet_PET also corresponded to the anatomic structure as shown by standard CT. **Conclusion:** The results presented in this pioneer upright brain imaging study show that low-dose PET pattern imaging in the upright position is feasible. Implications of this study suggest that low-dose longitudinal PET brain imaging in the upright position is possible, which could produce numerous research or clinical opportunities. In regards to the clinical perspective, this type of imager could assess the progress of post-stroke patients to see how well they have regained function over a series of time points. Different types of rehabilitation modalities could be compared for improvement efficacy, as well as neural plasticity, connectivity, or neurotransmitter modulation as measured by PET. On the basic research side, the motion tolerance of the device would allow one to better elucidate neural mechanics of motor and posture-related processes. In addition, we would be able to image less predictable emotional states without needing to return to baseline or to suppress related motion (e.g. laughter, crying, facial expression), and with the participant more naturally engaged in social and emotional scenarios in real or virtual reality settings.

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Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 658.14/VV17

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Sweetie: An optimized genetically-encoded fluorescent sensor for glucose

Authors: *J. P. KELLER[1], J. MARVIN[2], E. SCHREITER[2], L. L. LOOGER[2];
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Abstracts: Because of glucose's central role as the primary physiological source of energy, it is difficult to overstate the need for a clear and detailed understanding of glucose trafficking and usage to all branches of biological and medical sciences. To address this need, we created, optimized, and benchmarked several versions of genetically-encoded fluorescent protein sensors, all with large dynamic ranges and high specificity for glucose. We describe herein the methods used for initial design of both intracellular and extracellular versions of the sensor with both ratiometric and non-ratiometric capabilities, as well as strategies for optimization of dynamic range, tuning of affinity (Kd), and improvement of membrane targeting of the extracellular version of the sensor. Regarding specificity, assays for cross-reaction with glucose-related compounds determined that the highest affinity among them was galactose, whose affinity was ~100-fold weaker than that of glucose, thus demonstrating the sensor's high specificity for glucose. Regarding dynamic range, the df/f of the sensor was found to be ~3, which was robustly detectable in all of experimental settings tested. To further broaden the sensor's utility, we used targeted mutations to produce sensors with Kd's of approximately 10 uM, 250 uM, and 3 mM, allowing for measurements across a broad range of glucose concentrations. The last of these is intentionally approximately equivalent to the physiological serum glucose concentrations in many organisms, thus enabling continuous glucose monitoring (CGM) in living subjects when the extracellular version of the sensor is used. To improve this extracellular version, we optimized its membrane targeting through the insertion of endoplasmic reticulum export tags, which significantly improved targeting in all cell lines tested. Beyond *in vitro* benchmarking experiments, we also present herein examples of *in vivo* real-time imaging of glucose fluctuations, thus demonstrating the utility of the sensor in living subjects. We believe that these genetically-encoded glucose sensors will prove valuable tools for the scientific, medical, and pharmaceutical communities, especially when used in concert with the increasingly-powerful genetic manipulation techniques coming to fore.

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Poster

658. Techniques to Image or Modulate Neural Activity

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Topic: C.07. Epilepsy

Support: T32HD046388

Title: Optogenetic control of seizures through the nigrotectal pathway

Authors: *P. A. FORCELLI, C. SOPER, C. KULICK, S. GUTHERZ, J. ACCARDI, K. GALE;
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Abstracts: Optogenetic methods offer high levels of spatiotemporal, cell-type specific, and pathway specific methods for neuronal stimulation and silencing. Previous studies examining optogenetic methods of seizure control have focused on suppressing activity at the site of seizure initiation. Clinically, however, the site of seizure initiation is often unknown or multifocal. For this reason, exploiting brain networks that have broad-spectrum anticonvulsant effects (e.g., endogenous circuits that can impede pathological network synchronization) is an important new direction. For this purpose, we examined the nigrotectal pathway; pharmacological suppression of activity within substantia nigra pars reticulata (SNpr) or enhancement of activity within the deep and intermediate layers of superior colliculus (DLSC) has been previously shown to suppress a wide variety of seizure types. However, pharmacological manipulations do not allow for a high degree of temporal resolution, nor do they allow for an analysis of the contributions of specific terminal fields. To test the efficacy of optogenetics manipulations of this circuit, we injected rAAV-hSyn-ChR2 into DLSC to mediate activation or rAAV-hSyn-ArchT into SNpr into SNpr to mediate silencing. Fiber optic cannula were placed either at the site of virus injection (DLSC or SNpr) or in DLSC after virus injection into SNpr. Seizures were evoked by either: pentylenetetrazole (which activates both forebrain and hindbrain seizure networks), focal disinhibition of area tempestas (a site in piriform cortex that selectively evokes forebrain seizures), or by gamma butyrolactone (a drug that triggers thalamocortical, absence-like spike and wave seizures). Recordings of multi-unit activity within DLSC were used to confirm the efficacy of optogenetic activation. We found that activation of DLSC at high frequencies (constant stimulation or 100Hz stimulation) or low frequency (5Hz) was highly effective at reducing all seizure types examined. Similar patterns of seizure protection were found after optogenetic silencing of SNpr. Finally, preliminary results suggest that selective silencing of nigrotectal terminals within DLSC is sufficient to attenuate evoked seizure responses. These data demonstrate that optogenetic manipulation of the nigrotectal pathway can be exploited *in vivo* to control seizures evoked from diverse brain networks. This raises the prospect that optogenetic manipulation of endogenous seizure-controlling circuitry may hold promise for therapeutic intervention in epilepsy.

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Poster

658. Techniques to Image or Modulate Neural Activity

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: KAKENHI 25880021

Waseda Univ Grant 2013A-6503

Title: Optimization of micropattern geometry for long-term culture of isolated neurons and identification of excitatory-inhibitory cell types

Authors: *H. YAMAMOTO[1,2], S. KONO[4], T. KUSHIDA[4], A. HIRANO-IWATA[2], M. NIWANO[3,2], T. TANII[4];
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Abstracts: Using a micropatterned substrate as a culture scaffold, single neurons can be arrayed with controlled axon-dendrite orientation [1,2]. The neurons can further be wired *in vitro* using laser-assisted surface modification techniques [3]. Such surface engineering will allow us to construct a small neuronal network with pre-defined signal propagation direction. One major obstacle that hindered the progress of this work was the early death of neurons isolated on micropatterns. Thus we studied the geometrical factors that accelerate death of neurons and optimized the pattern geometry for long-term culture. Micropatterns of cell-permissive polylysine were fabricated in a polyethylene glycol background on a glass substrate by electron-beam lithography. A micropattern consisted of a circular island for soma adhesion, a single long pathway, and three short pathways: this pattern has been successful in directing axon-dendrite polarity of a single neuron, with the axon growing on the longest pathway [2]. Embryonic rat hippocampal neurons or cortical neurons were cultured on the patterned substrate, and axon lengths of each cell were monitored from 2 to 7 DIV. After 7 days, the cells were fixed and doubly stained for MAP2 (neuronal marker) and GAD67 (GABAergic neuron marker) to identify excitatory and inhibitory neurons. We found that constraint of axon elongation and soma

spreading was the primary cause of accelerated cell death, and that ~70% viability can be achieved at 7 DIV by optimization of micropattern geometry. Taking advantage of this pattern, we studied axon growth rate of micropattered cortical neurons to find that excitatory and inhibitory cells can be identified based on axon length by setting an appropriate threshold length. [1] Stenger DA et al. (1998) Microlithographic determination of axonal/dendritic polarity in cultured hippocampal neurons. J Neurosci Meth 82:167-173. [2] Yamamoto H et al. (2012) Differential neurite outgrowth is required for axon specification by cultured hippocampal neurons. J Neurochem 123:904-910. [3] Yamamoto H et al. (2011) In-situ guidance of individual neuronal processes by wet femtosecond-laser processing of self-assembled monolayers. Appl Phys Lett 99:163701.

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Poster

658. Techniques to Image or Modulate Neural Activity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 658.17/VV20

Topic: A.04. Stem Cells

Title: Toward efficient production of transgenic marmosets

Authors: *J. OKAHARA[1], H. OKANO[2], E. SASAKI[3];
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Abstracts: The common marmoset (marmoset) is a non-human primate, which is able to generate transgenic non-human primate with germ line transmission, which would be a useful and powerful experimental animal for neuroscience as well as human neuronal disease models. Moreover, their small body size, easier handling than macaque and fecundity are useful characteristics as a lab animal. Currently, 10 independent transgenic marmoset lines have been generated by lenti-viral vector method. In this method, to avoid obtaining non-transgenic animals by transgene integration failure, fluorescent protein marker gene is introduced with objective transgene, the fluorescence from which is to be confirmed around 8 cell stages before transplantation into the uterus of surrogate mother. While producing 10 transgenic marmoset lines including high cognitive disorder disease models it seemed that the promoters and fluorescent proteins showed tendencies to affect embryonic development. In this study,

influences of promoters and fluorescent proteins on the marmoset embryonic development were analyzed. Five promoters, EF1α (Elongation Factor1α), CAG (a modified chicken beta-actin promoter with CMV-IE enhancer), CMV (Cytomegarovirus), Synapsin (Syn), EOS (Early Transposond Sox2 enhancers), and 5 fluorescence protein markers, Green Fluorescent Protein (GFP), Venus, Kusabira Orange (KO), monomeric Red Fluorescent Protein (mRFP) and mCherry, were analyzed for influences on the embryonic development. The data set of total 444 transferred embryos to surrogate mothers and 23 newborn babies were used for Chi-square test analysis. As the results, the efficiency of generation rates of transgenic marmoset pups were 2.6% (EF1α), 7.0% (CMV), 4.1% (EOS), 8.8% (CAG) and 7.7% (Syn), respectively. The Chi-square showed that birth rates were no differences among the promoters. On the other hand, the efficiency of birth rate of transgenic marmoset pups in each fluorescence proteins marker was 9.4% (GFP), 7.7% (Venus), 5.4% (KO), 1.6% (mRFP) and 0% (mCherry) respectively. This result indicated that the transgenic birth rates were affected by fluorescent protein ($P< 0.05$). From these results, selection of fluorescent protein markers would be one of critical point for efficient transgenic marmoset production for neuroscience models.

Disclosures: **J. Okahara:** A. Employment/Salary (full or part-time):; full. **H. Okano:** A. Employment/Salary (full or part-time):; full. **E. Sasaki:** A. Employment/Salary (full or part-time):; full.

Poster

659. Optogenetics: Integration With Electrophysiology

Location: Halls A-C

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Program#/Poster: 659.01/VV21

Topic: G.04. Physiological Methods

Support: NIH R01 Grant MH085159

Title: Dopamine kinetics in discrete larval *Drosophila* brain regions

Authors: *E. PRIVMAN, J. VENTON;
Dept. of Chemistry, Univ. of Virginia, Charlottesville, VA

Abstracts: Dopamine has many evolutionarily conserved functions and neurotransmission mechanisms. Nanomolar concentration of monoamine neurotransmitters such as dopamine can be detected at a millisecond time scale in the *Drosophila* melanogaster central nervous system using an electrochemical technique known as fast scan cyclic voltammetry (FSCV). This study

uses a newly described modified channelrhodopsin called CsChrimson to stimulate release from dopaminergic neuronal subpopulations. CsChrimson is activated by red light avoiding the heat, tissue damage, and electrode surface changes caused when using conventional blue-light activated channelrhodopsins. Dopamine release is stimulated with 4 ms red light pulse trains to mimic the physiological phasic burst firing patterns of neurons. FSCV is used to monitor real time extracellular dopamine concentrations at the tip of an implanted 7 um carbon fiber electrode. The release of dopamine at a pulse frequency range of 10 to 120 Hz is tested in the protocerebrum and ventral nerve cord (VNC) of an *ex vivo* larval central nervous system (CNS) preparation. The subsequent uptake is modulated using the amine transporter blocker nisoxetine and uptake changes are quantified using Michaelis-Menten kinetics. Increasing the number and frequency of red light pulses predictably increased the extracellular dopamine concentration. We found that Vmax and Km are significantly different in the protocerebrum and ventral nerve cord of the larval *Drosophila*, as is seen with different brain regions in mammals. Nisoxetine was shown to act as a competitive inhibitor at the *Drosophila* dopamine transporter, as it does for its proposed evolutionary relative the human norepinephrine transporter. This novel combination of optogenetic and electrochemical techniques allows us to study the effects of basic pharmacological and genetic manipulations on dopamine neurotransmission in disparate brain regions of the *Drosophila*.

Disclosures: **E. Privman:** None. **J. Venton:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

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Topic: G.04. Physiological Methods

Support: New York Stem Cell Foundation-Robertson Award

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Google

DARPA HR0011-14-2-0004

NSF Center for Brains, Minds and Machines

IET Harvey

NSF Cognitive Rhythms Collaborative

Title: The sinusoidal probe- towards translation in the non-human primate

Authors: *H. SOHAL[1,2], F. YOSHIDA[1,2], N. P. BICHOT[1], K. PAYER[3], G. RIGGOT[3], E. S. BOYDEN[2,1], R. DESIMONE[1];
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Abstracts: Micromotion, attributable to the modulus mismatch between the moving brain and electrode materials, is a fundamental phenomenon contributing to generalized electrode failure for chronic brain implants. This failure hampers our ability to dissect neural circuits over chronic experimental paradigms and the ability to produce reliable signals for invasive brain-machine interfaces. We have recently shown that the sinusoidal probe is effective as a chronic implant, providing high fidelity, stable, neural recordings for up to two years with reduced overall gliosis when compared to conventional electrodes (Sohal et al, 2014), perhaps due to its flexibility, and thus intrinsic micromotion reducing measures. Such measures included a rounded recording tip, a sinusoidal shaft and a polyimide ball-anchor. The sinusoidal shaft accommodates the brain movement, while the recording sites are anchored in place with the ball anchor. All measures attempt to restrict electrode recording site movement relative to the surrounding brain tissue. The original probe was a proof-of-concept therefore comprised of a limited number of electrode recording sites and restricted to certain lengths. Further, the probes were optimized for chronic recordings in rodents, rather than non-human primates. Now, we have designed and microfabricated the next generation of the sinusoidal probe, substantially increasing the number of recording sites and allowing for multi-depth recording. Lengths of these novel probes, based on the original design, vary from 3-30 mm allowing for the specific targeting of brain regions in both rodents and non-human primates in highly customizable, 3D arrangements. Further, we have optimized insertion procedures, which may allow for the implantation of more sinusoidal probes using a single carrier, hence minimizing vasculature damage during the insertion process due to repeated brain penetrations. Here we report data from initial bench testing and implantation for the sinusoidal probe in the non-human primate. The initial results suggest that the sinusoidal probe can be successfully translated from rodents to accommodate the technological challenges associated with successfully implanting and chronically recording from the non-human primate brain with flexible devices. References: Sohal HS, Jackson A, Jackson R, Clowry GJ, Vaisilevskiy K, O'Neill A and Baker S (2014). The Sinusoidal Probe: a new approach to improve electrode longevity. Front. Neuroeng. 7:10. doi: 10.3389/fneng.2014.00010

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Poster

659. Optogenetics: Integration With Electrophysiology

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Program#/Poster: 659.03/VV23

Topic: G.04. Physiological Methods

Support: MIT Media Lab

Samsung Scholarship

MIT Synthetic Intelligence Project

New York Stem Cell Foundation-Robertson Award

Paul Allen Family Foundation

Simons Foundation

NIH 1R01DA029639

Title: Imaging from the inside out: A design of an implantable probe for imaging of single-cell physiological dynamics in deep brain tissue at large scales

Authors: *M. A. HENNINGER[1], Y.-G. YOON[1], J. DEGUCHI[1,2], J. SCHOLVIN[1], A. ZORZOS[1], R. HORSTMEYER[3], R. RASKAR[1], E. S. BOYDEN, III[1];
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Abstracts: We present the design of a system to image the neural activity of many individual neurons simultaneously, at arbitrary locations in the brain. We use numerical simulations to estimate the proposed system's performance, and predict how future advances would improve performance. Simulating widely available fluorescent reporters and current CMOS pixel designs, our results indicate that a microprobe shank (e.g., 10 microns thin, 60 microns wide) placed in the cortex could image the activity of many hundreds of neurons. While fluorescent indicators of physiological activity are powerful tools for monitoring brain activity, it remains difficult to use them to image the activity of individual cells at high speeds, especially in deep tissue. We propose a lensless microimager that is thin enough to implant with minimal tissue displacement. An array of such probes can record from many arbitrary sites in the mammalian brain. The system consists of a silicon probe that is densely arrayed with CMOS imaging pixels. On top of the CMOS pixels are a standoff layer, a light-modulating mask layer, and fluorescence emission

filters. This probe is implanted in conjunction with an excitation light delivery mechanism--such as a waveguide or optical fiber–to excite the fluorescent signal to be recorded. Whereas the lenses in a traditional imager alter the angles of incoming light such that the recorded image contains purely spatial information, our mask causes the imager to collect spatial and angular information, sampling from the 4D light field. The recorded angular information allows us to determine depth information; the imager records a full 3D image every frame. This reduces problems related to out of focal plane light without requiring techniques like confocal or two photon microscopy. While our technique does not allow diffraction-limited resolution, it does allow resolving to within a few microns, which is sufficient to correctly assign fluorescence to its cell of origin in most brain structures. Reconstructing the 3D volume from the recorded image can be cast as a straightforward linear algebra problem. While this problem appears to be underconstrained, there are several techniques for introducing additional information—such as sparsity, knowledge of cell locations, or positive-definiteness—that allow us to constrain the problem to a single valid solution. The end result is an activity trace for each neuron in the probe's field of view. We characterize the correlation between the fluorescent activity and the probe recording, and find that with full fluorescent labeling in the cortex, a single probe would record hundreds of neurons with over 90% fidelity to the underlying signal.

Disclosures: **M.A. Henninger:** None. **Y. Yoon:** None. **J. Deguchi:** None. **J. Scholvin:** None. **A. Zorzos:** None. **R. Horstmeyer:** None. **R. Raskar:** None. **E.S. Boyden:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 659.04/VV24

Topic: G.04. Physiological Methods

Support: HHMI

MIT Media Lab

Title: Red-shifted optogenetic neural manipulation in *Drosophila* melanogaster

Authors: S. S. KIM[1], S. R. PULVER[1], D. B. TURNER-EVANS[1], H. HABERKERN[1], R. FRANCONVILLE[1], A. S. CHUONG[2], N. C. KLAPOETKE[2], E. S. BOYDEN[2], *V. JAYARAMAN[1];

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Abstracts: Optogenetic tools have been used with considerable success in mammalian model organisms, but visual and thermal artifacts induced by activation light have limited their use in *Drosophila*. Here we introduce two new optogenetic reagents in *Drosophila*: CsChrimson and Jaws. CsChrimson is a red-shifted channelrhodopsin, a chimera between the N-terminus of CsChR and the transmembrane domain of Chrimson. It is 100 times more sensitive to orange light than the most sensitive variant of channelrhodopsin-2 previously used in *Drosophila*. With CsChrimson, we were able to activate neurons inside the brain of freely behaving flies through the cuticle. We have also used CsChrimson for other applications, including coarse functional connectivity mapping, triggering endogenous biogenic amine release, and neural stimulation using deep-red light (720nm) without visual-system-mediated behavioral artifacts. Jaws, a newly derived efficient red-shifted cruxhalorhodopsin, provides a complementary tool for neural inactivation. With CsChrimson and Jaws, the *Drosophila* research community now has a basic set of optogenetic tools for neural activation and inhibition with significantly higher temporal resolution than traditional thermogenetic tools such as dTrpA1 or Shibire(ts).

Disclosures: S.S. Kim: None. **V. Jayaraman:** None. **S.R. Pulver:** None. **D.B. Turner-Evans:** None. **H. Haberkern:** None. **R. Franconville:** None. **A.S. Chuong:** None. **N.C. Klapoetke:** None. **E.S. Boyden:** None.

Poster

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Google

NIH 2R44NS070453-03A1

NSF CBET 1053233

SkTech

NIH 1R01DA029639

Title: *In vivo* experimental testing of scalable 3-d microfabricated electrode array neural recording in mammalian brain

Authors: *J. P. KINNEY[1], J. BERNSTEIN[1], J. SCHOLVIN[1], C. MOORE-KOCHLACS[1,2], N. KOPELL[2], E. BOYDEN[1];
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Abstracts: We have developed (Scholvin et al., SFN 2012) 3-D micromachined silicon electrode arrays with ultradensely packed electrodes, targetable to defined sites distributed throughout the mammalian brain, and compatible with simultaneous use of optogenetic neural circuit perturbation. In order for such *in vivo* probes to be utilized in a scalable fashion, we have invented (Kinney et al., SFN 2012) a novel minimalist computer architecture for performing scalable neural signal acquisition, storage, and processing, appropriate for extracellular neural recording at large scale (i.e., in head-fixed animals in virtual reality environments). Finally, to enable the analysis of neural dynamics thus recorded, we have devised automated data analysis methods that can automatically spike sort -- unmixing signals acquired on our ultradense electrode arrays into well defined spike times attributed to individual source neurons, without human intervention, and with zero false positives, as assessed using modeled neural activity (Moore-Kochlacs et al., SFN 2013). Here we present the integration, and validation, of these three technologies - ultradense electrode arrays, minimalist computers for scalable data acquisition, and automated spike sorting -- into a scalable neural recording system for live mammals. We characterize the performance of our system to record neural activity on large numbers of densely packed electrodes simultaneously (e.g., aiming for 1000 channels of neural recording or more) in head fixed mice, aiming for both locally dense recording as well as recording from multiple sites at once. We also present strategies for optimally implanting and utilizing such probes, as well as strategies for technology dissemination to the neuroscience community.

Disclosures: J.P. Kinney: None. **J. Bernstein:** None. **J. Scholvin:** None. **C. Moore-Kochlacs:** None. **N. Kopell:** None. **E. Boyden:** None.

Poster

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Myhrvold Family Hertz Foundation Fellowship

New York Stem Cell Foundation-Robertson Award

NIH 1R01EY023173

Title: Mechanical and geometric principles of interfacing to the nervous system via the vasculature

Authors: *C. LINGHU[1], A. N. ZORZOS[1], G. T. FRANZESI[1], H. S. SOHAL[1], C. T. WENTZ[1], N. GROSSMAN[1], P. BLINDER[2], E. S. BOYDEN[1];
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Abstracts: Building a minimally-invasive chronic interface for recording and stimulating cells of the nervous system is of great importance to a broad range of topics in both basic neuroscience research and clinical treatment. In principle, threading electrodes through blood vessels could enable less invasive access to neurons. Many questions emerge from such a proposal: how much of the nervous system is accessible via introduction of probes into blood vessels? How many turns must probes make, and how much distance must they travel, to reach desired targets in the body from a given entry point? Should probes be actively steered or passively guided to targets? The answers to these questions could guide the development of practical probes for neural interfacing via the blood vessel network. Here we performed a theoretical analysis of the mechanical and geometrical constraints on neural interfacing via the vasculature. Using digitized structural data of neural vasculature and positional data of the neurons, we analyzed the statistical distribution of neuronal somata relative to vascular structures, as well as what fraction of the cells can be accessed from probes introduced at specific points in the vascular network. Based on fluid dynamic models of devices in the bloodstream, we performed Monte Carlo simulations to study the mechanical behavior of devices in the vasculature, as well as their influences on blood flow in vessels. These statistical and Monte Carlo simulations provide important mechanical and geometric constraints on how neurons could be interfaced to, via the vasculature.

Disclosures: **C. Linghu:** None. **A.N. Zorzos:** None. **G.T. Franzesi:** None. **H.S. Sohal:** None. **P. Blinder:** None. **E.S. Boyden:** None. **C.T. Wentz:** None. **N. Grossman:** None.

Poster

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Program#/Poster: 659.07/VV27

Topic: G.04. Physiological Methods

Title: Physical principles for brain activity mapping

Authors: ***A. H. MARBLESTONE**[1], B. ZAMFT[2], Y. MAGUIRE[2], M. SHAPIRO[3], T. CYBULSKI[4], J. GLASER[4], D. AMODEI[5], B. STRANGES[2], R. KALHOR[2], D. DALRYMPLE[6], D. SEO[7], E. ALON[7], M. MAHARBIZ[7], J. RABAEY[7], J. CARMENA[7], E. S. BOYDEN[8], G. CHURCH[2], K. KORDING[4];
[1]Harvard Univ., Boston, ; [2]Harvard Univ., Boston, MA; [3]Caltech, Pasadena, CA; [4]Northwestern Univ., Chicago, IL; [5]Stanford, Palo Alto, CA; [6]Nemaload, San Francisco, CA; [7]Univ. of California at Berkeley, Berkeley, CA; [8]MIT, Cambridge, MA

Abstracts: Simultaneously measuring the activities of all neurons in a mammalian brain at millisecond resolution is a challenge beyond the limits of existing techniques in neuroscience. Entirely new approaches may be required, motivating an analysis of the fundamental physical constraints on the problem. We outline the physical principles governing brain activity mapping using optical, electrical, magnetic resonance, and molecular modalities of neural recording. Focusing on the mouse brain, we analyze the scalability of each method, concentrating on the limitations imposed by spatiotemporal resolution, energy dissipation, and volume displacement. Based on this analysis, all existing approaches require orders of magnitude improvement in key parameters. Electrical recording is limited by the low multiplexing capacity of electrodes and their lack of intrinsic spatial resolution, optical methods are constrained by the scattering of visible light in brain tissue, magnetic resonance is hindered by the diffusion and relaxation timescales of water protons, and the implementation of molecular recording is complicated by the stochastic kinetics of enzymes. Understanding the physical limits of brain activity mapping may provide insight into opportunities for novel solutions. For example, unconventional methods for delivering electrodes may enable unprecedented numbers of recording sites, embedded optical devices could allow optical detectors to be placed within a few scattering lengths of the measured neurons, and new classes of molecularly engineered sensors might obviate

cumbersome hardware architectures. We also study the physics of powering and communicating with microscale devices embedded in brain tissue and find that, while radio-frequency electromagnetic data transmission suffers from a severe power-bandwidth tradeoff, communication via infrared light or ultrasound may allow high data rates due to the possibility of spatial multiplexing. The use of embedded local recording and wireless data transmission would only be viable, however, given major improvements to the power efficiency of microelectronic devices.

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Poster

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Topic: G.04. Physiological Methods

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NIH 1R01EY023173

New York Stem Cell Foundation-Robertson Award

Google

Title: Time course of subthreshold activity preceding spike generation in awake behaving mouse hippocampus

Authors: *A. C. SINGER[1], G. TALEI FRANZESI[1], S. B. KODANDARAMAIAH[1], M. TSITSIKLIS[1], S. SHARMA[1], D. BOZIC[1], S. BATIR[1], I. R. WICKERSHAM[1], G. L. HOLST[2], C. R. FOREST[2], C. BÖRGERS[3], N. J. KOPELL[4], E. S. BOYDEN[1];
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Abstracts: Neurons are often thought of as coincidence detectors that respond selectively to highly synchronized inputs. Indeed, research *in vitro* (e.g. in brain slices) has characterized how coincident synaptic inputs sum together within a neuron to drive well-timed spiking, and *in vivo* highly synchronized spiking across multiple presynaptic neurons is often assumed to drive activity in downstream neurons. However, patterns of inputs and intrinsic activity are very different between neurons *in vitro* and in awake behaving animals, leaving a major gap in our understanding of how neurons integrate incoming inputs and intrinsic activity to produce a spike in the awake brain. Accordingly, we have examined the time course of subthreshold depolarization preceding spiking in CA1 neurons in awake behaving mice. We performed whole cell patch clamp recordings using an optimized patch clamp robot (Awake Autopatcher) in head-fixed mice navigating through a virtual reality environment. We found that subthreshold depolarizations ramped up over extended periods - as much as fifty to a hundred milliseconds or more -- preceding the time of actual spike generation. Because these extended depolarizing ramps bring cells close to threshold, they could allow subsequent small inputs to rapidly result in spiking. Furthemore, these results may provide insight into the neural network patterns that drive individual neurons to fire in the living brain. (Singer and Talei Franzesi are co-first authors.)

Disclosures: A.C. Singer: None. **G. Talei Franzesi:** None. **S.B. Kodandaramaiah:** None. **M. Tsitsiklis:** None. **S. Sharma:** None. **D. Bozic:** None. **S. Batir:** None. **I.R. Wickersham:** None. **G.L. Holst:** None. **C.R. Forest:** None. **C. Börgers:** None. **N.J. Kopell:** None. **E.S. Boyden:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

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Human Frontiers Science Program

NIH 1R01DA029639

Title: Next-generation multiphoton optogenetic control via opsin engineering and computer generated holography

Authors: *E. RONZITTI[1], R. CONTI[1], N. KLAPOETKE[2], A. J. FOUST[1], E. PAPAGIAKOUMOU[1], E. S. BOYDEN[2], V. EMILIANI[1];
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Abstracts: The ability to perturb and manipulate the flow of excitation and inhibition, enabled by a rapidly developing repertoire of optogenetic actuators, is essential for elucidating causal relationships between neural circuit activity and function. Optogenetic tools have spurred a parallel revolution in optical technology to realize their full potential for brain circuit interrogation, specifically through the development of methods for light patterning. An ideal light delivery method should be: efficient, robust to scattering, span multiple spatial scales, and feature high spatial (micron) and temporal (millisecond) resolution. To accomplish these goals, the Emiliani laboratory utilizes computer generated holography (CGH), generalized phase contrast (GPC), and temporal focusing (TF) to send shaped single- and two-photon (2P) excitation volumes into neural tissue. Specifically, we have shown that wave front shaping, accomplished with a liquid crystal matrix, enables dynamic control of the light at the sample plane matching the geometry of structures or circuits of interest with micrometer lateral and axial resolution. Using these approaches we demonstrated efficient 2P optogenetic stimulation of single and multiple cell expressing ChR2, in culture and brain slices. One challenge to implementing parallel illumination methods for neural circuit stimulation is that available laser power is divided among selected targets. As a result, the number of opsin-expressing cells that can be stimulated in parallel is determined by a combination of total laser power, sensitivity and expression level of the specific opsin. Therefore, any improvement to opsin sensitivity can increase the number of cells that can be independently and simultaneously actuated with CGH patterns and reduce the total amount of light necessary for patterned photostimulation. The Boyden laboratory has recently demonstrated a series of new sensitivity-enhanced opsins including Chronos, Chrimson and CoChR. Here we characterize, in cultured cells and brain slices, the 2P action spectra and on/off kinetics of these new opsins under 2P holographic illumination and laser scanning excitation. We demonstrate that 2P CGH, combined with these new opsins, enables efficient generation of 2P photocurrents, highlighting the potential of this technology for *in vivo* cellular resolution optical stimulation of multiple targets in large 3D volumes.

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Poster

659. Optogenetics: Integration With Electrophysiology

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Topic: G.04. Physiological Methods

Support: NIH R01 DC009836

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NIH P30 DC5029

NIH T32 DC000038

HHMI International Student Research Fellowship

Title: Hearing the light: Perceptual and neurophysiological encoding of optogenetic stimulation delivered to the auditory midbrain

Authors: *A. E. HIGHT[1,2], W. GUO[1,5], J. X. CHEN[1,3], N. C. KLAPOETKE[6], B. G. SHINN-CUNNINGHAM[5], E. S. BOYDEN[6], D. J. LEE[1,4], D. B. POLLEY[1,5,4];
[1]Eaton-Peabody Labs., Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Speech and hearing bioscience and technology, [3]New Pathway MD Program, [4]Dept. Otology and Laryngology, Harvard Med. Sch., Boston, MA; [5]Ctr. for Computat. Neurosci. and Neural Technol., Boston Univ., Boston, MA; [6]McGovern Inst., MIT, Cambridge, MA

Abstracts: Optogenetics provides the means to manipulate specific neural circuits with high temporal precision. Neurons in the central auditory pathway encode temporal modulation of acoustic signals with submillisecond precision at rates as high as hundreds - or even thousands - of Hertz. Thus, to faithfully reconstruct auditory representations in the CNS, optogenetic stimulation must provide excitation that is both precise and fast. In this study, we used viral constructs to infect neurons in the mouse central nucleus of the inferior colliculus (ICc) with two types of channelrhodopsins: standard channelrhodopsin-2 (ChR2) or Chronos, an opsin recently demonstrated to have enhanced photosensitivity and rapid channel activation kinetics (Klapoetke 2014). *In vivo* extracellular recordings of ChR2+ ICc neurons revealed rapid spike adaptation to

stimulation rates > 40 Hz. By contrast, Chronos+ neurons synchronized spike timing to pulse rates as high as 140 Hz, with less synchronized, non-adapting responses at higher pulse rates. To address how these temporal coding differences might translate to perception, we trained mice having previously received ICc injections of ChR2+, Chronos+ or saline on a simple behavioral task in which they crossed sides of a shuttle box upon detecting pulse trains of acoustic or laser stimuli presented at various rates and amplitudes. As expected, the acoustic detection probabilities in all three groups were similar to one another and strictly dependent upon sound pressure level. Although saline-injected mice could not behaviorally detect the ICc photostimulation, we were surprised to discover that optogenetic detection functions between ChR2+ and Chronos+ mice were similar across amplitudes and pulse rates. To address how the psychometric functions derived from ChR2+ and Chronos+ mice could be so similar, yet their ICc spike coding be so different, we are now characterizing the downstream effects of ChR2 and Chronos activation in ICc on pulse rate encoding in the auditory cortex. Our preliminary findings suggest the precisely synchronized midbrain coding has been reformatted to a simplified rate code in the cortex that appears grossly similar between the two opsins, which may explain the similar behavioral detection functions. Our ongoing experiments address the hypothesis that the cortical encoding of ICc optogenetic stimulation with either opsin can predict the behavioral detection functions in individual mice.

Disclosures: **A.E. Hight:** None. **W. Guo:** None. **J.X. Chen:** None. **N.C. Klapoetke:** None. **B.G. Shinn-Cunningham:** None. **E.S. Boyden:** None. **D.J. Lee:** None. **D.B. Polley:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

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MIT MINT Program

Title: Optogenetic tool operation with extracellular vs. intracellular ionic sources

Authors: A. YANG, *Y. K. CHO, E. S. BOYDEN;
Media Lab, McGovern Inst., Dept. of Biol. Eng., Dept. of Brain and Cog. Sci., MIT, Cambridge, MA

Abstracts: We recently showed (Perea et al., 2014) that optogenetic tools not only transport ions across the plasma membrane, between the outside and inside of the cell, but also can drive release of ions from intracellular stores. Astrocytes in brain slices or in culture, upon intracellular calcium store depletion via thapsigargin application, no longer showed channelrhodopsin-2 (ChR2)-mediated calcium responses. Others have also shown that optogenetic tools can operate in intracellular organelles, for example altering mitochondrial function and metabolism through altering proton exchange (Hara et al., 2013). Given that channelrhodopsins transport many ionic species, including Na+, K+, Ca+2, and H+, we recently generated a channelrhodopsin ChromeQ (which stands for channelrhodopsin that omits Ca+2 and H+, quadruple mutant), that presents ~10x lower Ca+2 and H+ currents than wild-type, while preserving Na+ and K+ conductances. We show that ChromeQ, upon expression in astrocytes, does not result in light-driven intracellular store release of calcium. Thus, for some applications where calcium- and proton-dependent effects, ranging from plasticity to changes in cell survival to changes in cell signaling, are undesired, ChromeQ may present a useful reagent for the study and engineering of cellular membrane potential.

Disclosures: **A. Yang:** None. **E.S. Boyden:** None. **Y.K. Cho:** None.

Poster

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Support: MIT Media Lab

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Hertz Fellowship

Jeremy and Joyce Wertheimer

Google

NIH 2R44NS070453-03A1

New York Stem Cell Foundation-Robertson Award

Title: Super-resolution microscopy across arbitrary scales

Authors: *F. CHEN, P. W. TILLBERG, E. S. BOYDEN;
MIT, Cambridge, MA

Abstracts: With existing super-resolution microscopy methods, there are fundamental tradeoffs between imaging volume, imaging speed, and resolution. Furthermore, existing methods also can be difficult to implement, requiring expensive or complex optical components and/or specialized fluorophores (e.g. capable of photoswitching). Here we report a novel super-resolution imaging method which is potentially capable of imaging large volumes, at high speeds, with excellent resolution. Our method can achieve ~60 nm lateral and ~200 nm axial resolution, imaging at a rate of 2.5*10^6 super-resolved voxels per second (i.e., a 50 μm x 50 μm x 1 μm sample volume can be imaged in ~1 second). This method is relatively inexpensive to implement, and requires relatively modest computational resources, compared to previous microscopes. We anticipate that our new method may enable nanoscopic investigation of neural structures across essentially arbitrarily large sample volumes.

Disclosures: **F. Chen:** A. Employment/Salary (full or part-time):; Massachusetts Institute of Technology. **P.W. Tillberg:** A. Employment/Salary (full or part-time):; Massachusetts Institute of Technology. **E.S. Boyden:** A. Employment/Salary (full or part-time):; Massachusetts Institute of Technology.

Poster

659. Optogenetics: Integration With Electrophysiology

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MIT Synthetic Intelligence Project, NSF Center for Brains Minds and Machines at MIT

IET Harvey Prize, New York Stem Cell Foundation-Robertson Award

Title: Simultaneous whole-animal 3D-imaging of neuronal activity using light-field microscopy

Authors: *Y. YOON[1,2], R. PREVEDEL[7,8,9], M. HOFFMANN[7,8,9], N. PAK[2,3], G. WETZSTEIN[2], S. KATO[7], T. SCHRÖDEL[7], R. RASKAR[2], M. ZIMMER[7], E. S. BOYDEN[2,4,5,6], A. VAZIRI[7,8,9];
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Abstracts: 3D functional imaging of neuronal activity of entire organisms at the single cell level and on physiologically relevant time scales poses a major challenge in neuroscience. Here, using light-field microscopy in combination with 3D deconvolution, we demonstrate intrinsically simultaneous volumetric functional imaging of neuronal population activity at single neuron resolution for an entire organism, the nematode Caenorhabditis elegans, at up to 50Hz. This owes to a system-level optimized light field microscope, a fast deconvolution algorithm, and an appropriate genetically-encoded calcium indicator (GECI). This data combined with the full wiring diagram, or connectome, of *C. elegans* may allow the study of the nervous system at the circuit level in a systematic way. By performing whole-brain imaging of neural activity in larval zebrafish, we demonstrate the ability of our technique to capture dynamics of spiking neurons in volumes of ~700x700x200µm at 20Hz. The optical resolution for this volume is below single neuron resolution, but computational techniques based on independent component analysis enabled us to extract the activity of large numbers of neurons. The simplicity of our technique makes it an attractive tool for high-speed volumetric calcium imaging, and potentially voltage imaging, of neural activities in intact neural networks. (Prevedel and Yoon are co-first authors; Vaziri and Boyden co-corresponding authors).

Disclosures: **Y. Yoon:** None. **R. Prevedel:** None. **M. Hoffmann:** None. **N. Pak:** None. **G. Wetzstein:** None. **S. Kato:** None. **T. Schrödel:** None. **R. Raskar:** None. **M. Zimmer:** None. **E.S. Boyden:** None. **A. Vaziri:** None.

Poster

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New York Stem Cell Foundation-Robertson Investigator Award

Human Frontiers Science Foundation

NIH 1R01DA029639

Title: Optogenetic inactivation of the frontal eye field (FEF) increases error rates during all epochs of the memory-guided saccade task

Authors: ***L. ACKER**, E. BOYDEN, R. DESIMONE;
MIT, Cambridge, MA

Abstracts: Pharamacological inactivation studies have shown that the frontal eye field (FEF) is critical for executing saccades to remembered locations. Additionally, neurons within the FEF increase their firing rate during all three epochs of the memory guided saccade task: visual stimulus presentation, the delay interval, and motor preparation. It is unclear, though, whether FEF activity is necessary during all of those times for memory-guided saccade execution. A red-shifted halorhodopsin (JAWS) and a novel large-volume tissue illuminator were used in two rhesus macaques to inactivate part of the FEF at different times during a memory-guided saccade task. Neuronal recordings showed that the inactivated tissue volume spanned several cubic millimeters, which is consistent with histological findings. Error rates (e.g., failures to execute

memory-guided saccades to the proper target location) increased in both subjects with inactivation during either the target, delay, or motor period for targets in the inactivated receptive field. This implies that FEF neuronal activity contributes to performance throughout the memory-guided saccade task.

Disclosures: L. Acker: None. **E. Boyden:** None. **R. Desimone:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 659.15/VV35

Topic: G.04. Physiological Methods

Support: NIH 1R01DA029639

NIH 1DP1NS087724

New York Stem Cell Foundation-Robertson Award

NIH 1R01NS067199

NIH 1R01EY023173

New York Stem Cell Foundation-Robertson Award

Google

Title: Automated exploration of intracellular mechanisms of *in vivo* neural computation

Authors: *G. TALEI FRANZESI[1], A. SINGER[2], I. KOLB[3], S. SHARMA[2], S. KODANDARAMAIAH[2], M. TSITSIKLIS[2], I. WICKERSHAM[2], G. HOLST[3], D. BOZIC[2], S. BATIR[2], C. FOREST[3], C. BORGERS[4], N. KOPELL[5], E. S. BOYDEN[2];
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Abstracts: Many molecular, synaptic, and cellular mechanisms have been discovered and explored *in vitro* (e.g., in brain slices) or in anesthetized animals. Whether these mechanisms play a role in awake brain functions, however, often remains to be determined, in part because

performing intracellular recordings in awake behaving animals - necessary for verifying the real-time contribution of a specific synaptic or ionic conductance to a specific network function, for example - is technically challenging. Accordingly, we recently developed an awake autopatching robot to perform whole cell patch clamp recordings in awake behaving animals. We are now working on strategies for automatically exploring whether specific types of cellular mechanisms observed *in vitro*, also contribute to neural computations in the awake behaving animal. To achieve this, we are building informatics approaches to mine complex patterns of subthreshold and suprathreshold activity within single neurons undergoing whole cell patch clamp *in vivo* via our autopatcher robot. We can then mine the recordings that emerge for specific subthreshold patterns of synaptic activity or intrinsic conductance in an unbiased fashion, applying pharmacological (and even optogenetic) perturbation as needed to validate the findings. For example, one question amenable to this approach is the investigation of the fundamental timescales over which subthreshold activity patterns might be observed to repeat in a stereotyped fashion. By taking patch clamp recording traces, and cross-correlating each part of a trace with other parts, we can in an unbiased fashion survey whether specific patterns of activity repeat over time. These automated and unbiased methods of recording and analyzing intracellular activity in awake behaving animals may yield important insights into the computations that occur within neurons and neural networks. Moreover, combined with automated methods for feedback and causal hypothesis testing, as well as automated pharmacology and optogenetic approaches, this approach could enable rapid and systematic exploration in the awake brain of the specific roles played by the vast number of mechanisms identified *in vitro*. (Talei Franzesi, Singer, Kolb, and Sharma are co-first authors.)

Disclosures: **G. Talei Franzesi:** None. **A. Singer:** None. **I. Kolb:** None. **S. Sharma:** None. **S. Kodandaramaiah:** None. **M. Tsitsiklis:** None. **I. Wickersham:** None. **G. Holst:** None. **D. Bozic:** None. **S. Batir:** None. **C. Borgers:** None. **N. Kopell:** None. **E.S. Boyden:** None. **C. Forest:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

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Topic: G.04. Physiological Methods

Support: NIH 1R01DA029639

NIH 1DP1NS087724

NIH 1R01NS067199

NIH 1R01EY023173

New York Stem Cell Foundation-Robertson Award

R01 GM10498

Title: Automated multiple-cell patch clamp assessment of multineuron subthreshold dynamics in waking and anesthetized states

Authors: *S. B. KODANDARAMAIAH[1,2,3], F. J. FLORES[7,2], G. TALEI FRANZESI[4], A. C. SINGER[5,2], G. HOLST[8], I. R. WICKERSHAM[2], C. BORGERS[9], N. J. KOPELL[10], C. R. FOREST[8], E. N. BROWN[7,2,11,6], E. S. BOYDEN[4,2,3];
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Abstracts: We are developing a robot capable of patch-clamp whole cell neural recording of many cells at once (the "multipatcher"), in the waking and anesthetized brain, in order to reveal the synaptic and ion channel conductances that generate neural dynamics that support different brain states. Building from our past work (Kodandaramaiah et al., Nature Methods, 2012), we are both perfecting the technology and applying it to a major question: investigating the cellular mechanisms of general anesthesia. Despite its clinical importance, as well as its importance in basic-science studies of consciousness, perception, awareness, arousal, and other brain functions, little is known about how anesthetics act on specific cell types in the live brain, despite overt similarities in anesthetized behavioral states arrived at through administration of different drugs. In this study we are using our multipatching robot to study neural responses to multiple kinds of anesthetic drug: ketamine, an NMDA receptor antagonist; dexmedetomidine, an alpha-2 adrenoceptor agonist; and propofol, a GABA-A receptor agonist. We have performed single and multiple whole-cell patch clamp recordings in the somatosensory cortex of awake head-fixed mice, while tracking the changes in membrane potential and spiking activity as the awake animals transition into sedated states. We observe that the membrane potential of both inhibitory and excitatory cortical neurons in awake animals are characterized by slow oscillations during quiet wakefulness, interspersed by persistent depolarization during movement. Systemic infusion of both ketamine and dexmedetomidine results in the abolishment of the persistent depolarization, with the slow oscillation remaining. These membrane potential slow oscillations

are highly coherent across nearby (<200 microns) and more distant neuron pairs (200-500 microns). However, infusion of propofol produces a strong, constant hyperpolarization of the membrane potential. Our results indicate that ketamine and dexmedetomidine which are two very different drug classes have similar effects on the membrane potential of cortical neurons, possibly through their actions in brainstem arousal nuclei; whereas propofol might be inducing inhibition, perhaps in part by enhancing GABA-mediated chloride currents in pyramidal neurons in the cortex.

Disclosures: **S.B. Kodandaramaiah:** None. **F.J. Flores:** None. **G. Talei Franzesi:** None. **A.C. Singer:** None. **G. Holst:** None. **I.R. Wickersham:** None. **C. Borgers:** None. **N.J. Kopell:** None. **C.R. Forest:** None. **E.N. Brown:** None. **E.S. Boyden:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 659.17/VV37

Topic: G.04. Physiological Methods

Support: Simons Center for Social Brain Seed Grant

Title: Automated image-guided whole-cell patch clamp technology for mapping functional neuronal circuitry

Authors: ***A. A. CHUBYKIN**[1,3], I. KOLB[4], B. M. CALLAHAN[1], G. HOLST[4], W. STOY[4], C. R. FOREST[4], E. S. BOYDEN[2], M. F. BEAR[1];
[1]Picower Inst. for Learning and Memory, [2]Media Lab., MIT, Cambridge, MA; [3]Biol. Sci., Purdue Univ., West Lafayette, IN; [4]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstracts: One of the critical questions in neuroscience is how brain neural networks perform computations necessary for higher level cognitive functions. To answer this question one needs to record electrical activity of individual neurons with synaptic resolution. The tool best suited to address this question is the whole-cell patch clamp technique with which multiple aspects of excitatory and inhibitory synaptic currents, cellular excitability, and interneuronal connectivity can be characterized. However, this method is slow and currently requires the high level of expertise of the experimenter to achieve good recordings. We have developed a new image-guided Autopatcher system for brain slices and primary neuronal cultures, extending the “blind” *in vivo* automated whole-cell patch clamp prototype published previously. The system presented

here is an image-guided whole-cell patch clamp electrophysiology suite for analyzing synaptic currents and electrophysiological properties of single neurons *in vitro*, and can be combined with Channelrhodopsin-Assisted Circuit Mapping (CRACM) to map functional connectivity in different brain areas.

Disclosures: **A.A. Chubykin:** None. **I. Kolb:** None. **B.M. Callahan:** None. **C.R. Forest:** None. **E.S. Boyden:** None. **M.F. Bear:** None. **G. Holst:** None. **W. Stoy:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

Location: Halls A-C

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Program#/Poster: 659.18/VV38

Topic: G.04. Physiological Methods

Support: Jerry and Marge Burnett; Human Frontiers Science Program

IET A. F. Harvey Prize

McGovern Institute; MIT Media Lab

George W. Woodruff School of Mechanical Engineering, Georgia Tech

NIH Director's New Innovator Award (1DP2OD002002) and other NIH grants

NSF EFRI0835878

CISE 1110947

Title: High-throughput fully-automated patch clamp robot for in-vivo electrophysiology and morphology

Authors: *G. HOLST[1], S. B. KODANDARAMAIAH[3], I. KOLB[2], W. STOY[2], I. WICKERSHAM[4], A. SINGER[3], L. LI[5], E. S. BOYDEN[3], H. ZENG[5], C. R. FOREST[1]; [1]Mechanical Engin., [2]Wallace H. Coulter, Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]McGovern Inst. for Brain Research, Media Lab., MIT, Cambridge, MA; [4]McGovern Inst. for Brain Res., MIT, Cambridge, GA; [5]Allen Inst. for Brain Sci., Seattle, WA

Abstracts: Patch clamp recording is the gold-standard for measuring single ion channel currents, synaptic inputs, and whole cell currents in neurons. However, patch clamp recordings are still

something of an art form requiring great skill to record from only a few cells per day. For the last 20 years patch clamping was only performed by highly skilled technicians who have undergone months of training. Kodandaramiah et. al. recently developed a robot and neuron detection algorithm to autonomously record from neurons in-vivo. This robot, called the autopatcher, performs the skilled tasks previously performed by trained technicians and enables autonomous simultaneous recordings that have historically been extremely difficult to obtain. This work demonstrates the use of the autopatcher combined with a pipette exchange robot to obtain high-throughput in-vivo whole cell recordings in a fully automated fashion. To use the system, the operator only needs to prepare the mouse and perform the craniotomy at the correct stereotaxic location. After the mouse is placed in the robot, the operator locates the craniotomy with the first pipette and the autopatcher performs all the steps to obtain a gigaseal. Next, the pipette changing robot controls the automated break-in process by applying pulses of suction or voltage and assessing the quality of the seal after a whole cell configuration is obtained. Upon obtaining a successful recording, the robot waits for the experimental protocol to run and assesses the quality of the seal between repeats of the experimental protocol. After the recording has been completed or the seal with the cell has failed, the robot replaces the pipette for another recording attempt. For simultaneous biocytin staining, the robot slowly retracts the pipette after the recording has completed to reform the gigaseal for a high quality fill. The pipette exchange robot stores up to 20 pipettes that can be used as necessary throughout the experiment. For example, if a pipette is broken or clogged after being lowered into the brain, the robot detects it and immediately exchanges it with a fresh pipette without any human interaction. This kind of automated troubleshooting and correction throughout the experiment eliminates the need for constant supervision of the operator and enables parallel operation of multiple robots. We will also report progress of a large experiment (n>30 cells) using this robot to record both electrophysiology and morphology data on single cells in the visual cortex of mice. This data may begin to determine metrics for differentiating between types of neurons based on their intrinsic properties, morphology, and some basic visual stimulus protocols.

Disclosures: **G. Holst:** F. Consulting Fees (e.g., advisory boards); Neuromatic Devices Inc.. **S.B. Kodandaramaiah:** None. **I. Kolb:** None. **W. Stoy:** None. **I. Wickersham:** None. **A. Singer:** None. **L. Li:** None. **E.S. Boyden:** None. **H. Zeng:** None. **C.R. Forest:** None.

Poster

659. Optogenetics: Integration With Electrophysiology

Location: Halls A-C

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Program#/Poster: 659.19/VV39

Topic: D.18. Brain-Machine Interface

Support: DARPA Contract No: N66001-11-C-4171

Title: Optimizing unsupervised spike sorting using heuristic spike sort tuner

Authors: *D. A. BJANES[1], R. A. GAUNT[2,3], D. J. WEBER[3,2];
[1]Electrical and Computer Engin., [2]Physical Med. and Rehabil., [3]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstracts: Extracellular microelectrodes frequently record neural activity from multiple sources in the vicinity of the electrode. Spike sorting generally describes the process of labeling each recorded spike waveform with the identity of its source neuron, which is required to conduct any further analysis of the neuronal spiking patterns. This process for spike sorting or isolating neural activity is often approached from an abstracted mathematical perspective such as calculating the Euclidean distance between spike waveform features in some lower dimensional space or using probability distributions to describe the isolation of neural activity or recorded spikes. However, these approaches ignore neurophysiological realities and result in the loss of important information that could improve the accuracy of these methods. Furthermore, standard algorithms typically require manual selection of at least one free parameter, which can have significant effects on the ultimate quality of the spike sorting and all resulting neurophysiological inferences. We describe a Heuristic Spike Sorting Tuner (HSST), a spike sorting framework which can determine the optimal choice of the set of free parameters for any given spike sorting algorithm. A set of heuristic metrics computes a neurophysiologically-based qualification score of an algorithm's output across a range of parameters. This qualification score measures unit isolation and signal discrimination, allowing HSST to select the best set of parameters for a sorting algorithm, resulting in high sort quality. With simulated datasets, we compare performance of many existing sorting algorithms, while using HSST to select their free parameters. We show HSST's ability to reliably select the optimal set of free parameters, demonstrating robust performance over varied data (signal-to-noise ratio, number of units, relative size of units to each other, etc). Surprisingly, simple algorithms such as K-Means (when HSST is selecting its parameters) are shown to out-perform more complex supervised algorithms. Rather than being a spike sorting algorithm in its own right, HSST is a general framework that can incorporate any existing spike sorting algorithm, has an extendable set of heuristics and can be integrated in any existing neural signal processing stream. HSST makes use of known neurophysiological priors while simultaneously taking advantage of the power of abstract mathematical tools. We believe that this approach enables unsupervised spike sorting that exceeds the performance of previous methods, thereby enabling principled processing of large data sets without the significant confound of human intervention.

Disclosures: D.A. Bjanes: None. **R.A. Gaunt:** None. **D.J. Weber:** None.

Poster

660. Cellular Electrophysiological Methods

Location: Halls A-C

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Program#/Poster: 660.01/VV40

Topic: G.04. Physiological Methods

Support: NIH Grant MH094839

McKnight Foundation

Title: MATLAB-based automated patch clamp system for awake behaving mice

Authors: *N. S. DESAI, J. J. SIEGEL, R. A. CHITWOOD, D. JOHNSTON;
Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX

Abstracts: Automation has been an important part of biomedical research for decades; and the use of automated and robotic systems is now standard for such tasks as DNA sequencing, microfluidics, and high-throughput screening (including *in vitro* electrophysiology in cell lines). Recently, Kodandaramaiah and colleagues (Nat. Methods, 2012) demonstrated, using anesthetized animals, the feasibility of automating blind patch clamp recordings *in vivo*. Blind patch is a good target for automation because it is a complex but highly stereotyped process that revolves around analysis of a single signal (electrode impedance) and movement along a single axis. Here, we introduce an automated system for blind patch clamp recordings from awake behaving mice running on a wheel. In its design we were guided by three requirements: easy-to-use and easy-to-modify software; simple integration of behavioral signals and online data acquisition and analysis; and efficient use of time (important because of the typically short durations of intracellular recordings in moving animals). The resulting system employs equipment that is either standard for patch recording rigs, moderately priced, or simple to make. It is written entirely in MATLAB, a programming environment that has an enormous user base in the neuroscience community and many available resources for analysis and instrument control. The system includes not only components for obtaining patch recordings *in vivo* but a complete data acquisition system suitable for current clamp, voltage clamp, and field potential recordings; image acquisition for MATLAB-compatible cameras; and triggering signals for behavioral equipment. In pilot studies, we obtained 6 recordings (~40 attempts) from deep-layer neurons in prefrontal cortex of 8-9 week old mice, which ran at speeds between 0 and 20 cm/sec. Our procedures are still being optimized, but the success rate (~15%) is already comparable to that of experienced electrophysiologists working manually. The recordings were good by several criteria: membrane potential (-59.6 ± 2.3 mV), input resistance (48.7 ± 8.0 MΩ), and

spontaneous firing rate (0.3 ± 0.1 spikes/sec). The durations averaged 5.7 ± 1.0 min, but we have yet to explore several straightforward options for improving stability. Together with complementary approaches, this system should be useful to many cellular electrophysiologists who wish to study (head-fixed) mouse behavior. The system is general enough that it could be used as is in a variety of brain areas and with a variety of behaviors, but, written in a simple and familiar language, it also allows individuals to tailor procedures for the particulars of their own experiments.

Disclosures: **N.S. Desai:** None. **J.J. Siegel:** None. **R.A. Chitwood:** None. **D. Johnston:** None.

Poster

660. Cellular Electrophysiological Methods

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Program#/Poster: 660.02/VV41

Topic: G.04. Physiological Methods

Support: Wellcome Trust

Title: Automated intracellular recording with multiple sharp micropipettes

Authors: *G. F. COLLINS, S. N. BAKER;
Inst. of Neurosci., Newcastle Univ., Newcastle-upon-Tyne, United Kingdom

Abstracts: Intracellular recording from neurons allows access to sub-threshold potentials and permits the characterisation of single cell properties. Using multiple electrodes, synaptic and gap-junctional connections between cells can be characterised. Such recordings are made using either patch or sharp pipette electrodes. Advantages of patch electrodes are low access resistance and high seal resistance. Several groups have recently reported automated systems for patching neurons, either 'blind' or under visual guidance. However, patch electrodes can only be used once; after a cell has been successfully recorded, pipettes must be replaced. By contrast, sharp electrodes have higher access resistance and form less effective membrane seals but can be repeatedly driven into and out of cells, allowing many neurons to be recorded over the course of a single track, thereby increasing the data yield. We have built a novel system to automate recording with sharp micropipettes. Brain slices (450μm) are maintained in an interface chamber at 34°C, perfused with artificial CSF and exposed to 95% O_2/5% CO_2. Six micropipettes (resistance 50-100MΩ) are positioned over the slice using three-axis manual micromanipulators. Z-axis movement is provided by piezoelectric microdrives (Newport PZA12) with 12.5mm

travel and 30nm resolution under computer control. Pipettes are connected to bridge amplifiers (NPI, model BA03X), the output of which are digitised by a data acquisition card (National Instruments). A microcontroller-based circuit provides computer control of current pulses delivered to each bridge amplifier, as well as the 'buzz' and electrode impedance measurement functions. Software written in the Dephi environment provides real-time display of recordings, and control of pipette movement. With one click, pipettes may be driven down until they just touch the surface of the slice (detected by the sudden change in offset potential). Height is then zeroed, and electrode offset nulled. Automated cell finding then commences. Electrodes are advanced in small steps (~2μm); after each step, the electrode is buzzed. Cell penetration is detected by a reduction in membrane potential, and appearance of spikes in response to a depolarising current step. Electrodes continue until either a cell is found, at which point movement is stopped, or a maximum travel limit is reached. Once cells have been located, screens within the software guide the user through measurement of intracellular properties. We are now applying this system to record from primate brain slices, where the high value of tissue especially benefits from the increased yield which an automated approach provides.

Disclosures: G.F. Collins: None. **S.N. Baker:** None.

Poster

660. Cellular Electrophysiological Methods

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 660.03/VV42

Topic: G.04. Physiological Methods

Support: Paul G. Allen

Title: Creation of an *in vitro* slice electrophysiology platform to characterize human and mouse electrophysiological cell classes

Authors: A. OLDRE, J. BERG, T. JARSKY, C. ANASTASSIOU, A. ARKHIPOV, S. CALDEJON, P. CHONG, C. CUHACIYAN, S. DATTA, N. DEE, C. FARRELL, K. GODFREY, D. HILL, L. LI, S. MIHALAS, L. NG, H. PENG, J. PERKINS, S. PARRY, C. SLAUGHTERBECK, G. SOLER-LLAVINA, S. SORENSEN, S. SUNKIN, N. TASKIN, C. TEETER, J. TING, K. TRETT, W. WAKEMAN, R. YOUNG, C. DANG, M. HAWRYLYCZ, E. LEIN, J. W. PHILLIPS, C. KOCH, H. ZENG, *A. BERNARD;
Allen Inst. Brain Sci., SEATTLE, WA

Abstracts: The Allen Institute has initiated a multi-year program to understand the diverse neuronal cell classes in both human and mouse. We expect the properties of these cell classes to provide a foundation for the understanding of higher cortical function. To this end, we are investigating one of the most fundamental aspects of neuronal behavior - intrinsic electrophysiological properties. To develop a comprehensive electrophysiological classification of neuronal populations from both mouse and human, we have established a core platform to perform *in vitro* electrophysiology at scale. This platform will integrate and optimize technical expertise, instrument engineering, data acquisition standards and information management to maximize data output and quality. Quality control standards and systematic experimental workflows are under development to ensure that the datasets are reproducible and robust. Initial scientific objectives include gathering data from broad populations of cortical neurons from adult mouse and explanted human tissue from surgical specimens. A core set of electrophysiological stimuli suitable for a range of computational analysis, from detailed biophysical to generalized linear models, has been implemented using a custom acquisition software package. Ultimately, a data-driven cell classification scheme will be developed using features extracted from subthreshold and action potential waveforms as well as model parameters. Neurons will be labeled with biocytin while recording and the subsequent image acquisition and stereological analysis will provide for the pairing of morphological and electrophysiological data. Once established, this robust platform for gathering intrinsic electrophysiological properties from slice recordings should allow for the acquisition of ever more complex cell classification attributes, including paired cell recordings and post-hoc transcriptional profiling. Data, analysis tools, and model parameters will be made accessible via an online portal.

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Poster

660. Cellular Electrophysiological Methods

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 660.04/VV43

Topic: G.04. Physiological Methods

Title: A minimally invasive method for the analysis of sleep/wake behavior in rats

Authors: *J. BAUTISTA[1], D. H. MALIN[1], H. L. MATHEWS[2], D. M. NGHIEM[1], J. J. IZYGON[1], J. C. SHAHIN[1], C. A. MADISON[1], D. MCGHIEY[1], C. P. WARD[1];
[1]Psychology, Univ. of Houston Clear-Lake, Houston, TX; [2]Inst. for Behavioral Genet., Univ. of Colorado Boulder, Boulder, CO

Abstracts: Electroencephalography (EEG) is a procedure routinely used in sleep research to measure sleep-wake behavior. Non-invasive techniques are available for both humans and larger mammals. However, in rodents, long-lasting, invasive surgical procedures are required to implant screw electrodes for EEG recording purposes. This method uses a novel minimally invasive procedure (using surface electrodes). Specifically, the objective of this project is to minimize the invasiveness of the standard surgery. Adult Sprague-Dawley rats (n=3) were briefly anesthetized (isoflurane) and fitted with a spandex jacket attached to a cable tether. During a four-day period prior to polysomnography (PSG) recordings, rats were habituated to the jackets. During this period, rats were monitored for weight and locomotor activity. At the end of the fourth day, six customized electrodes were pasted on the scalp of each rat with a customized, plastic-made, protective cap glued on top of the EEG electrodes in order to prevent tampering. Each electrode was individually connected to a tethering cable affixed to the back of each rat jacket. In order to test the relative effectiveness of the minimally invasive procedure, the sleep/wake behavioral states of each rat were scored using polysomnographic recordings based on EEG and electromyography (EMG) data for a period of 24 hours. Following the recording period, data was analyzed using sleep-scoring software (SleepSign for Animals). The habituation data suggests that there was no fluctuation in weight or locomotor activity after the four-day recording period. Results from PSG data analysis show that over 24 hours there was a 49.9 % of total time spent in wake (W), 40.6 % of total time spent in NREM sleep, 4.8 % of total time spent in REM sleep. This was compared to control rats (n=6) using standard surgical methods to attached electrodes to the skull. Controls spent 48.90 % in W, 43.45 % in NREM, and 7.59 % in REM over 24 hours. Wake and NREM sleep was not significantly different between the two techniques ($t(7)=0.430$, $p<.05$ and $t(7)=0.289$, $p<.05$ respectively). There was significantly more REM scored in control rats ($t(7)=0.0231$, $p<.05$). In addition, there was a total of 4.6 % of unscorable epochs using surface electrodes due to mainly to artifacts occurring during W period as indicated by EMG patterns.

Disclosures: J. Bautista: None. **D.H. Malin:** None. **H.L. Mathews:** None. **D.M. Nghiem:** None. **J.J. Izygon:** None. **J.C. Shahin:** None. **C.A. Madison:** None. **D. McGhiey:** None. **C.P. Ward:** None.

Poster

660. Cellular Electrophysiological Methods

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Topic: G.04. Physiological Methods

Title: Automated neuronal spike detection and discrimination

Authors: *E. B. MONTGOMERY JR[1], H. HUANG[1], A. BARBORICA[2], F. HAER[3]; [1]Greenville Neuromodulation Ctr., Greenville, PA; [2]FHC Europe, Bucharest, Romania; [3]FHC, Inc., Bowdoin, ME

Abstracts: Objective: Neuronal spike train analysis is fundamental to neurophysiology. Electrode arrays vastly increases the amount of electrophysiological data that can be simultaneously collected, risking overwhelming the investigator. Particularly difficult are distinguishing neuronal spike from noise and artifact and discriminating different signals that can be attributed to individual neurons in the recording. Novel algorithms, initially developed for remote intraoperative neurophysiological monitoring during surgery, are presented that automate this process. Methods: The statistical analyses derive from the fact that noise is the largest portion of the recording and dominates the statistical distributions of signal amplitudes. The amplitude of a subsequent signal relative to the prior signal should follow the principle of regression towards the mean (1). A signal amplitude near the maximum of the noise likely would be followed by a signal amplitude closer to the mean. If the signal amplitude is just above the noise and not at the maximum of the true signal, the next signal amplitude will not regress towards the mean of the noise but towards the mean of the signal. A new algorithm developed plots the distribution of the difference between the ith and the ith+n amplitudes and should show a Gaussian distribution except at the tails. This distribution can be compared to the distribution where the order of amplitudes were randomized. The difference determines the amplitudes that distinguish the signal from the noise. Waveforms of the neuronal signals are discriminated by using a set of six heuristics previously described (1) where the value of the heuristics are plotted in a six dimensional space and unsupervised n-dimensional cluster analyses (2) is able to partition the waveforms into discrete clusters that can be assigned to individual neurons. The methods were applied to neuronal spike trains recorded during intraoperative neurophysiological monitoring. Recording were modified to create differing signal-to-noise ratios to determine the sensitivity of neuronal detection. Spike discriminations were tested by both auto-correlograms and cross-correlograms between simultaneously recorded neurons Results and Conclusions: The completely automated system (Distance Expert) was highly effective in detecting neuronal activity from noise and in discriminating neuronal waveforms attributable to individual neurons.

1. Methods for identifying neuronal spikes US patent no. 7,957,793 B2 2. Methods and devices for analysis of clustered data, in particular action potentials US patient 8,150,795 B2

Disclosures: **E.B. Montgomery Jr:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalities from Wisconsin Alumni Research Foundation. F. Consulting Fees (e.g., advisory boards); FHC, Inc. **H. Huang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalities from Wisconsin Alumni Research Foundation. **A. Barborica:** A. Employment/Salary (full or part-time):; FHC Europe. **F. Haer:** A. Employment/Salary (full or part-time):; FHC, Inc..

Poster

660. Cellular Electrophysiological Methods

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Topic: G.04. Physiological Methods

Support: Wellcome Trust

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ERC

EMBO

People Programme (Marie Curie Actions, FP7/2007-2013, Grant 328048)

Title: Electrophysiological properties of neurons in the mouse claustrum

Authors: *N. L. PETTIT, A. M. PACKER, S. CHUN, M. HAUSSER;
Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom

Abstracts: The claustrum is a thin nucleus lateral to the striatum that may be involved in inter-hemispheric coordination of cortical processing. Modern studies of the mouse connectome have confirmed widespread connectivity between the claustrum and the cortex and highlight the need for a more thorough investigation of the claustrum's internal elements. To address this, we have employed retrograde tracing from mouse primary visual cortex (V1) to reliably identify the claustrum in acute slices and carry out whole-cell patch-clamp recordings from confirmed

claustral neurons *in vitro* at physiological temperatures. Recovery of cell morphology with biocytin staining allowed us to assign electrophysiological properties to morphologically-defined claustral cell types. We observed substantial variability in passive membrane properties, action potential waveforms, and degree of spike-frequency adaptation, consistent with previous intracellular recordings in the claustrum of anaesthetized rats. We have also expressed C1V1, a red-shifted opsin, in motor cortex and performed subsequent retrobead injections and targeted *in vitro* claustral recordings to investigate the potential of channelrhodopsin-based cortico-claustral circuit mapping. The heterogeneity in the firing properties of recorded claustral neurons suggests the presence of functionally distinct subtypes of principal claustral neurons.

Disclosures: **N.L. Pettit:** None. **A.M. Packer:** None. **S. Chun:** None. **M. Hausser:** None.

Poster

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Topic: G.04. Physiological Methods

Support: NIH Grant R01-EB016407

Title: The Real-Time eXperiment Interface: A closed-loop data acquisition system with sub-millisecond latencies for electrophysiology

Authors: *A. GEORGE[1], Y. PATEL[2], F. ORTEGA[1], J. WHITE[3], D. CHRISTINI[1], A. DORVAL[3], R. BUTERA[2];
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Abstracts: To understand causal interactions between neural activity and function, both function and neural activity need to be monitored with timing precision at the sub-millisecond scale. This requires enabling of closed-loop real-time technologies that are capable of controlling stimulation (optogenetic, electrical, thermal, etc) dependent upon some functional measure (behavior, motor action, etc) within a deterministic period. While monitoring neural activity at precise time scales is commonplace, there is an important unmet need in a cost-effective sub-millisecond closed-loop real-time framework capable of interacting with neural activity and function at the appropriate timescales. To enable investigation of neural activity and function at these time scales, we have developed the Real-Time eXperiment Interface (RTXI) - a versatile

interface based off of real-time Linux that enables deterministic closed-loop monitoring, stimulation, and control of single-cell, network, animal, and human electrophysiology experiments. RTXI is a free and open source platform currently employed by over 65 labs around the world. Essential modules such as a high-speed oscilloscope, signal generators, and common filters are included in the base of RTXI. An expanding library of community-developed modules is maintained and available for all users to enable resource sharing and maximizing reproducibility of experimental setups and results. In its present state, the RTXI allows for up to 32 16-bit I/O channels, with worst-case jitter of 10 microseconds relative to the input, and enables control of a wide variety of stimulation methods (optical, electrical, thermal, etc). Current and future development is focused on interfacing with Intan bioaquisition headstages to enable higher channel counts as well as scaling of hardware. Addtional information is available on our website (http://www.rtxi.org), and our code repository is open on GitHub (https://github.com/RTXI).

Disclosures: **A. George:** None. **Y. Patel:** None. **F. Ortega:** None. **J. White:** None. **D. Christini:** None. **A. Dorval:** None. **R. Butera:** None.

Poster

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Topic: G.04. Physiological Methods

Support: JST, ERATO

Title: Differentiation induction of neural stem cell microfibers

Authors: ***H. ONOE**[1,2,3], M. KATO-NEGISHI[2,3], S. TAKEUCHI[2,3];
[1]Keio Univ., Yokohama-Shi, Kanagawa, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]ERATO, JST, Tokyo, Japan

Abstracts: Cell-laden hydrogel microfibers have recently studied for scaffolds or building blocks for 3D tissue constructions. One of the attractive targets using these cell-laden hydrogel microfibers is artificial reconstruction of 3D neural network *in vitro* for tissue engineering and regenerative medicine. As a standpoint of such practical applications, (i) the use of stem cells and (ii) the control of differentiation induction are essential factors. Here we report differentiation induction of mouse primary neural stem cells (NSCs) in a fiber-shaped three-

dimensional (3D) microenvironment created in a core-shell hydrogel microfiber. Formation of NSC-encapsulating core-shell hydrogel fiber (inner diameter: ~100 μm, length: > meter) was conducted by using double coaxial microfluidic device. The NSC microfibers were composed of the core of NSCs with collagen matrix and the shell of mechanically stable calcium alginate. The differentiation of the fabricated NSC-encapsulating hydrogel microfibers was induced by controlling growth factors in medium. We successfully demonstrated the differentiation of NSCs to neurons and glial cells in the fiber-shaped 3D microenvironment where the diameter of the core NSC cells and the shell of calcium alginate were varied. We found that the spatial configuration of the differentiated neurons is not uniform: neurons were clustered and localized at the surface of the fibers. These results indicate that our technology would provide a confined fiber-shaped 3D microenvironment as a tool for neural stem cell biology and also could provide differentiated fiber-shaped neural tissues that can be applicable to tissue engineering and medical transplantation.

Disclosures: H. Onoe: None. **M. Kato-Negishi:** None. **S. Takeuchi:** None.

Poster

660. Cellular Electrophysiological Methods

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Topic: G.04. Physiological Methods

Title: A scalable system for large spherical treadmills

Authors: *A. K. LEE, N. J. SOFRONIEW, K. SVOBODA, J. COHEN;
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Abstracts: Recent advances in the use of the awake, head-fixed preparation for small rodents (e.g., rats and mice) have led to an array of new interactive tools for studying mammalian brain and behavior. Here we present a method for creating large, lightweight spherical treadmills of arbitrary size. In order to accommodate different treadmill sizes, we provide a point-based floatation mechanism that is highly scalable. Moreover, its simple, sparse features leave the treadmill surface quite accessible for motion-tracking and other potential manipulations. For mice (8-12 wk, 20-30 g), we employ a 16” (40.64 cm, approx. 70 g) diameter spherical treadmill. For young-adult rats (3-6 wk, 60-150 g), we employ a 24” (60.96 cm, approx. 225 g) diameter sphere. We show that with little-to-no training, rodents are able to comfortably perform a variety of simple behaviors on these large spheres, such as running, walking, grooming, resting, and

sleeping. In addition, we demonstrate that this behavioral apparatus can be used in conjunction with sensitive electrophysiological and optical imaging techniques to obtain high-quality, long-duration recordings. Examples shown include long-duration multichannel extracellular and whole-cell patch-clamp recordings in the hippocampus of young-adult rats and adult mice behaving in visual-based virtual environments, and optical imaging of the somatosensory cortex of mice behaving in a tactile virtual reality.

Disclosures: **A.K. Lee:** None. **N.J. Sofroniew:** None. **K. Svoboda:** None. **J. Cohen:** None.

Poster

660. Cellular Electrophysiological Methods

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Program#/Poster: 660.10/VV49

Topic: G.04. Physiological Methods

Title: Evaluation of positive allosteric modulators on ligand gated ion channels using automated electrophysiology and fast fluidic exchange

Authors: J. WEBBER[1], A. YEHIA[2], J. COSTANTIN[1], *X. JIANG[1];
[1]Mol. Devices, LLC, SUNNYVALE, CA; [2]Fluxion Biosci., South San Francisco, CA

Abstracts: Ligand gated ion channels (LGICs) mediate fast synaptic transmission in the nervous system, and they represent a class of highly attractive drug targets due to the pivotal role they play in many physiological functions. At the molecular level, the binding of ligands such as neurotransmitters to LGICs transduce chemical signals into electrical signals, by enabling the passage of ions across the membrane. Functional impairment of these LGICs represents a variety of disease states, and positive allosteric modulators (PAMs) are proven therapeutic tools which alter the function of LGICs. Such modulation includes altering the channel conductance or the gating properties in the presence of ligand. After activation, many LGICs quickly enter a desensitized state, often within milliseconds. The transient nature of the LGIC activation and subsequent desensitization is crucial for fast synaptic signaling. Over the last decade automated electrophysiology has become an indispensable tool for analyzing ion channel activities. Here we evaluate the fluidic performance envelope in automated patch clamp, and its impact on LGIC assays both in the presence and absence of PAMs. We examined three ligand gated ion channels: acid sensing ion channel 1a (ASIC1a), gamma aminobutyric acid (GABA A), and nicotinic alpha 1. CHO-K1 cells stably expressing pore-forming subunit of human ASIC1a channel, HEK293 cells stably expressing GABA A, and TE671 cells with endogenous expression of nicotinic alpha

1 were used for these experimental models. In one series of experiments, we examined the speed of tonic ligand wash-in and wash-out to examine the activity and kinetics of ion channels, as well as multiple channel modulators and compared these results to those obtained with historical data generated from a conventional patch clamp. Next, we examine recovery from desensitization using a paired ligand application method. The interval between first and second application of ligand is varied and the amplitude of the second ligand application is compared to the first. In conclusion, we demonstrated the fluidics performance envelope in automated electrophysiology, and its successful applications in high-throughput LGIC assays offering a high throughput, robust platform for LGIC screening in drug discovery.

Disclosures: **J. Webber:** A. Employment/Salary (full or part-time):; Molecular Devices, LLC. **A. Yehia:** A. Employment/Salary (full or part-time):; Fluxion Bioscience. **X. Jiang:** A. Employment/Salary (full or part-time):; Molecular Devices, LLC. **J. Costantin:** A. Employment/Salary (full or part-time):; Molecular Devices, LLC.

Poster

660. Cellular Electrophysiological Methods

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Topic: G.04. Physiological Methods

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NRC/DTRA CBD RAP Fellowship

Title: Functional measures of synaptic transmission in stem cell-derived neurons intoxicated with Clostridial neurotoxins: An ultra-sensitive cell-based platform with shared pathophysiologies to *in vivo* intoxication

Authors: *P. H. BESKE, A. B. BRADFORD, M. E. LYMAN, P. M. MCNUTT;
US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstracts: Following internalization into presynaptic termini, the two families of Clostridial neurotoxins (CNTs), botulinum neurotoxin (BoNT) and tetanus neurotoxin (TeNT), cleave SNARE proteins associated with synaptic vesicle release, preventing the exocytosis of neurotransmitters. To date, no cell-based model used in CNT research has been shown to form

functioning synapses, limiting the ability to conduct target discovery or therapeutic screening. Stem cell-derived neurons have recently been proposed as a next-generation cell-based platform for neurotoxicity research. We have shown that mouse embryonic stem cell-derived neurons (ESNs) replicate the unique neuronal responses to intoxication that occur *in vivo*, including serotype-specific persistences, activity-enhanced intoxication, and differential serotype potencies. ESNs are highly sensitive to TeNT and the seven classical BoNT serotypes, with EC_{50} values that are similar to primary neurons. Whole-cell patch clamp electrophysiology indicates that ESNs develop mature electrical responses and form a complex, synaptically coupled network with an excitatory:inhibitory balance by 18 days *in vitro* (DIV). Using RNAseq, electrophysiology and immunocytochemistry, we show that ESNs produce exclusively glutamatergic (75%) and GABAergic (25%) synapses and demonstrate abundant spontaneous miniature excitatory and inhibitory post-synaptic currents, indicative of monosynaptic activity. Hypothesizing that synaptically coupled ESNs may undergo inhibition of synaptic transmission in the presence of CNTs, we evaluated the effects of intoxication with BoNT/A through BoNT/G as well asTeNT on functional measurements of synaptic activity at DIV 24. We found that electrophysiologic-based measurements of mEPSC frequency provided a more sensitive metric of CNT intoxication compared to immuno-based assays for SNARE protein cleavage. Collectively, these studies demonstrate that intoxicated ESNs exhibit the same pathophysiology that manifests as clinical intoxication, suggesting that they are suitable models for therapeutic screening. Additionally, this approach suggests that electrophysiological characterization of trans-synaptic activity may comprise a novel, rapid screen for the presence of functional toxin in forensic, pharmaceutical, environmental and/or food samples, as well as provide an *in vitro* model for central neuron research involving CNT induced synapse silencing. Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

Disclosures: **P.H. Beske:** None. **A.B. Bradford:** None. **M.E. Lyman:** None. **P.M. McNutt:** None.

Poster

660. Cellular Electrophysiological Methods

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Topic: G.04. Physiological Methods

Support: SRFDP & RGC ERG Joint Research Scheme (M-CUHK409/13; 2900703)

Title: Spatial tracking and volume reconstruction for *in vivo* blind whole cell patch-clamp recordings

Authors: *D. C. CHAN, H. KO, W. YUNG;
Sch. of Biomed. Sci., The Chinese Univ. of Hong Kong, Hong Kong, China

Abstracts: The *in vivo* application of the whole cell patch-clamp technique is fundamental to understanding the subthreshold computational dynamics of individual neurons in an ethologically relevant setting. Recordings of deep cortical and subcortical neurons typically employ a blind patch approach, in which spatial resolution is lost due to variability in pipette fabrication and mounting. We developed an automated tracking system that rapidly registers the micromanipulator position of each freshly mounted pipette relative to a fixed reference frame, prior to insertion into brain tissue. Biocytin labelling of recorded neurons and subsequent volume reconstruction enables us to match neuron morphology with recordings in a post-hoc fashion. Accurate localization of the pipette tip also enables us to approach a volume of interest in close proximity (~30μm), and derive local spatial relationships in microcircuit computation. We demonstrated the system in anaesthetized as well as awake and mobile head-fixated C57BL/6 mice, with the latter conducted on a one-dimensional treadmill paradigm. We performed whole cell patch-clamp recordings and volume reconstruction in the hippocampus, and the caudal forelimb area (CFA) and hindlimb area (HA) of the primary motor cortex (M1), and demonstrated specific micromanipulator trajectories to record from intracolumnar and local intralaminar volumes relative to neocortical layer V pyramidal neurons.

Disclosures: D.C. Chan: None. **H. Ko:** None. **W. Yung:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Howard Hughes Medical Institute

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Title: Fast scan cyclic voltammetry in *Drosophila* melanogaster for dopamine measurement

Authors: R. FRANCONVILLE[1], E. PRIVMAN[2], B. BARBARITS[1], P. AHAMMAD[1], M. BARBIC[1], V. JAYARAMAN[1], T. HARRIS[1], J. VENTON[2], *S. KIM[1];
[1]Janelia Farm Res. Campus / HHMI, Ashburn, VA; [2]Dept. of Chem., Univ. of Virginia, Charlottesville, VA

Abstracts: The amount of dopamine released by dopaminergic neurons is not a simple function of their activity level. Thus, neither electrophysiological recordings nor calcium imaging of dopaminergic neurons is sufficient to monitor dopamine concentration in the brain. In mammals, Fast Scan Cyclic Voltammetry (FSCV) has been successfully used to measure dopamine concentration *in vivo*, but, in *Drosophila*, the large size of carbon fiber probes relative to the size of the fly brain have limited their use. Here, we successfully apply FSCV in the adult *Drosophila* brain using a positioning technique with improved z-resolution, and a sharp carbon fiber electrode small enough to be placed inside tiny compartments of the *Drosophila* brain. We used a two-photon laser-scanning microscope to precisely position the electrode in the peduncle or the medial bulb of the mushroom body in an isolated brain. We optogenetically stimulated CsChrimson-expressing dopamine neurons with orange light (590nm) and used FSCV to measure dopamine concentration. We were able to detect dopamine concentration as low as 10nM at a temporal resolution of 10Hz. Combined with 2-photon calcium imaging, our new technique will provide a more detailed and fine-grained understanding of dopaminergic modulation in the fly brain during behavior.

Disclosures: R. Franconville: None. **S. Kim:** None. **E. Privman:** None. **B. Barbarits:** None. **P. Ahammad:** None. **M. Barbic:** None. **V. Jayaraman:** None. **T. Harris:** None. **J. Venton:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

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Topic: G.04. Physiological Methods

Support: NIH DC02260

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Bakar Fellows Program

Title: Simultaneous multi-scale electrophysiological measurement and optical manipulation of *in vivo* cortical networks

Authors: *K. BOUCHARD[1], P. LEDOCHOWITSCH[4], L. MULLER[2], E. A. K. PHILLIPS[3], B. SEYBOLD[3], M. M. MAHARBIZ[5], A. HASENSTAUB[3], C. E. SCHREINER[3], E. F. CHANG[1]; [1]Neurosurg. and Physiol., [2]Neurosurg., [3]Otolaryngology, UCSF, San Francisco, CA; [4]Allen Brain Inst., Seatle, WA; [5]Electrical Engin. and Computer Sci., UCB, Berkeley, CA

Abstracts: Here, we describe an electrophysiological recording system that combines micro-eletrocorticography (μECoG) to record neural activity from the cortical surface over extended areas with mesoscale spatial resolution, with laminar polytrodes to densely record neural activity across cortical layers with microscale spatial resolution. Combining these high-temporal resolution, multiscale electrophysiological recordings with optical manipulations of neural activity further allows causal inference into the role of specific neural populations in local and distributed cortical computations. We highlight this system in the context of auditory cortex recordings from anesthetized rats and mice. We demonstrate that μECoG arrays placed on the surface of rodent auditory cortex record sound evoked field potentials with high signal-to-noise ratio primarily in the 70-170 Hz range (high-gamma) but extending up to 300-800Hz. Examination of recorded signals >1kHz reveals neural events with timing and amplitude characteristics indicative of multi-unit action potentials evoked by sounds. Focusing on the activity in the high-gamma range, we demonstrate that μECoG recorded field potentials have sufficient spatial resolution and selectivity to derive functional organization of rat auditory cortex (tonotopy), and thus provide a method for rapid, non-destructive mapping of cortical function. We show that auditory evoked high-gamma responses provide sufficient temporal resolution to derive spectro-temporal receptive fields that are qualitatively similar to those derived from multi-unit spiking activity. Direct recordings of action potentials with laminar polytrodes inserted through perforations in the μECoG array suggest that functional tuning derived from μECoG (70-170Hz) reflects a spatial average of multiunit spiking activity immediately beneath the μECoG contacts. Finally, we combined our multiscale electrophysiological recording system with optogenetic manipulation of neural activity in mice. Preliminary results in PV-Cre x AI32 animals, in which parvalbumin-positive (PV+) interneurons are activated by light, demonstrate the ability of μECoG to record mesoscale changes in sound-evoked neural activity with high temporal resolution during optical manipulation of specific neuronal populations. Together, these results demonstrate high-temporal resolution, multi-scale electrophysiological measurements with simultaneous optical manipulation of *in vivo* cortical networks. Translating this system to behaving animals promises to reveal mechanisms of distributed cortical processing underlying complex tasks.

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Poster

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Support: This work was supported in part by the Defense Advanced Research Projects Agency, Microsystems Technology Office (DARPA, MTO) Reliable Neural Interface Program through an Interagency Agreement with the U.S. FDA (FDA-DARPA 224-10-6006).

Title: Longitudinal evaluation of the safety and reliability of peripheral nerve electrode technologies

Authors: *S. VASUDEVAN, C. WELLE;
Office of Sci. and Engin. Laboratories, Div. of Physics, FDA, Silver Spring, MD

Abstracts: There are over a million amputees in the United States alone, many of whom can benefit from neuroprosthetic devices. Human studies have shown the ability to translate signals from the peripheral nerves into motor commands even after chronic amputation. However, the long-term performance, both safety and reliability, of peripheral nerve interface technology remains to be fully demonstrated. To investigate peripheral nerve electrode technologies, we have developed surgical, electrophysiological, behavioral, histological and data analysis protocols to characterize peripheral nerve-machine interface technology using established rodent models. Utilizing existing research electrode technology including intraneural floating multi-electrode arrays (Microprobes), life arrays and Utah-style arrays (Blackrock) and extraneural cuff electrodes (Microprobes, Ardiem), implanted in the rat sciatic nerve, we obtained neural signals. Connectors were anchored onto the back of the animals using 3D printed custom pedestals. Beginning two weeks after surgical implantation, electrophysiological recordings and impedance spectroscopy were performed weekly for the study duration. The quality and temporal dynamics of the recorded electrophysiological signals were compared for extraneural and intraneural electrode types. Upon obtaining stable recordings from the implanted electrodes, electrode impedance characteristics were correlated with electrophysiology recording metrics. To study the extent of motor and sensory deficits associated with electrode implantation, we used

walking track analysis and sensory stimulation thresholds measured bi-weekly over the course of the experiment. These behavioral and functional assessments were used to demonstrate recovery trajectory following electrode implantation. Following termination of the *in vivo* experiment, we harvested nerve tissue for assessment using histology and histomorphometry. Using histological techniques, the average axonal distance from the recording electrodes and the total number of myelinated axons were quantified across electrode type. This regulatory science research study serves as a test platform to understand the principles that influence peripheral nerve interface device performance. DISCLAIMER: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

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Poster

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Fondation Motrice

Fondation Nanosciences

Fondation de l'Avenir

Fondation Philanthropique Edmond J. Safra

Title: A fully implantable ECoG recording device WIMAGINE® for human BCI applications: Toward a clinical trial

Authors: ***G. CHARVET**, C. MESTAIS, F. SAUTER, M. FOERSTER, A. LAMBERT, N. TORRES-MARTINEZ, T. COSTECALDE, D. RATEL, A. BENABID;
CEA/LETI/CLINATEC - MINATEC Campus, Grenoble, France

Abstracts: The WIMAGINE® implant (Wireless Implantable Multi-channel Acquisition system for Generic Interface with NEurons) was developed to record ECoG (ElectroCorticoGram)

signals for long term clinical applications, such as BCI. The implant is composed of an array of 64 biocompatible electrodes, a hermetic titanium housing including the electronic boards and biocompatible antennae for wireless transmission of data and remote power supply. During the surgical procedure, the implant will be inserted into a 50 mm craniotomy so that the electrode array is in contact with the dura mater, and the implant recovered by the skin. The design of the WIMAGINE® implant takes into account all the constraints of long term implantable medical devices: low power, miniaturization, safety and reliability. WIMAGINE® was designed to satisfy the Essential requirements of the European Medical Device Directives 93/42/CEE and 90/385/EEC. A risk analysis according to ISO 14971 standards has been conducted, and risk management actions were set up. The implant manufacturing was achieved according to a qualified industrial process under ISO certification 13485. In order to increase the reliability, all electronic boards undergo a burn-in procedure to avoid early in-use system failures, and functional tests. The electronic board is encapsulated into a dedicated titanium packaging with hermetic feedthrough. The hermeticity is achieved by laser welding and tested in terms of helium leakage and yield 10-9 bar.cm3.s-1. Then, each implant is tested, cleaned and sterilized according to a validated process. The first qualification tests of the implant according to standards were achieved by certified bodies. In particular mechanical and electrical test according the ISO 45502-1 were successfully achieved such as resistance to mechanical forces, vibrations and shocks, electrodes leakage current less than 1µA, ElectroStatic Discharge (ESD) immunity, heating at the surface of the implant less than 2°C. Likewise, the electrical security tests and electromagnetic compatibility (EMC) tests according to the EN 60601-1 were performed on the implant and its external unit. Finally, the long-term biocompatibility has been evaluated according to the ISO 10993, in particular consisting of the local tolerance and systemic effects after 26 weeks contact duration in animals. The next step is the submission of the protocol for clinical trial authorization to the French authorities (ANSM) in the context of Brain Computer Interface for tetraplegia. Other neurological applications requiring wireless ECoG recording such as presurgical evaluation of epilepsy, or post stroke rehabilitation can be addressed.

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Poster

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Topic: G.04. Physiological Methods

Title: Simultaneous electrophysiology and calcium imaging in hippocampal slices using transparent electrodes

Authors: D. KUZUM[1], *H. TAKANO[7,2,3], E. SHIM[4], J. C. REED[4], H. JUUL[2], M. DICHTER[2], D. A. COULTER[7,5,3], E. CUBUKCU[4,6], B. LITT[1,2];
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Abstracts: Graphene has recently emerged as the most investigated 2D material, owing to its superior electrical, mechanical and thermal properties. Graphene's flexibility, transparency, its excellent electrical conductivity and low noise characteristics make it attractive for neural sensing and stimulation applications. Transparency of graphene is particularly important for developing neural electrode arrays that enable simultaneous functional optical imaging and electrophysiological recording from the same population of neurons. Combining high spatial resolution of calcium imaging with high temporal resolution of electrical recording, neuronal circuit dynamics can be studied with high spatiotemporal resolution. We demonstrated that hippocampal slices can be imaged through transparent graphene electrodes by confocal microscopy, while the neural activity was simultaneously recorded by the graphene electrode. Both excitation and emission light penetrated through the graphene electrode without causing any light induced artifacts in the electrical recordings. Recordings by the graphene electrode and calcium transients measured by the confocal microscopy were found to be consistent, showing short population bursts during induced epileptic form of activity. The temporal resolution of the recordings with the graphene electrode enabled detection of high frequency population discharges, which could not be resolved by the calcium fluorescence responses. In contrast, calcium imaging was able to capture complex network contributions of individual neurons which were not evident in the electrical recordings. Experiments with the slices have shown that the graphene electrode was able to measure very fast population spikes with durations less than 5 ms, as well as slow field potentials that were not detectable by calcium imaging.

Disclosures: **D. Kuzum:** None. **H. Takano:** None. **E. Shim:** None. **J.C. Reed:** None. **H. Juul:** None. **M. Dichter:** None. **D.A. Coulter:** None. **E. Cubukcu:** None. **B. Litt:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.05/VV57

Topic: G.04. Physiological Methods

Support: DoD, Defense Advanced Research Projects Agency (DARPA) #N66001-12-1-4026

Multidisciplinary University Research Initiative (MURI) #N00014-10-1-0198

Title: Assessing novel materials to improve chronic cortical implants

Authors: *D. G. MCHAIL[1], H. CHARKHKAR[2], G. L. KNAACK[1], H. S. MANDAL[3], J. S. KASTEE[3], J. F. RUBINSON[4], J. J. PANCRAZIO[3], T. C. DUMAS[1];
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Abstracts: Implantable microelectrode arrays (MEAs) detect neuronal signals, which can be utilized in neural prosthetics or other brain-machine interface applications. Failure in chronic neuronal recordings is considered the most challenging issue with implantable MEAs. Such failures are either biotic (neuroinflammation), abiotic (delamination or corrosion of device), or a combination of both. Recent efforts seek to extend the longevity of these implants by utilizing novel materials. In this work, we have studied the performance of two candidate materials for chronic brain implants. Prior work has shown that coating *in vivo* probes with the conducting polymer (CP) poly(3,4-ethylenedioxythiophene) (PEDOT) lowers impedance and might improve the recording quality compared to gold electrodes, likely due to the increased effective surface area and reduced noise levels provided by PEDOT. The performance and stability of the PEDOT coatings can be further enhanced by replacing the common counter ion e.g. poly(styrene sulfonate) (PSS) with a smaller counter ion such as tetrafluoroborate (TFB). Alternatively, non-metallic probes might offer benefits over metallic probes by eliciting lower inflammatory response. Shape memory polymer (SMP) softens at body temperature to accommodate the brain's natural mechanical stiffness. In addition to the probes with PEDOT:TFB coated electrodes, functional SMP probes were implanted into the primary motor cortex of rats. Single unit activity and electrochemical impedance were monitored on probes with PEDOT:TFB coated electrodes for four to twelve weeks post-implant to assess recording quality and device integrity respectively. Single unit activity and electrochemical impedance were also monitored weekly for implanted SMP MEAs. Consistent with our previous *in vitro* findings, PEDOT:TFB coated electrodes exhibited decreased impedance and consistent single unit activity compared to gold coated controls over a 12 week period. In ongoing experiments, SMP probes have sustained unit activity for up to two weeks following implant, which demonstrates proof-of-concept for SMP probes in chronic neural recordings *in vivo*. Our findings suggest that the quality and durability

of brain-machine interfaces can be further improved by employing novel materials such as PEDOT:TFB and SMP for brain implants.

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Poster

661. Electrophysiology Recording Tools and Techniques

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Topic: G.04. Physiological Methods

Support: 973 program: 2012CB825503

NSFC: 81100976, 91132306

Grant No. 2012BAI01B08

XDB02050003

Guangdong Innovation Research Team Fund for Low-cost Healthcare Technologies

Title: Multi-unit recording and labeling with iridium oxide modified stereotrodes in *Drosophila* melanogaster

Authors: ***C. ZHONG**, Q. MONTARDY, X. LIU, L. WANG;
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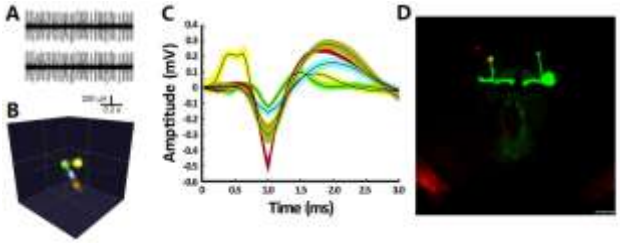
Abstracts: Background: *Drosophila* is a very favorable animal model for the studies of neuroscience. However, it remains a great challenge to employ electrophysiological approaches in *Drosophila* melanogaster to study the neuronal assembly dynamics *in vivo*, partially due to the small size of the *Drosophila* brain. Small and sensitive microelectrodes for multi-unit recordings are greatly desired. New Method: We fabricated micro-scale stereotrodes for electrical recordings in *Drosophila* melanogaster. The stereotrodes were modified with iridium oxide (IrO2) under a highly controllable anodically deposition procedure to improve their electrochemical properties. Electrical recordings were carried out using the IrO2 stereotrodes to detect spontaneous action potentials and LFPs *in vivo*. The recording site was labeled by the red Nissl fluorescent in a transgenic *drosophila*, which mushroom body is marked by green

fluorescence. Results: After deposition, the IrO2 electrodes exhibited significantly higher capacitance and lower electrochemical impedance at 1 kHz. Electrical recording with the IrO2 stereotrodes in the brains of *Drosophila* demonstrated an average signal-to-noise ratio (SNR) of 7.3 and a significantly improved LFP sensitivities. 5 types of different neurons recorded were clearly separated. Electrophysiological responses to visual and odor stimulation were detected, respectively. The Nissl fluorescent labeled recording site should be mushroom body. Conclusions: The IrO2 stereotrodes are capable to meet the requirements of multi-unit recording and spike sorting, which will be a useful tool for the electrophysiology-based researches especially in *Drosophila* and other small animals. Figure 1 Spike sortings of multi-unit recordings in *Drosophila* melanogaster using iridium oxide (IrO2) stereotrodes. (A), Representative examples of raw spike data. (B), Three-dimensional views of unit clusters. (C), Averaged spike waveforms. (D), The brain of *Drosophila* melanogaster (labeled by green) and recording site (labeled by red). The white line, 75μm.



Disclosures: **C. Zhong:** None. **Q. Montardy:** None. **X. Liu:** None. **L. Wang:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

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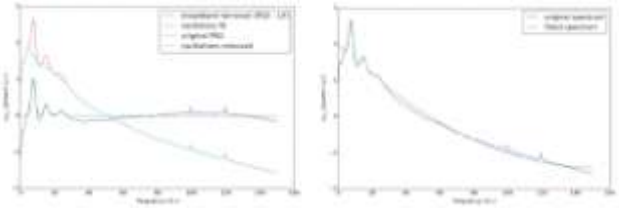
Program#/Poster: 661.07/VV59

Topic: G.04. Physiological Methods

Title: Automated “spectral fingerprinting” of electrophysiological oscillations

Authors: M. HALLER[1], P. VARMA[1,2], T. NOTO[4], R. T. KNIGHT[1,3], A. SHESTYUK[1,3], *B. VOYTEK[4,5,6];
[1]Helen Wills Neurosci. Inst., [2]Electrical Engin. and Computer Sci., [3]Psychology, Univ. of California, Berkeley, Berkeley, CA; [4]Cognitive Sci., [5]Neurosciences Grad. Program, [6]Inst. for Neural Computation, UCSD, La Jolla, CA

Abstracts: Neuronal oscillations play an important role in neural communication and network coordination. Low frequency oscillations are comodulated with local neuronal firing rates and correlate with a physiological, perceptual, and cognitive processes. Changes in the population firing rate are reflected by a broadband shift in the power spectral density of the local field potential. On top of this broadband, 1/f^α field, there may exist concurrent, low frequency oscillations. The spectral peak and bandwidth of low frequency oscillations differ among people, brain regions, and cognitive states. Despite this widely-acknowledged variability, the vast majority of research uses a priori bands of interest (e.g., 1-4 Hz delta, 4-8 Hz theta, 8-12 Hz alpha, 12-30 Hz beta). Here we present a novel method for identifying the oscillatory components of the physiological power spectrum on an individual basis, which captures 95-99% of the variance in the power spectral density of the signal with a minimal number of parameters. This algorithm isolates the center frequency and bandwidth of each oscillation, providing a blind method for identifying individual spectral differences. We demonstrate how automated identification of individual oscillatory components can improve neurobehavioral correlations and identify population differences in spectral and oscillatory parameters. 

Disclosures: **M. Haller:** None. **B. Voytek:** None. **T. Noto:** None. **P. Varma:** None. **A. Shestyuk:** None. **R.T. Knight:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.08/VV60

Topic: G.04. Physiological Methods

Title: Quantum wells embedded in semiconductor microtubes as optical sensors for action potentials

Authors: ***A. KOITMÄE**[1], J. HARBERTS[1], G. LOERS[2], C. BAUSCH[1], D. DIEDRICH[1], D. SONNENBERG[1], C. HEYN[1], W. HANSEN[1], R. BLICK[1];

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Abstracts: We suggest an advanced method of optical readout of action-potentials using semiconductor microtubes as resonators. The fabrication process of GaAs/InGaAs microtubes is based on the principle of self-organized rolling-up of strained semiconductor layers. The multilayer starts rolling-up during selective etching of the sacrificial layer (AlAs). The reason for this phenomenon is the slight difference of the lattice constants of InGaAs and GaAs [1,2]. The diameters of the tubes are kept in the magnitudes of the diameters of the axons (Fig. 1) and can be tuned to support a certain optical resonance mode. The toxicity issue of As is overcome using CVD deposition of Parylene-C. The optical activity of the microtubes is ensured by quantum wells (QW) embedded in the microtube multilayers. By illuminating the tubes with a green (532 nm) laser the tubes emit light at a certain wavelength (photoluminescence). Applying an external electrical field (action potential) to the QW, a shift in the emission spectrum can be observed (Stark effect). To guide the axons selectively only through the tubes, the areas of tube entrances are patterned with poly-l-lysine (PLL) using a material printer. [1] Koitmäe et al. Direct Transfer of GaAs Microtube Arrays onto Transparent Substrates for Imaging Neuron Outgrowth, Soft Nanoscience Letters 3, 79-82 (2013) [2] Bausch et al. Guided Neuronal Growth on Arrays of Biofunctionalized GaAs/InGaAs Semiconductor Microtubes, Applied Physics Letters 103, 173705 (2013)

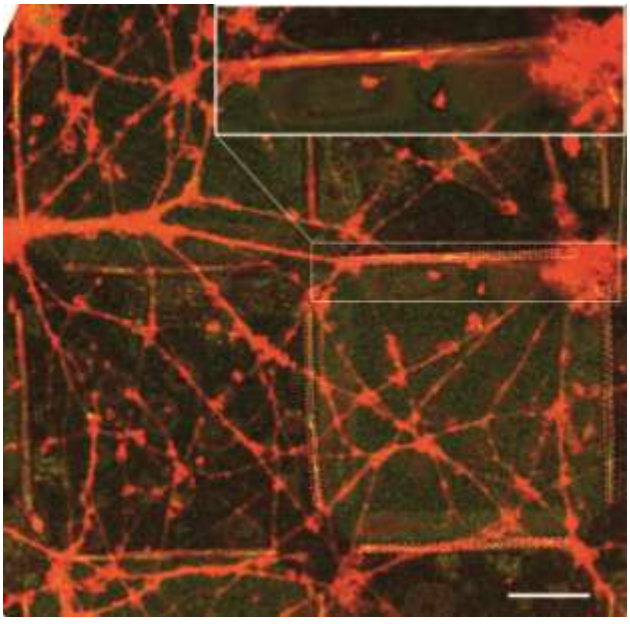

Fig. 1 Neurite outgrowth through semiconductor tubes (dashed white lines). Scale bar 20 µm.

Disclosures: **A. Koitmäe:** None. **J. Harberts:** None. **G. Loers:** None. **C. Bausch:** None. **D. Diedrich:** None. **D. Sonnenberg:** None. **C. Heyn:** None. **W. Hansen:** None. **R. Blick:** None.

Poster

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Topic: G.04. Physiological Methods

Support: OTKA K81354

ANR-TÉT Neurogen

ANR-TÉT Multisca

TÁMOP-4.2.1.B-11/2/KMR-2011-0002

EU FP7 600925 NeuroSeeker

Title: Two-dimensional, high-resolution current source density analysis of neuronal action potentials

Authors: *R. FIÁTH[1], P. BEREGSZÁSZI[3], K. KOCSIS[4], S. MUSA[5], P. RUTHER[6], I. ULBERT[2,3];
[2]Inst. of Cognitive Neurosci. and Psychology, [1]Res. Ctr. For Natural Sciences, Hungarian Acad. of Sci., Budapest, Hungary; [3]Fac. of Information Technol. and Bionics, Pázmány Péter Catholic Univ., Budapest, Hungary; [4]Eötvös Loránd Univ., Budapest, Hungary; [5]Interuniversity Microelectronics Ctr. (IMEC), Leuven, Belgium; [6]Dept. of Microsystems Engin. (IMTEK), Univ. of Freiburg, Freiburg, Germany

Abstracts: Action potentials (APs) are the basic computational elements of neuronal networks. The mechanisms of generation and propagation of APs in neurons are well described. However, *in vivo* two-dimensional extracellular recordings with high-resolution could reveal valuable new findings. One of the objectives of the Neuroseeker EU FP7 project is to develop ultra-high density, programmable silicon probes with thousands of small (~5 μm diameter), closely spaced (~1 μm) contacts. We used the first design of the probes in acute and chronic *in vivo* experiments to record the activity of cortical, hippocampal and thalamic neurons and to investigate the spatiotemporal distribution of transmembrane currents flowing during their APs. The silicon probe used contains sixteen recording sites with 10 μm in diameter arranged in four rows and another ten smaller contacts with 5 μm in diameter placed in two rows. The effective recording area is approximately 70x40 μm. Rats were anesthetized with ketamine/xylazine, and wideband (0.1-7000 Hz) neuronal activity was recorded from multiple regions of the brain. Spike sorting

was performed on the recorded data to isolate single neurons. Based on the sorted data, average AP waveforms were constructed from the raw recordings and the two-dimensional current source density (CSD) was estimated and visualized with Matlab-based softwares implementing the inverse CSD (iCSD) and kernel CSD (kCSD) methods. The CSD distributions calculated by the two methods were compared with each other. After that, we analyzed the two-dimensional propagation of the APs and the distribution of their transmembrane currents in different cell types, in consecutive spikes of spike bursts and in different brain states. With this method we could detect the initiation sites of APs near the soma of the neurons and backpropagation of the action potentials into the dendrites. We found significant differences in the current distribution between consecutive spikes of bursts during the afterhyperpolarization phase of APs. Both the iCSD and the kCSD method resulted in similar CSD distributions. However, the non-regular recording contact arrangement of this 26-contact probe makes the kCSD method more suited for the estimation of CSD, because it can manage data recorded from arbitrarily distributed electrode contacts, while the iCSD method can handle only regular grids and needs the interpolation of the local field potential. Based on our results we can conclude, that the two-dimensional, high-resolution CSD may be a useful tool in the future.

Disclosures: **R. Fiáth:** None. **P. Beregszászi:** None. **K. Kocsis:** None. **S. Musa:** None. **P. Ruther:** None. **I. Ulbert:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.10/VV62

Topic: G.04. Physiological Methods

Title: Flexible multi-electrode arrays for acute and chronic recordings

Authors: *C. M. LEWIS[1], E. FIEDLER[2], T. STIEGLITZ[2], P. FRIES[3];
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Abstracts: Understanding of brain function is currently limited by our ability to record both densely within regions of interest and simultaneously sample across many areas. Techniques that allow variable sampling of space, especially over extended periods of time, promise to expand

our understanding of brain function. We have developed flexible, polyimide-based multi-electrode arrays for acute and chronic preparations. These arrays allow the placement of many recording sites in arbitrary configurations and enable precise targeting of superficial and deep stereotaxic targets. Further, their size and flexibility allows minimally invasive implantation and an enhanced lifetime. We have implanted these arrays through the dura mater of monkeys, cats and rats, avoiding the necessity to make large craniotomies, or open the dura during implantation. Recordings have yielded isolated single units, as well as multi-unit activity and local field potentials, both in acute preparations, as well as in chronically implanted animals. In chronically implanted rats, we have successfully recorded unit and LFP activity from S1 and M1 for over 6 months. Arrays can be custom tailored in order to sample areas of interest with variable resolution: dense recordings of local populations, targeted sampling of laminae, or widespread coverage of multiple areas simultaneously. It is additionally possible to electrically stimulate through the arrays, or couple them with light guides for optogenetic manipulation. The flexible, polyimide substrate is also compatible with miniature FPC connectors, overcoming a major constraint on chronic implants: the necessary size of amplifier interfaces. Ours, and similar technologies promise increasing access and appreciation of brain dynamics across spatial and temporal scales.

Disclosures: **C.M. Lewis:** None. **E. Fiedler:** None. **T. Stieglitz:** None. **P. Fries:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

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Program#/Poster: 661.11/VV63

Topic: G.04. Physiological Methods

Support: Swedish Research Council, Linnaeus Grant 60012701

The Knut and Alice Wallenberg Foundation Grant KAW 2004.0119

Title: Development and initial characterizations of a novel 3D ultra-flexible neural interface that is implantable in brain tissue with preserved structure

Authors: *J. AGORELIUS, F. TSANAKALIS, A. FRIBERG, P. PETTERSSON, L. M. E. PETTERSSON, J. SCHOUENBORG;
Neuronano Res. Center, Exp Med. Sci., Lund Univ., Lund, Sweden

Abstracts: A major challenge in the field of Brain-Machine-Interface is to obtain stable and high quality neural recordings from minimally affected nervous tissue in the awake animal. Recent studies indicate that micromotions, caused by mechanical differences between tissue and implanted electrodes, play a key role for the glial reactions to the implant and thereby for the stability of recordings and survival of nearby neurons. To reduce these micromotions, we have here developed a new, ultra-flexible electrode array and methods to implant such delicate electrodes with preserved structure. In short, we have designed an ultra-thin electrode array consisting of eight gold leads (4 μm thick) insulated with parylene-C (4 μm), which are individually flexible in three dimensions and equipped with a distal uninsulated protrusion serving a dual role of anchorage and recording site. The electrodes had a mean impedance of 832 kΩ (SD=230 kΩ) at 1 kHz. To enable implantation into soft tissue of such delicate structures, the electrodes were embedded in a stiff, but dissolvable gelatin based material. The mechanical buckling force of the embedded electrodes was measured in a compression test to 0.373 N (SD=0.177 N), i.e. more than 20 000 times higher than for the non-embedded electrodes. Clarification of brain tissue was made post mortem using Methyl salicylate to visualize the structure of the implanted electrodes *in situ*. This analysis revealed that the structure of the electrode array was indeed preserved in the brain after up to 3 weeks of implantation. To verify electrode functionality, in-vivo recordings were made up to three weeks after implantation, and showed sufficient signal-to-noise ratios for high quality single unit recordings. In conclusion, we have developed a new type of ultra-flexible electrode designed to meet the needs for a stable, chronically implanted brain machine interface, confirmed that it can be implanted into the brain with retained conformation, as well as provide high quality recordings of single unit activity.

Disclosures: J. Agorelius: None. **F. Tsanakalis:** None. **A. Friberg:** None. **P. Pettersson:** None. **L.M.E. Pettersson:** None. **J. Schouenborg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Jens Schouenborg is a stake holder in Neuronano AB that has a patent on the electrode.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.12/VV64

Topic: G.04. Physiological Methods

Support: Melon

Title: Integrative physiobehavioral monitoring for health patterns feedback and research: Chronic wireless and computer vision analysis in an open-source platform

Authors: *T. A. NICK, E. D. LUNDQUIST, L. M. BERMAN, A. Z. BARNEHAMA; Neurosci., Melon, Santa Monica, CA

Abstracts: Clinical snapshots of physiological measurements, such as heart rate and electroencephalogram (EEG), have revealed early biomarkers for various disorders of the nervous system, as well as other body systems. Increasing evidence indicates that time-evolving patterns of physiological measurements may provide even earlier signs of disease and/or behavioral patterns that may lead to disease. For example, clock genes may have a role in autism (Nicholas et al., 2007, Mol Psychiatry, 12:581-92) and circadian rhythm disruption may increase cardiovascular risk factors (Arble et al., 2010; Best Pract Res Clin Edocrinol Metab, 24(5):785-800). Personal measurements of physiological cycles or trajectories over days and longer periods may assist clinicians in diagnoses and lifestyle/treatment recommendations. A user-friendly, non-invasive, self-monitoring platform is therefore required to provide long-term, even chronic, measurements of basic physiological metrics. If the software were also open-source, the platform could be specialized and extended for each person and/or researcher to enable focused and lightweight analysis of particular metrics of interest. We have built such a platform with these basic and modifiable metrics: resting pulse, heart rate variability, and pulse wave velocity using computer vision; brain frontal lobe oscillatory activity (EEG) and eye blinks with forehead electrodes; and muscle contraction with a resizable band that can be strapped to limbs. Temporal integration of these various metrics may provide additional information that is not available through isolated measures. Moreover, daily (and longer) patterns of these and other measures can be saved and viewed later by the user, physician, or researcher. The current system is built on the Apple OS X desktop and iOS mobile platforms. Raw signals from the hardware and/or processed waveforms and metrics can be displayed in a configurable display panel. The software also contains user-configurable filter settings, as well as real-time export capability for on-line analysis and feedback. The EEG hardware employs a low-power, 24-big analog front-end (Texas Instruments, ADS1294), coupled with a low-power Bluetooth SoC (Nordic nRF51822). Low-noise, differential recordings employ four forehead electrodes. We will present (1) proof-of-concept data that indicate the feasibility of this system and (2) preliminary findings on circadian variability and correlations of measured physiological parameters in healthy adults.

Disclosures: **T.A. Nick:** A. Employment/Salary (full or part-time):; Melon (full-time). **E.D. Lundquist:** A. Employment/Salary (full or part-time):; Melon (full-time). **L.M. Berman:** A. Employment/Salary (full or part-time):; Melon (full-time). **A.Z. Barnehama:** A. Employment/Salary (full or part-time):; Melon (full-time).

Poster

661. Electrophysiology Recording Tools and Techniques

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Program#/Poster: 661.13/VV65

Topic: G.04. Physiological Methods

Support: NSF CAREER Award 1149446

Title: Whole-brain electrophysiological recording in the isolated naked mole-rat brain

Authors: *D. P. MCCLOSKEY[1,3,2], T. BUDYLIN[3,2], M. ZIONS[3,2];
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Abstracts: Electrophysiological recording of the whole brain *ex vivo* has been performed in a number of mammalian species, with the guinea pig preparation developed by Mühlethaler, de Curtis, Walton and Llinás (1993) among the most successful. In this technique, the basilar artery is catheterized and perfused with oxygenated artificial cerebrospinal fluid. Arterial pressure is maintained with the use of a peristaltic pump and the ligation of severed arteries. This approach can provide neuronal viability for a number of hours, and allows access to many different areas of the brain simultaneously. The guinea pig is particularly well-suited for this preparation because of the large diameter of the posterior communicating arteries relative to the basilar artery. Here, we have discovered that the fossorial rodent species known as the naked mole-rat (Heterocephalus Glaber)has similar cerebral vascular to the guinea pig, and is therefore well-suited for the isolated whole brain preparation. Naked mole-rats are hypoxia resistant and seizure-prone, so they provide a great opportunity to understand circuitry involved in seizure generation and hypoxia-response. Transcardial perfusion of the naked mole-rat with india ink demonstrates relatively large diameter posterior communicating arteries. Catheterizing the basilar artery in this species allows for stable electrophysiological recordings for at least 6 hours *ex vivo*. We have recorded field potentials in the piriform cortex following bipolar electrode stimulation of the olfactory bulb or lateral olfactory tract. We have also recorded field potentials in the hippocampus following stimulation of the entorhinal cortex. In the absence of stimulation, we have observed spontaneous epileptiform events in the hippocampus similar to what we have recorded in hippocampal slices of naked mole-rats. These events were enhanced with the perfusion of 10μM bicuculline. We are combining this approach with multi-electrode array recording and graph theory-based network analysis to map circuitry between areas of the brain.

Disclosures: **D.P. McCloskey:** None. **M. Zions:** None. **T. Budylin:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

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Program#/Poster: 661.14/VV66

Topic: G.04. Physiological Methods

Support: NIH Grant 7R43DA035545-02

NIH Grant 1R43AA022030-01

Title: High spatial and temporal resolution chemical measurements with functionalized neural probe

Authors: ***E. BIGELOW**[1], C. WHITE[1], J. FEINBERG-SOMERSON[2], K. PLAXCO[2], B. G. JAMIESON[1];
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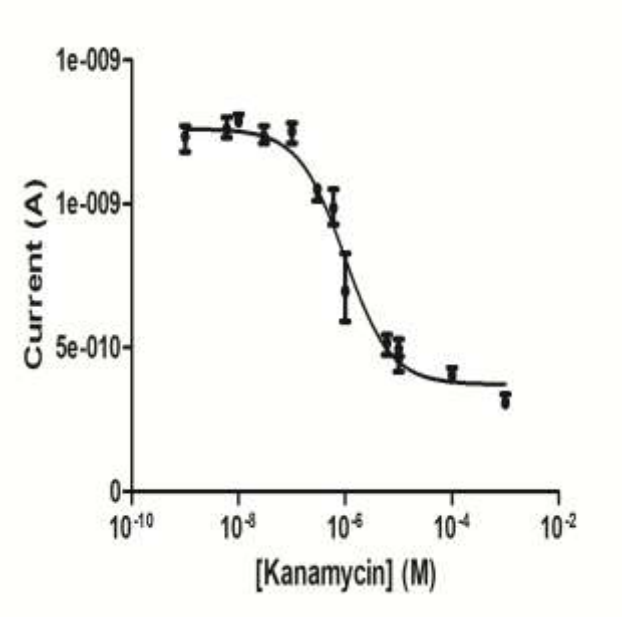
Abstracts: This study demonstrates proof-of-concept for aptamer-based biosensing on microfabricated neural probes (array of 400 μm^2 sites). The techniques described will be applied to the *in vivo* detection of multiple chemicals at high temporal and spatial resolution. Current *in vivo* chemical detection techniques are limited to microdialysis, with low temporal resolution (measurements every 2-20 minutes), or enzyme-based sensors and fast scan cyclic voltammetry, which are limited to single analyte detection and small range of detectable target molecules. Aptamer functionalized microfabricated neural probes are predicted to collect chemical concentration data every 5 seconds *in vivo* with detection sites every 50 μm. Aptamers are particularly well suited as a bio-recognition element for sensing *in vivo*, as they are non-immunogenic, do not consume the analyte, can be tuned for a variety of binding kinetics, and can be used for detection in hypoxic conditions. In this study, neural probe sites (array of 16 400 μm^2 sites) were functionalized with aptamers by forming a self-assembled monolayer (SAM) through a 5' alkanethiol modification. Additionally, these aptamers have a 3' methylene blue modification for current-based readout and *in vivo* stability. Aptamers designed to detect kanamycin were used in this proof of concept study. Square wave voltammetry (SWV) was used to detect changes in current modulated by the 3' methylene blue groups moving closer or further away from the gold site when unbound and bound, respectively, to the analyte. Signals of up to

1.0 nA, or an 80% signal gain, were detected. The sensor was evaluated for sensitivity at various physiologically relevant kanamycin concentrations, and exhibits a linear response range of 100 nM – 10 uM. This study demonstrates a proof-of-concept for aptamer-based biosensor fabrication and readout techniques on a microfabricated electrode. Applying these methods with neurotransmitter aptamers, such as those for dopamine, GABA, and substance P, will produce a powerful new chemical detection system with high temporal and spatial resolution.



Disclosures: **E. Bigelow:** A. Employment/Salary (full or part-time):; Diagnostic Biochips. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Diagnostic Biochips. **C. White:** A. Employment/Salary (full or part-time):; Diagnostic Biochips. **J. Feinberg-Somerson:** None. **K. Plaxco:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Diagnostic Biochips. **B.G. Jamieson:** A. Employment/Salary (full or part-time):; Diagnostic Biochips. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Diagnostic Biochips.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.15/VV67

Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research (A) JSPS [Ishida]

Grant-in-Aid for Young Scientists (A) JSPS [Kawano]

PRESTO program JST [Kawano]

Title: *In vivo* recordings via microscale diameter neuroprobe block devices

Authors: *H. SAWAHATA[1], S. YAMAGIWA[1], A. MORIYA[1], H. OI[2], Y. ANDO[2], R. NUMANO[2], M. ISHIDA[1], K. KOIDA[2], T. KAWANO[1];
[2]Electronics-Inspired Interdisciplinary Res. Inst. (EIIRIS), [1]Toyohashi Univ. of Technol., Toyohashi, Aichi, Japan

Abstracts: We have proposed a fine (<10 μm in diameter) and high-density multi-electrode array fabricated by the selective vapor−liquid−solid (VLS) growth of silicon 'whisker' wires (Fujishiro et al., 2014). Such fine electrodes potentially offer both low invasive and high spatial resolution of neuronal recordings *in vivo*. However, the fabrication of the electrode arrays with 100% yield is problematic. In addition, varied characteristics of each electrode in the array might degrade the performance of the device. To overcome the device issues, here we devised a manufacturing technique by using single whisker electrode modules. To fabricate the electrode block as a module, the silicon whisker needle electrode (3 μm in diameter, 160 μm in length) was fabricated on a low resistance one-millimeter square silicon block by the VLS growth technology. As the metallization, platinum was deposited over the needle shaft, which was then insulated with an insulating layer of parylene-C except for the electrode tip (1.5 μm in height). Such small diameter platinum-electrode exhibits a high electrolyte/electrode interfacial electrical impedance, which was decreased to a lower impedance of ~100 kΩ at 1 kHz in saline by platinum-black plating. An amplifier was connected to the backside of the electrode block via an IC-socket pin connector. To evaluate the *in vivo* recording capability of the device, fabricated electrode block modules were tested in the acute animal experiments. To access to the occipital lobe of a mouse brain, where visual areas exist, the skull and dura mater were opened under anesthesia. The electrode was stereotaxically defined and inserted into the primary visual area. Signals from the needle electrode were amplified using an amplifier with 300 Hz and 5 kHz cut-off frequencies. As a result, spike activities were detected in response to the visual stimulation (white LED flash, 500 ms in duration) for the contralateral eye. The signal/noise ratio was sufficiently large for detection of unit activity. In conclusion, we demonstrated *in vivo* recordings via microscale diameter neuroprobe block devices. The proposed electrode devices can be assembled as high yield electrode arrays in future multi-site recordings. In addition, we are currently working on the assembly of an array of heterogeneous electrodes including various lengths, shapes and materials of whisker needle electrodes for recording from various types of cortical neurons.

Disclosures: **H. Sawahata:** None. **S. Yamagiwa:** None. **A. Moriya:** None. **H. Oi:** None. **Y. Ando:** None. **R. Numano:** None. **M. Ishida:** None. **K. Koida:** None. **T. Kawano:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

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Program#/Poster: 661.16/VV68

Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research (A)

Grant-in-Aid for Young Scientists (A)

PRESTO program

Title: Fabrication of microscale diameter neuroprobe block devices

Authors: ***S. YAMAGIWA**[1], H. SAWAHATA[1], A. MORIYA[1], M. ISHIDA[1,2], K. KOIDA[2], T. KAWANO[1];
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Abstracts: We have proposed a microfabricated needle-like penetrating 'whisker' electrode (needle diameter = < 5 μm) array, and the *in vivo* recording capability has been demonstrated [A. Fujishiro et al, Sci. Rep., 4, 4868, 2014]. However, the low-yield fabrication process of the electrode arrays is still problematic for multi-site recordings via a large number of electrodes. Here we propose the simplified fabrication process and assembly of a single whisker electrode device for the electrode arrays with 100% yield. As a piece of electrode, we design the single whisker electrode on a one-millimeter square block of silicon. Such pieces of electrode block can be assembled into numerous designs of electrode arrays with 100% yield; herein we report the fabrication process of the single whisker electrode. First of the fabrication process, a vertical microneedle of silicon (~3 μm in diameter) was fabricated on a silicon substrate by vapor-liquid-solid (VLS) crystal growth technology. After the microneedle fabrication, platinum was deposited on the top- and bottom-sides of the substrate. After the metal patterning, an insulating layer of parylene-C was deposited on the substrate and the tip section of the needle was exposed by oxygen plasma. Finally, each section of the needle electrode was separated by silicon wafer

dicing. For *in vivo* recordings, each electrode block was packaged with an IC socket pin, which can be connected to a head-amplifier of a recording system. The sidewall of the electrode block and the IC socket pin were insulated by silicone. The block size of the electrode will be minimized (< one-millimeter square) in future design. In our previous study, nanoscale-tipped probes [Y. Kubota et. al., IEEE MEMS 2014 conf., 2014] and multi-functional (e.g., drug delivery) tube probes [K. Takei et. al., Biomed. Microdevices, 11, 3, 2009] have been proposed and demonstrated in animal experiments. As a future work, these probes will be integrated in one chip, enhancing the performance of the electrode device.

Disclosures: **S. Yamagiwa:** None. **H. Sawahata:** None. **A. Moriya:** None. **M. Ishida:** None. **K. Koida:** None. **T. Kawano:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

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Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research (A)

Grant-in-Aid for Young Scientists (A)

PRESTO program

Title: An array of vertically aligned nanoscale tipped microprobe electrodes

Authors: ***Y. KUBOTA**[1], H. OI[2], H. SAWAHATA[1], A. GORYU[1], Y. ANDO[2], R. NUMANO[2,3], M. ISHIDA[1,2], T. KAWANO[1];
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Abstracts: Electrophysiological methodology is a way to understand the communication between neurons in a tissue. Especially, multi-channel intracellular recording within a tissue becomes a powerful methodology, in terms of the large amplitude (~100 mV) and the signal quality (synaptic potential measured) compared to extracellular recording (< 100 μV). Nanoscale bioprobe devices have been proposed for intracellular recordings. However these nanodevices cannot be used for thick tissues due to the short probe length of 100-μm-long microprobe electrodes (NTEs) array and the electrical characteristics for intracellular applications. We have

proposed nanoscale tipped high-aspect-ratio silicon-microwire array fabricated by vapor-liquid-solid (VLS) growth of silicon-microwire and the nanotip formation. After the silicon-nanowire formation, the metallization for both the nanowire and the device interconnection was conducted by metal sputtering (e.g., iridium and platinum). The sidewall of the nanowire and the interconnection were insulated with parylene, while the nanotip-section was exposed by plasma etching. The fabricated NTE exhibited the tip diameter of < 500 nm. The height/area of the nanotip exposed from the parylene-shell can be controlled by changing the time of the plasma etching, resulting in the exposed tip-height of < 5 μm. The electrolyte/metal interfacial impedance of the electrode is an important characteristic of the intracellular electrode. The impedance of the fabricated NTE measured in saline exhibited < 5 MΩ at 1 kHz, with the output/input signal amplitude ratio of > 50%. Since the measured noise of the recording system was tens of microvolts, ~ 50% attenuated intracellular potentials (>> noise level) can be obtained by the NTE. The attenuation of the O/I ratio was due to the set of the impedance of the NTE and parasitic impedances embedded in the recording system. The improving the ratio will be important for investigating intracellular signals. However, we believe that the fabricated NTE array device can be used for numerous biological samples including brain slice and perform multi-channel intracellular potential recordings deep within the tissue.

Disclosures: Y. Kubota: None. **H. Oi:** None. **H. Sawahata:** None. **A. Goryu:** None. **Y. Ando:** None. **R. Numano:** None. **M. Ishida:** None. **T. Kawano:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.18/VV70

Topic: G.04. Physiological Methods

Title: Neural correlates of tourette syndrome within the centromedian thalamus, premotor and primary motor cortices

Authors: *J. B. SHUTE[1,2], N. MALING[5], J. ROSSI[3], C. DE HEMPTINNE[6], K. FOOTE[4], M. OKUN[3], A. GUNDUZ[2];
[1]BioMedical Engin., [2]J. Crayton Pruitt Family Dept. of BioMedical Engin., [3]Dept. of Neurol., [4]Dept. of Neurolog. Surgery, UF, Gainesville, FL; [5]Case Western, Cleveland, OH; [6]UCSF Sch. of Med., Neurological Surgery, CA

Abstracts: Tourette Syndrome (TS) is a neurological disorder characterized by undesired motor and vocal tics. Treatment of TS varies depending on symptom severity and individual therapy effectiveness. Deep brain stimulation (DBS) is an emerging therapy for the treatment of many neurological disorders, including severe TS through the electrical stimulation of deep brain nuclei. A patient with Tourette's Syndrome was implanted at University of Florida (UF) Shands Hospital with two bilateral subdural 4-contact cortical strip electrodes and two bilateral 4-contact subcortical depth electrodes with the aim of capturing tic-related physiology. All procedures were approved by the FDA and UF IRB. The depth electrodes were placed bilaterally within the centromedian nucleus of the thalamus (Cm) and the cortical subdural strips were placed bilaterally over the premotor (PM) and primary motor (M1) cortices. Local field potentials (LFP) from the depth electrodes, electrocorticograms (ECoG) from the cortical electrodes, and electromyograms (EMG) from electrodes placed over the forearm and cheeks were recorded intra-operatively while the patient was awake. The patient was asked in interleaved trials to suppress tics (baseline), make right and left hand movements, imitate tics, and tic freely. The patient's tics presented as right hand and right facial twitches, along with right arm flexion-extension. In our preliminary analysis, we sought to discover the neural correlates of tics as related to TS and to differentiate them from voluntary movements and from imitated tics. The initial results suggest that when compared to baseline, broadband gamma activity in PM, M1 and Cm were correlated with contralateral tic behavior compared to baseline. This observation was different than what was observed in voluntary movements, which were associated with contralateral cortical activity but not thalamic activity. Local and subcortical-to-cortical phase amplitude coupling (PAC) suggested regionally specific activation patterns across PM, M1 and Cm. Future analysis of PAC between the thalamus and motor cortex during baseline, tic, and motor activity will likely provide additional spatial and temporal features important to understanding the physiology underpinning TS. Further refinement of these findings may lead to the identification of neural signatures for TS, and pave the way for the development of responsive stimulation paradigms.

Disclosures: J.B. Shute: None. **A. Gunduz:** None. **C. De Hemptinne:** None. **K. Foote:** None. **M. Okun:** None. **N. Maling:** None. **J. Rossi:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.19/VV71

Topic: G.04. Physiological Methods

Title: Intraoperative functional mapping of hand premotor cortex for chronic implantation of subdural strip electrodes

Authors: *N. MALING[1], J. B. SHUTE[1], P. J. ROSSI[1], C. DE HEMPTINNE[2], J. C. SANCHEZ[3], B. KRETZMAN[1], A. W. SHUKLA[1], K. D. FOOTE[1], M. S. OKUN[1], A. GUNDUZ[1];
[1]Univ. of Florida, Gainesville, FL; [2]Neurolog. Surgery, UCSF, San Francisco, CA; [3]Univ. of Miami, Miami, FL

Abstracts: A patient with Tourette's Syndrome was implanted at University of Florida Health Hospital with bilateral subdural 4-contact cortical strip electrodes and bilateral 4-contact subcortical depth electrodes with the aim of capturing tic related physiology. These electrodes were connected to a chronic sensing and stimulation device Activa PC+S (Medtronic Inc, Minneapolis, MN). All procedures were approved. The patient exhibited strong hand motor tics and thus the premotor and primary hand motor cortices (PM, M1) were target locations for the cortical strips. While implantation of bilateral cortical and subcortical electrodes is a great step forward in deep brain stimulation (DBS) and in recording technology, surgical placement of chronic, small subdural strips (44mm x 8mm x 1.8mm paddle, 10.2mm center to center contact distance, 4mm contact diameter) requires precise localization to obtain relevant signals, given the uncertainty in the representation of tic phenomena in the cortex. Herein we describe a confluence of techniques, the combination of which were effective in targeting the motor and pre-motor cortex. Preoperatively, the patient underwent transcranial magnetic stimulation (TMS) for hand motor cortex mapping. Areas that induced maximal thenar group activation were labeled with fiducial markers on the scalp. 3T MRI scans were acquired with these markers to aid in identifying the structural 'hand knobs' approximately 4mm lateral to the longitudinal fissure, and anterior to the central sulcus. Subdural strips were placed through a bur-hole such that the anterior contacts were approximately over M1, and the posterior contacts were over primary somatosensory cortex (S1). Somatosensory evoked potentials (SSEPs) were elicited and recorded from the implanted ECoG strip. Phase reversal was delineated the boundary between M1 and S1. We then partially retracted the ECoG strips based on the boundary location, such that the posterior contact was placed at the posterior border of M1, and the anterior contact presumably lay over PM. To verify final placement, real-time functional mapping was carried out to verify the location of the hand representation in the underlying cortices. This mapping utilized changes in high gamma range oscillatory activity coupled to behavior to calculate the probability that the activity in the underlying cortex was specifically related to the respective motor behavior. This combination of methods ensured that the strip electrodes were optimally placed, with posterior contacts over M1, and the anterior contacts over premotor cortex. Finally, post-operative CTs were co-registered with pre-operative MRIs to confirm placement.

Disclosures: **N. Maling:** None. **J.B. Shute:** None. **P.J. Rossi:** None. **C. de Hemptinne:** None. **J.C. Sanchez:** None. **B. Kretzman:** None. **A.W. Shukla:** None. **K.D. Foote:** None. **M.S. Okun:** None. **A. Gunduz:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.20/VV72

Topic: G.04. Physiological Methods

Support: Allen Institute for Brain Science

Title: End of anonymity for single units in extracellular recordings

Authors: *P. LEDOCHOWITSCH, D. DENMAN, A. CHENG, G. SOLER-LLAVINA, H. ZHENG, T. J. BLANCHE;
Neural Coding, Allen Inst. For Brain Sci., Seattle, WA

Abstracts: Single-unit electrodes remain the principal technology for *in vivo* electrophysiological studies. Compared to conventional single- or multi-wire electrodes, silicon multielectrode arrays ('polytrodes') provide better single-unit isolation and enable simultaneous recording from dozens of neurons. However, in practice, large-scale recordings are typically analyzed without regard for spatial interrelationships, or sufficient appreciation of sampling bias towards larger or more active neurons – due to the lack of robust techniques to extract cell type, anatomical location, morphology, or functional connectivity from extracellular electrophysiology data. Accurate, automated spike sorting remains a tremendous challenge, especially for high channel-count arrays. We are creating tools to exploit the unique electrical 'signature' of neurons recorded on polytrodes of unprecedented density to estimate cell type and soma location. To achieve this, we are combining electrophysiology with optical physiology: polytrodes with up to 900 recording sites spaced 20 μm apart, genetically targeted *in vivo* 2-photon resonant Ca^{2+} imaging in triple-transgenic mice of the type PRM-Cre x Camk2a-tTA x Ai93-GCamP6f (where PRM indicates a cell type or layer-specific promoter such as e.g. Scnn1a for layer 4), and the targeted simultaneous excitation of neurons transfected with ReaChR (a red-shifted channelrhodopsin derivative) in a 3D volume via digital holography with a spatial light modulator (SLM). From these cell type- and location-specific signatures, we extract the parameters for a hybrid monopole-dipole field model of extracellular spike potentials to test how well such a neuron localization model can be generalized to arbitrary neuron orientation, tissue

anisotropy, and cell morphology. We can also determine, for the first time, the cell type specific biases of extracellular electrodes. Compared to the currently available optical techniques for imaging population activity, polytrodes provide higher temporal resolution, allow access to all cortical layers, do not require genetic intervention, and are more readily applicable to awake behaving animals. We aim to provide complementary information about the functional and anatomical properties of a large number of neurons *in vivo*, bridging the divide between structure and function. In addition, our approach will provide ground truth data for validating spike sorting algorithms, for optimizing the design of next generation polytrodes to achieve more complete neural sampling, and allow tracking of neural ensembles over time in the event of electrode drift in chronic large scale neuronal recordings.

Disclosures: P. Ledochowitsch: None. **D. Denman:** None. **T.J. Blanche:** None. **A. Cheng:** None. **H. Zheng:** None. **G. Soler-Llavina:** None.

Poster

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Program#/Poster: 661.21/VV73

Topic: G.04. Physiological Methods

Support: NIH Pioneer Award

Title: A wireless transmission neural interface system for non-human primates

Authors: *J. FERNANDEZ-LEON[1,2], A. PARAJULI[1], M. MULAS[3], M. HU[1], R. FRANKLIN[4], M. SORENSON[4], B. HANSEN[5], V. DRAGOI[1];
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Abstracts: Research over the last few years has opened up the possibility to study brain dynamics in animals freely moving in their natural environment. However, experimentation with unconstrained large animals, such as non-human primates, requires compact and robust devices in order to acquire neural data during free movement. To address this issue we developed a high fidelity wireless transmission set up to record extracellular spikes and local field potentials from

a range of cortical areas. A compact wireless transmitter (a 96-channel wireless broadband, 0.03 Hz-7.5k Hz; Blackrock CerePlex Radio) attaches onto a skull-mounted pedestal that connects to a microelectrode Utah array (Blackrock Microsystem Inc.). The wireless transmitter (IEEE 802.11n) integrates several low power, custom integrated circuits that requires two 3V CR123A batteries, and +5V on the receiver. For flexible management of the mobile subject's electromagnetic environment, the RF receiver transmission range (radio power 15dBn) can be extended from 32ft by in-line positioning of additional antennae in 6.5ft increments. The electronic operating environment ranges from 10°C to 40 °C, 5 to 95% R.H. (non-condensing) with a sampling frequency of 20ksps. The wireless device interfaces with the Blackrock Cerebus Neural signal processor. We tested our system by acquiring and decoding neuronal responses in real time from cells populations in area V4 and in dorsolateral prefrontal cortex (dlPFC) in freely behaving monkeys (Macaca mulatta). Animals performed different foraging tasks in the presence of randomly placed objects with and without food reward. In addition, we tested the wireless system by recording in dlPFC in an 'unfairness payment experiment'. We tracked the recorded neural population over days by analyzing single unit waveforms, principal component clusters, and response properties of single units and multi-unit clusters. The wireless neural recording system that we have developed represents a step forward in our attempt to study adaptive behavior in naturalistic tasks while recording wirelessly from many neurons across multiple brain areas simultaneously.

Disclosures: J. Fernandez-Leon: None. **A. Parajuli:** None. **M. Mulas:** None. **M. Hu:** None. **R. Franklin:** None. **M. Sorenson:** None. **B. Hansen:** None. **V. Dragoi:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Wellcome Grant 095668

Wellcome Grant 062379

EPSRC Grant K015141

Title: Spike sorting for large, dense electrode arrays

Authors: *S. N. KADIR[1], C. ROSSANT[1], D. F. GOODMAN[2], K. D. HARRIS[1];
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Abstracts: Understanding how the brain processes information will require simultaneous recordings of large number of neurons, both locally within structures, and distributed over wide areas of brain tissue. The only technology currently capable of such recordings is extracellular electrophysiology. Recent developments in microfabrication have enabled the production of electrodes with hundreds of low-impedance recording sites, and electrodes with thousands of sites are currently under development. The problem of using the raw data recorded by these probes to decipher the firing times of the recorded neurons - known as "spike sorting" - represents a fundamental technical challenge for systems neuroscience. Techniques for the spike sorting of data from small-scale electrode arrays such as "tetrodes" are now mature, but do not scale to next-generation, large, dense electrode arrays. Current techniques typically operate in three stages: 1) detection of spikes and extraction of a feature vector summarizing each spike's waveform across channels; 2) automatic clustering of feature vectors into groups putatively corresponding to single neurons; 3) manual inspection and correction of these groups using graphical software. Although these techniques perform close to optimally for small electrode arrays, none of these three stages scale to the large, dense geometry of next-generation electrode arrays. We present an integrated system for spike sorting next-generation electrode arrays, based on algorithms enhanced to work with large dense arrays. Our system has four components: Ï SpikeDetekt, a program for detection and analysis of action potential waveforms from large-scale arrays, based on a flood-fill algorithm that detects each spike only from spatially localized channels; Ï KlustaKwik 3.0, a program designed for automatic clustering of high-dimensional data using a novel "Masked EM" algorithm; Ï KlustaViewa, a graphical program for manual verification of machine performance, in which fast machine performance is made possible by GPU optimization, and human time is minimized by a "Wizard" that guides the operator through the manual verification process. Ï KwikFormat, an HDF5-based file format for efficient processing, storage, and sharing of large-scale electrophysiology data. All software is freely available at http://klusta-team.github.io/. Testing on data from large-scale cortical recordings suggests that performance is close to an estimate of the upper bound achievable.

Disclosures: S.N. Kadir: None. **C. Rossant:** None. **D.F. Goodman:** None. **K.D. Harris:** None.

Poster

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Program#/Poster: 661.23/VV75

Topic: G.04. Physiological Methods

Title: Model-based measurement of eeg data from linear high-density array

Authors: *R. KOZMA[1], W. J. FREEMAN, III[2], J. J. DAVIS[3], C.-T. LIN[4];
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Abstracts: Aim: Our goal is to validate hypotheses about the existence of metastable amplitude patterns and propagating phase gradients using a linear array of high-density noninvasive scalp electroencephalogram (EEG) array. Introduction: Intermittent transitions between brain states with and without large-scale synchronous oscillations carrying spatial patterns are hallmarks of higher cognition, which were first identified using invasive intracranial ECoG measurements. Recent results by noninvasive scalp EEG measurements indicate that it is feasible to measure these amplitude and phase patterns using scalp EEG as well [1]. Methods: Based on theoretical considerations and experimental studies, we optimize the array design, to extract cognitively relevant attributes from the measured neural fields [2]. We employ a network theory-based model called neuropercolation, which approximates neural processes in brains as a generalized percolation process through the layered sheet of the cerebral cortex [3]. Heterogeneous neuropercolation models are developed incorporating structural connectivity patterns identified by brain EEG imaging techniques [1]. Results: High temporal and spatial resolution allows studying canonical power spectral densities (PSDs) in time (PSDt) and space (PSDx) [1, 4, 5]. The canonical PSDs at rest are generally self-similar with slope indicative of the cognitive state. The self-similar range is bounded with a plateau at low frequencies up to a break frequency determined by the time of the impulse response, linear decrease in log power with log frequency determined by the decay rate of the impulse response, and a plateau at high frequencies due to the background noise level [6]. Neuropercolation models reproduce the experimental behavior near critical states and used for the extraction of cognitively relevant information from the data. Conclusions: We develop a novel experimental technology with wireless array that allows monitoring cognitive activity without significant interference with the daily activity of the participants. The experiments are interpreted using our graph-theoretical model and lead to the conclusion that the data by the high-density array manifest neural correlates of cognition.
References: 1 Freeman WJ, Quian Quiroga R (2013) Imaging Brain Function with EEG, Springer. 2. Liao L-D (2012) Proc IEEE 100 (13): 1553-1566 3. Kozma, R., Puljic, M., Freeman, W. J. (2012). arXiv preprint arXiv:1206.1108. 4. Ramon, C., & Holmes, M. D. (2013). Brain Topography, 26(1): 1-8. 5. Freeman et al. (2003) http://escholarship.org/uc/item/67x7q43f 6. Freeman WJ, Zhai J (2009) Cognitive Neurodynamics 3(1): 97-103.

Disclosures: **R. Kozma:** None. **W.J. Freeman:** None. **J.J. Davis:** None. **C. Lin:** None.

Poster

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Topic: G.04. Physiological Methods

Support: NIH Grant EY022730

Title: Quantitative methods for determining spike sorting quality in neonatal rodent cortex

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Abstracts: During development neuronal circuits produce unique patterns of activity that contribute their formation. An understanding of this early activity requires examination at the level of single units embedded in the ongoing network activity, a task requiring spike sorting from high-density multi-electrode arrays. Spike sorting in young brains has a unique set of difficulties that include low spike amplitudes, a high prevalence of bursts, low firing frequencies, and less variation of action potential shapes between neuron classes. Rigorous comparison of nascent and adult networks based on spike-sorted data will require quantitative metrics of sorting quality that can be compared between groups. Here we quantify the quality of individual clusters using established measures of inter-cluster separation (L-ratio & ISO distance) complemented by a new measure of intra-cluster spike similarity ('Similarity Index') based on the mean distance between all spike waveforms. Addition of the Similarity Index allows for automatic and objective splitting of clusters tentatively identified using the masked EM algorithm (Klustakwick), as well as merging of clusters with similar waveforms. By setting a threshold for the separation and similarity metrics to define good clusters, we establish a metric of overall quality for each tet/poly-trode, the percentage of spikes assigned to usable clusters, called 'Coverage'. Quantification of the average L-ratio/ISO distance, Similarity Index, and Coverage in a population for a given set of experimental parameters can be used to quantify differences between ages. We are testing these methods using single shank parallel electrode arrays in visual cortex of unanaesthetized rats, where preliminary results show we can achieve close to 90% coverage in animals from P9 and older. These validation metrics may be useful when comparing

any potentially variant neural networks, for example seizing vs non-seizing tissue, or in models of neurological disorders that could affect activity patterns.

Disclosures: **C. Lai:** None. **J. Berzhanskaya:** None. **M.T. Colonnese:** None.

Poster

661. Electrophysiology Recording Tools and Techniques

Location: Halls A-C

Time: Tuesday, November 18, 2014, 1:00 PM - 5:00 PM

Program#/Poster: 661.25/VV77

Topic: G.04. Physiological Methods

Support: KIST Institutional Fund 2E24184

Title: MEMS neural probe for local drug delivery to mouse brain with simultaneous recording of neural signals

Authors: ***I.-J. CHO**[1], H. LEE[2], Y. SON[2], E.-S. YOON[2], J. KIM[3], C. J. LEE[3], D. KIM[4], Y. KIM[4];
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Abstracts: We present a multi-functional silicon neural probe that consists of a microfluidic channel for local delivery of a precise quantity of a drug and multiple recording electrodes to simultaneously monitor the effects of drug delivery. The new fabrication method developed for this integrated drug delivery probe allows the channel to be embedded in the probe substrate. Thus, no additional thickness is required due to the presence of the channel. The successfully fabricated 40-m-thick and 100-m-wide neural probe consists of a single shank with 16 multiple recording sites (10 m 10 m), a silicon channel enclosed by glass and two reservoirs (inlet and outlet with radius of 500 m and 30 m, respectively). The recording sites are located near outlet port to record neural spike signals stimulated by delivered drug. The probe was characterized for its flow rate and electric impedance. The flow rate of 0.2 l/min was obtained at the air pressure of 200 kPa and linearity confirms precise quantity control. The impedance of 16 microelectrodes was measured using electrochemical impedance spectroscopy, where an average impedance value of 1.0 M at 1 kHz was measured, which is low enough to measure neural spikes. In this work, we also demonstrate a successful *in vivo* recording of neural action potentials from the thalamus of an anesthetized mouse. The fabricated MEMS neural probe was robust enough to penetrate the mouse's pia without bending or fracturing. We moved the implanted neural probe

to the target position (AP: 3.2, ML: 1.2, DV: -4.56) and delivered 0.6 l of Baclofen (100 mM) to induce absence seizure in a wild type mouse through the integrated microfluidic channel. And, we were able to monitor synchronous spike-and-wave discharges (SWDs) using integrated microelectrodes. Also, the SWDs were disappeared and basal neural spikes were appeared again in 1 hour after drug injection. These results are similar to the results of intraperitoneal injection and it confirms that Baclofen was successfully delivered to the mouse brain. In this abstract, we present an improved multi-functional silicon neural probe that is capable of selectively delivering a chemical to a highly localized region of interest and recording neuron responses *in vivo* simultaneously. By embedding the channel in the substrate, the thickness of the probe is no longer limited by the presence of the channel and thus the cell damage induced by such probe can be further minimized. In addition, the presented probe has a wide range of uses in the neuroscience including monitoring the effects of drugs for treatment of physiological disorders and tracing neural pathways.

Disclosures: **I. Cho:** A. Employment/Salary (full or part-time):; Korea Institute of Science and Technology. **H. Lee:** None. **Y. Son:** None. **E. Yoon:** None. **J. Kim:** None. **C.J. Lee:** None. **D. Kim:** None. **Y. Kim:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: CAPES

CNPq

FAPERN

Title: A comparison of metrics for phase-amplitude coupling

Authors: *C. RENNÓ-COSTA, A. B. L. TORT;
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Abstracts: Phase-amplitude cross-frequency coupling (PAC) occurs when the amplitude of a higher frequency oscillation is modulated by the phase of a lower frequency oscillation. Recent studies in neurobiology have linked PAC to aspects ranging from anatomical structure to

cognitive function. For example, theta-gamma coupling in the hippocampus has been suggested to constitute a neuronal correlate of learning. Also, altered PAC has been linked to brain disorders such as Parkinson's disease and schizophrenia. As a consequence, the assessment of PAC has become standard in the analysis of electrophysiological signals such as EEG, ECoG and LFP. However, different groups have developed different metrics to measure PAC that differ in the way PAC is defined and thus may not necessarily produce similar outcomes. For instance, while some groups define PAC intensity as the phase consistency between the amplitude and phase time series, others consider PAC as the magnitude of the amplitude variation within a phase cycle. These methodological aspects should be taken into account when interpreting PAC measurements, especially when comparing results from different labs. To provide a guide for interpreting PAC measures, we performed a systematic analysis using synthetic signals, which were parameterized by: the frequency of the modulatory and modulated signals; the amplitude of the modulatory and modulated signals; the magnitude of the amplitude variation of the modulated signal; the modulatory phase; the modulation width; the signal-to-noise ratio; and the consistency of the modulation across phase cycles. We have studied 12 published PAC metrics including: modulation index (MI); height ratios; power spectral density (PSD) of the amplitude envelope; mean vector length (MVL); phase-locking value (PLV); correlation coefficients; the general linear model (GLM); coherence between the amplitude envelope and the modulatory signal; and amplitude weighted phase locking factor (wPLF). Our analysis reveals that PAC metrics are differently affected by variations in signal parameters. Nevertheless, we could still classify the metrics into clusters according to their robustness to specific parametric changes. Based on these results, we propose the use of complementary metrics from different clusters for a more accurate assessment of PAC. As a by-product of this research, we organized the source code into a single MATLAB/Octave toolbox, named COMODO, which is freely available at http://neuro.ufrn.br/comodo. COMODO allows PAC analysis with a single-line command and the quick switching between metrics. Finally, new metrics can be added into COMODO using available templates.

Disclosures: C. Rennó-Costa: None. **A.B.L. Tort:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research on Innovative Areas "Bio Assembler" (23106008) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Research Fellowship of the Japan Society for the Promotion of Science (JSPS) for Young Scientists, Japan

Title: Construction of *in vitro* neuronal networks by assembling single neural cells using a microfabricated cell-handling device

Authors: *S. YOSHIDA, S. TAKEUCHI;
The Univ. of Tokyo, Tokyo, Japan

Abstracts: This paper describes a method for assembling *in vitro* neural cells with control over morphology to engineer geometrically controlled neuronal networks. Engineering of *in vitro* neuronal netoworks has long been pursued in the microengineering field by controlling chemical/physical microenvironments around neural cells using microfabrication techniques. The advantages of the microengineering-based *in vitro* neuronal network construction are that we can define network structures by micropatterns of cell-adhesive regions and record electrical signal by embedded microelectrodes, which facilitate analyses of neural information processing. However, it is still challenging to engineer neuronal networks with full control over morphology and connectivity of individual neural cells that is essential for individual cell-based analyses. Here, we propose a method for engineering individual cell-based neuronal networks by controlling morphology and connections of single neural cells using a microfabricated reconfigurable plate array. We fabricated the cell-adhesive micro-plates in the shape of a soma-sized circle and neurite-sized lines radiated from the circle, arrayed on a cell non-adhesive background. When we seeded neural cells on the micro-plate array, some single cells were isolated on single micro-plates with their somas localized on the circle parts and their neurites grown along the line parts. Consequently, some single neural cells which were morphologically defined by the shape of the microplates could be found in the microarray. Morphologically defined single neural cells were able to be selectively assembled into desired geometries by micromanipulation. We observed that assembled neural cells continued growth to form cell-cell contacts that are necessary for synaptogenesis and neural circuit formation. The advantages of this method over previous microengineering methods are controllability of individual soma position and neurite number. We envision that our technique would be a useful tool in individual cell-based analyses of synaptogenesis and neural information processing.

Disclosures: **S. Yoshida:** None. **S. Takeuchi:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Fondation Bertarelli

Nano-tera (20NA21_145923)

Title: Stretchable array for epidural and subdural electrocorticogram recordings in freely moving rodents

Authors: *A. HIRSCH[1], N. PAVLOVA[2], I. MINEV[3], Q. BARRAUD[2], J. GANDAR[2], G. COURTINE[2], S. P. LACOUR[3];
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Abstracts: Electrocorticography (ECoG) uses electrode array placed over the cerebral cortex to record electrophysiological signals. Short-term implantation of ECoG arrays is commonly used at the clinic for brain mapping and pre-surgical localization of epileptic seizure. Recent studies have demonstrated that ECoG arrays may support the design of brain machine interfaces with stable performances for extended durations. These new developments have created a need for chronic, biostable arrays with high electrode density. Micron scale ECoGs, termed μ-ECoG, are miniaturized array prepared with microfabrication techniques and implemented on thin plastic films. Implants prepared on <15 μm thick plastic substrate conform the complex surface of the brain. However, their local mechanical mismatch with delicate brain tissue may impede long-term, chronic use. Here, we introduce an alternative design where the electrode array is made of ultra-thin and elastic metal film embedded in a soft, elastic silicone. The soft μ-ECoG array hosts 3x3 electrodes distributed over 2.25mm^2. The microfabrication approach also allows the implant design to be customized and scaled up to larger surface areas. Combined with optimized surgical techniques for chronic epidural and subdural implantation in rats and mice, we obtained robust recording of neuronal activity in awake, freely behaving animals for extended periods of time. Post-mortem histological evaluations showed improved biotolerance of the soft implant with brain tissue compared to standard μ-ECoG technologies. Moreover, the transparency of the silicone elastomer allows to combine soft μ-ECoG recordings with local photostimulation. The resulting chronic, bi-directional optoelectronic neural interface opens new avenues for the design of closed-loop neuromodulation therapies. Funding: Fondation Bertarelli and FNS nano-tera.ch

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Poster

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Program#/Poster: 661.29/VV81

Topic: G.04. Physiological Methods

Title: Bio-coating to improve long-term performance of chronic intracortical implants

Authors: ***S. DE FAVERI**[1], E. MAGGIOLINI[1], E. CASTAGNOLA[1], A. ANSALDO[1], D. RICCI[1], L. FADIGA[1,2], F. BENFENATI[1,3];
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Abstracts: Intracortical microelectrodes have been widely used in animal neurophysiology to chronically record neural activity, providing high precision in detecting particular groups of neurons and allowing single unit activity (SUA) recording. Unfortunately, it is widely known that intracortical implants lose their recording ability over time because of the inflammatory reaction that occurs around the implanted device and therefore the quest to solve or, at least reduce, their invasiveness is very strong. In a previous work, we were able to assemble a biocompatible device by seeding living cells on the microelectrode shaft and embedding it in a soft fibrin layer. Moreover, we demonstrated that fibrin 'per se' is highly biocompatible, forms a uniform coating of controllable thickness, does not swell, does not alter the electrochemical properties of the microelectrode and allows good quality recordings. Finally, we demonstrated that fibrin reduces the number of reactive astrocytes and is fully reabsorbed by the surrounding tissue within seven days after the implant. In the present work, we evaluate the chronic performance of our bio-inspired hybrid microelectrode after the implant in rat cerebral cortex. The device is coated with autologous fibroblasts embedded in a fibrin layer. Autologous fibroblasts are used to mimic the host tissue and immunologically hide the microelectrode to further increase the lifetime of the implant. We investigate the tissue reaction due to the presence of bio-coating by immunofluorescence at increasing time intervals from the implant and the quality of neural recordings over time. The results show that the electrochemical properties of the microelectrode are not affected by the bio-coating, the bio-inspired hybrid microelectrode markedly reduces the extent of the tissue reaction and its chronic performances are improved. In

conclusion, the present method shows a good potential to improve the biological integration of neural devices for achieving successful long-term neural implants.

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Poster

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Program#/Poster: 661.30/VV82

Topic: G.04. Physiological Methods

Title: *In vitro* recording of hippocampal gamma oscillations with multi-electrode arrays for CNS drugs characterization

Authors: *E. STEIDL, R. TEYSSIE, M. GLEYZES, F. MADDALENA, H. SAVINEL, B. BUISSON;
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Abstracts: The need of translational biomarkers is an urgent need for many CNS Drug Discovery programs. Neuronal network oscillations in the gamma range contribute to cognitive function, whereas it has been shown that they are disturbed in some psychiatric disorders, such as Schizophrenia. Gamma Oscillations are recorded both in the Electro-encephalogram (EEG) of human subjects and rodents. A few publications have illustrated that Gamma Oscillations could be triggered in acute brain slices recorded *in vitro* with glass electrodes. Using Multi-Electrode Arrays (MEA) recordings we demonstrate that Kainate elicits Gamma Oscillations in acute rat brain hippocampal slices in a dose-dependent manner, for concentrations ranging from 10 to 300 nM. Maximal Kainate-induced oscillatory activity is observed at 100 nM, and strongly decreases at 300 nM. Oscillations in the gamma range are characterized by calculating their power spectrum (using a fast Fourier's transform algorithm, and considering the 20-80 Hz range) and their amplitude. Thanks to MEA recordings, with electrodes covering the whole hippocampal slice area, we illustrate that Gamma Oscillations are of larger amplitude specifically in the CA3 region. Under 100 nM Kainate exposure, the oscillations develop over a 20 minute time-window and become steady for at least a 1-hour additional period. Network oscillations depend on synchronous and rhythmic fluctuations of membrane potentials of populations of neurons, defining temporal windows of increased and reduced excitability. GABAergic interneurons play a key role in the generation of Gamma Oscillations, by synchronizing the activity of the

ensemble of neurons of the hippocampal network. In accordance with these findings, 1 μM Picrotoxin (a selective GABA-A receptor antagonist) strongly inhibits Gamma Oscillations elicited by 50 nM Kainate. In addition, compounds that modulate the excitation-inhibition balance also modulate Kainate-induced Gamma Oscillations: NMDA receptors antagonists, such as Ketamine (100 μM) and MK-801 (20 μM) potentiate Gamma Oscillations. Finally, we demonstrate that Clozapine (30 μM; a Serotonin and Dopamine receptors antagonist used as antipsychotic) decreases the oscillations amplitude and also strongly decreases the strength of Kainate-induced Gamma-Oscillations. In conclusion we have established a robust and rapid *in vitro* assay based on MEA recording of acute hippocampal slices that allows documenting the activity of well-known CNS drugs and to profile new chemical entities for CNS-related disorders.

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Poster

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Program#/Poster: 661.31/VV83

Topic: G.04. Physiological Methods

Title: High fidelity biopotential recordings in mice using a novel telemetry implant

Authors: *S. MALPAS, S. LAU, D. RUSSELL, D. MCCORMICK, S.-J. GUILD, D. BUDGETT, M. KONDO;
Millar Inc, Auckland, New Zealand

Abstracts: We have developed a fully implantable telemetry device for use in mice. The device is capable of sampling biopotential signals (EEG,EMG,ECG) at 2000 Hz. The device has a volume of 1.45 cc meaning it is suitable for mice over 20 gm in weight. An novel aspect to the device is that it contains no battery. Power to run the implant is provided by a wireless power pad placed under the cage of the animal. This provides continual operation of the telemeter during a range of normal animal behaviors. We have assessed the ability of this device to record biopotential signals over many weeks. We believe this technology offers new experimental paradigms to be explored.

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